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The journal deals with all areas of plant pathology, including epidemiology, disease control, biochemical and physiological aspects, and utilization of molecular technologies. All types of plant pathogens are covered, including fungi, nematodes, protozoa, bacteria, phytoplasmas, viruses, and viroids. Papers on mycotoxins, biological and integrated management of plant diseases, and the use of natural substances in disease and weed control are also strongly encouraged. The journal focuses on pathology of Mediterranean crops grown throughout the world.

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E-mail: laura.mugnai@unifi.it

Richard Falloon – Bio-Protection Research Centre, PO. Box 84, Lincoln University, Canterbury, New Zealand
Phone: +64 (3) 325 6400 - Fax: +64 (3) 325 2074
E-mail: richard.falloon@lincoln.ac.nz

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DiSPAA - Sez. Patologia vegetale ed entomologia, Università degli Studi, Firenze, Italy
E-mail: phymed@unifi.it, Phone: +39 055 2755863/861

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Rice blast forecasting models and their practical value: a review

Dimitrios Katsantonis1, Kalliopi Kado glutou1, Christos Dramalis1 and Paui Puigdollers2

1 Hellenic Agricultural Organization - DEMETER, Plant Breeding and Genetic Resources Institute, Thermiti-Thessalonikis, Ellinikis Georgikis Scholis, Greece
2 IRIS Parc Mediterrani de la Tecnologia, Avda, Castelldefels, Barcelona, Spain

Summary. Rice, after wheat, is the second largest cereal crop, and is the most consumed major staple food for more people than any other crop. Rice blast (caused by Pyricularia oryzae, teleomorph Magnaporthe grisea) is the most destructive of all rice diseases, causing multi-million dollar losses every year. Chemical control of this disease remains the most effective rice blast management method. Many attempts have been made to develop models to forecast rice blast. A review of literature of the rice blast forecasting models revealed that 52 studies have been published, with the majority capable of predicting only leaf blast. The most frequent input variable has been air temperature, followed by relative humidity and rainfall. Critical factors for the pathogenesis, such as leaf wetness, nitrogen fertilization and variety resistance have had limited integration in the development of these models. This review reveals low rates of model application due to inaccuracies and uncertainties in the predictions. Five models are part of current operational forecasting systems in Japan, Korea and India. Development of in-field rice-specific weather stations, along with integration of leaf wetness and end-user interactive inputs should be considered. This review will be useful for modelers, users and stakeholders, to assist model development and selection of the most suitable models for the effective rice blast forecasting.

Key words: leaf disease, neck disease, pathosystem, prediction, leaf wetness.

Introduction

Rice (Oryza sativa L.), is one of the main world staple food crops. Although it is predominant in Asia, this crop has also been cultivated in Europe since the 15th century, mainly in Mediterranean countries including Italy, Spain, Portugal, Greece, and France (FAO, 2016). Rice blast, caused by the fungus Pyricularia oryzae Cavara [synonym P. grisea Sacc, teleomorph Magnaporthe grisea (Hebert) Barr], has been identified as one of the major rice cultivation constraints worldwide (Wang et al., 2015). The blast fungus is capable of infecting rice at any stage of the host life cycle. The disease appears early as white to grey/brown leaf spots or lesions, followed by nodal rot and as neck blast, which can cause necrosis and frequently breakage of the host panicles (Katsantonis et al., 2007). As rice production expanded through Asia, Latin America and Africa, the disease followed the expansion, and now occurs in more than 85 countries (Wang and Valent, 2009; Bregaglio et al., 2016). Under favourable conditions, rice blast can be the most important rice disease in China, Japan and the USA, causing severe damage to rice yields (Groth, 2006; Noguchi et al., 2006; Zeng et al., 2009). Severe blast has expanded due to use of susceptible cultivars, irrigation, large amounts of nitrogen fertilization, sandy light soils and rice fields surrounded by sheltering trees (Long et al., 2000; Greer and Webster, 2001; Groth, 2006). Moderate field infections can cause approx. 50% grain yield reductions. It has been estimated that P. oryzae destroys rice grain each year that would feed 60 million people (Devi and Sharma, 2010). Based on scientific/economic importance, the pathogen was characterized in 2012 as the most destructive fungus in the world. This was based on
The rice blast pathosystem

The rice blast pathosystem consists of two interrelated subsystems: the leaf blast pathosystem and the neck blast pathosystem (Teng et al., 1991; Teng, 1994; Sirithunya et al., 2002; Savary et al., 2006). Within each subsystem, vertical and horizontal host resistance operates. Thus, alloinfection from non-rice hosts and rice hosts that initiate epidemics is important for rice blast forecasting and disease management. Many leaf blast and neck blast simulation models have been reported, although their validation in diverse environments is still not definitive. Many empirical damage functions for blast losses are known, but their validation and use in disease management requires further analyses.

While the leaf blast and neck blast have common features, they have usually been treated separately, because of time discontinuity and because their relationship is not clearly defined. Separate models and forecast systems have therefore been developed for each pathosystem, since leaf blast predictions do not always cover neck blast. Alloinfection in each subsystem is thought to occur with inoculum from rice plants in the immediate vicinity, which have been successfully infected, or from non-rice hosts of the pathogen. Once alloinfection has occurred with an initial amount of disease, then disease severity increases via autoinfection (Van der Plank, 1963).

Relationships between leaf and neck blast have been partially documented, while many questions still remain unanswered since conclusions are controversial (Ou, 1985; Hwang et al., 1987; Bonman, 1992; Zhu et al. 2005; Puri et al., 2009; Ghatak et al., 2013). One reason for contradictions in the correlation between leaf and neck blast is that very severe leaf blast, which causes plant senescence and panicle death, reduces the chances of developing neck blast. Although quantitative resistance against leaf blast is positively correlated with quantitative resistance to neck blast, some cultivars may be resistant to the disease on leaves, and relatively susceptible on panicles. Pyricularia oryzae conidia depositing onto panicle spikelets are the blast epidemic event considered to be more stochastic, driven by chance, than deterministic (Ishiguro and Hashimoto, 1991; Koizumi and Kato, 1991). Ishiguro and Hashimoto (1988) reported that although large numbers of conidia are released from lesions on leaves, they may or may not produce panicle blast infections even under favourable environmental conditions.

Environmental conditions and meteorological variables

Rice blast, is favoured by particular air and soil temperatures, relative humidity (RH), hours of continuous leaf wetness (LW), degree of light intensity and duration and timing of dark periods, all of which have been considered as very important for disease development. Many studies have reported ranges...
and optimum conditions for the development of the disease. An overview of these conditions outlined in different studies is presented in Table 1.

The life cycle of *P. oryzae* begins with the deposition of conidia on rice plants. The conidia become tightly attached to the hydrophobic rice leaf surfaces in LW conditions (El Refaei, 1977). Mature lesions can produce conidia when RH is greater than 89%. High sporulation potential is possible at 20°C (Kato *et al.*, 1970; Kato, 1974; Kato and Kozaka, 1974; El Refaei, 1977). Sporulation is also favoured by cultivation of rice in aerobic soils or wetlands by long duration of LW due to drizzle or dew disposition, by little or no wind at night and by night temperatures between 17 and 23°C (Webster and Gunnell, 1992). Suzuki (1969c) observed that water is necessary for conidium discharge; the more water droplets retained on infected leaves, the more conidia are released. Manandhar *et al.* (1998) concluded that seedlings grown under low temperature conditions (15 to 20°C) did not develop blast lesions, but when the same plants were transferred into warmer temperatures (25 to 30°C), blast lesions were detected. Numbers of conidia produced varied from 80,000 per spikelet lesion to 280,000 per neck node lesion, and sporulation potential is also related to the level of partial resistance in the host (Yeh and Bonman, 1986; Castaño *et al.*, 1989). Released conidia float under the rice plant canopy and then escape into the air above the canopy. After successful host invasion, the fungus colonizes host tissue, and visible symptoms appear in 5 d under favourable conditions (Öu, 1985).

**Table 1.** Range and optimum environmental conditions which favour rice blast development, as reported in the literature.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Stage</th>
<th>Range</th>
<th>Optimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf wetness</td>
<td>All stages</td>
<td>Always required</td>
<td></td>
</tr>
<tr>
<td>Air Temperature</td>
<td>Appressorium germination</td>
<td>10–33 °C</td>
<td>25–28 °C</td>
</tr>
<tr>
<td></td>
<td>Appressorium formation</td>
<td>21–30 °C</td>
<td>28 °C</td>
</tr>
<tr>
<td></td>
<td>Lesion formation (wet leaves)</td>
<td></td>
<td>4–5 d at 25–28 °C</td>
</tr>
<tr>
<td></td>
<td>Mycelium growth</td>
<td>8–37 °C</td>
<td>28 °C</td>
</tr>
<tr>
<td></td>
<td>Mycelium survival for 18 months</td>
<td></td>
<td>-30 °C</td>
</tr>
<tr>
<td></td>
<td>Sporulation</td>
<td>9–35 °C</td>
<td>25–28 °C</td>
</tr>
<tr>
<td></td>
<td>Dispersal of conidia</td>
<td></td>
<td>20.5–21.8 °C</td>
</tr>
<tr>
<td></td>
<td>All stages at night</td>
<td>17–22 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td></td>
<td>Host blast susceptibility</td>
<td>10–30 °C</td>
<td>25–28 °C</td>
</tr>
<tr>
<td>Soil temperatures</td>
<td>Rice seedlings</td>
<td>20–30 °C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult plants</td>
<td>18–24 °C</td>
<td></td>
</tr>
<tr>
<td>RH (air)</td>
<td>Mycelium growth</td>
<td></td>
<td>93 %</td>
</tr>
<tr>
<td></td>
<td>Conidium germination</td>
<td>89–96 %</td>
<td>93 %</td>
</tr>
<tr>
<td></td>
<td>Dispersal of conidia</td>
<td></td>
<td>90 %</td>
</tr>
<tr>
<td></td>
<td>Disease development</td>
<td></td>
<td>93.95 %</td>
</tr>
<tr>
<td>Rainfall</td>
<td>All stages (direct effect)</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
<tr>
<td>Sunlight</td>
<td>Lesion formation</td>
<td>Night hours</td>
<td></td>
</tr>
<tr>
<td>Near-UV light</td>
<td>Germtube length</td>
<td>366–340 nm</td>
<td>366 nm</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td></td>
<td></td>
<td>Ambient +200–300 μmol mol⁻¹</td>
</tr>
</tbody>
</table>
Rice blast management

Modern rice cropping practices in Europe include application of highly active nitrogen (N) fertilizers, such as urea (46% N). However, in conventional rice cropping, such highly active fertilizers are not recommended due to their breakdown effects on field resistance to blast (Ou, 1985; Freitas et al., 2010). Management of blast has been extensively investigated, where different disease management strategies have been examined. These include: applying antagonistic *Pseudomonas*, *Bacillus* and *Streptomyces* spp. for biological control, (Prabavathy et al., 2006; Tendulkar et al., 2007; Karthikeyan and Gnanamanickam, 2008; Goud and Muralikrishnan, 2009; Filippi, et al. 2011; Khalil et al., 2014; Meng et al., 2015); using disease-resistant cultivars (Tokunaga, 1965; Villareal et al., 1981; Koizumi and Kato, 1987); reducing N fertilizers (Ou, 1985; Long et al., 2000); treating seed grains with chemicals (Yokoyama, 1981; Teng, 1994); using organic manure (Obilo et al., 2012); applying triterpenoid glycosides derived from alfalfa (Abbruscato et al., 2014); using neem seed extracts (Sireesha and Venkateswarlu, 2013), and using essential oils or extracts with antifungal properties (Sun et al., 2014). Furthermore, other disease management methods have been reported, even when some exceptional techniques were introduced. For example, fan-forced wind into rice crop canopies to favour leaf dryness (Taguchi et al., 2014), and intercropping with wild species (Wang et al., 2007) have been tested. However, rice blast has never been eliminated from a region where rice is grown. A single change in crop management or in the way host resistance genes are deployed can result in significant disease losses, even after many years of successful disease control (TeBeest et al., 2007).

Fungicide applications remain the dominant practice for controlling rice blast, sometimes using environmentally harmful chemicals or inducing fungicide resistance among pathogen populations (Todorova and Kozhuharova, 2010). However, the number of the available fungicidal active ingredients is limited (Prabhu et al., 2003; Kunova et al., 2014; Chen et al., 2015), since rice blast control does not attract appropriate interest of agrochemical companies. In a study in India, ten common active ingredients were tested for efficacies against rice blast, including dithane, carbendazim, propiconazole, mancozeb, wettable sulphur, thiophanate methyl, benomyl, edifenphos, kitazine and tricyclazole. Only edifenphos, kitazine and tricyclazole were effective for rice blast control, and only tricyclazole increased crop yield (Ganesh et al., 2012). This chemical is a melanin biosynthesis inhibitor (Chen et al., 2015), and was released in 1975 by Eli Lily/Dow for rice blast control, although initially suspected to have limited success because fungicide resistance in *P. oryzae* had been observed in China and Italy (Zhang et al., 2006; Titone et al., 2015). Nevertheless, this chemical remains the most efficient and most widely used blasticide among European rice growers, although it had to be withdrawn from EU use in March 2009, with a grace period expiring in March 2010.

Several concerns and questions have been raised regarding the environmental and human health impacts of tricyclazole along with the existing EU MRL. The fungicide is toxic (oral acute LD$_{50}$ in mice = 245 mg kg$^{-1}$), and it has a long label-recommended residual period (54 d before harvesting; Froyd et al., 1976; Tokousbalides and Sisler, 1978; Morton and Staub, 2008; EFSA, 2013; Gosetti et al., 2014; Arora et al., 2014; Fattahi et al., 2015). In the EU, tricyclazole is banned but remains in circulation through the issue of 120 d short registrations at national levels, after demonstration of the effectiveness presented in the Commission. Currently, tricyclazole is banned from use in European rice cultivation. The EU MRL is 1.0 mg kg$^{-1}$, while in USA tricyclazole is banned. However, the USA import tolerance for the chemical is 3.0 mg kg$^{-1}$ (http://globalmlr.com, assessed in 2016). Nevertheless, systemic fungicides are widely used to protect rice against leaf and neck blast when applied at the correct stage, to give optimum control with reduced environmental impact. The pesticide rate, and time and method of application depends on the information derived from accurate and timely forecasting of environmental conditions that are favourable for rice blast development.

Rice blast forecasting models

Disease forecasting allows prediction of probable outbreaks or increases in disease intensity, allowing if, when, and where a particular disease management practice should be applied (Agrios, 2005). Disease forecasting systems are based on assumptions concerning the particular pathogen’s interactions with the host and the environment, the “disease triangle” of “virulent pathogen”, “susceptible host” and “favourable environmental conditions”.

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There is no comprehensive way to classify all the disease models and modelling approaches used in agriculture. Researchers have initially indicated that most epidemic models are either analytic or simulations (Teng, 1985; Berger, 1989). An analytic model is simple, often with one equation with few biological variables, which can frequently be mathematically solved. Simulation models usually each comprise a series of equations that describe the behaviour of subsystems, and explicitly account for the influence of the environment at the subsystem level. They cannot commonly be solved using analytical (mathematical) techniques and require numerical solution with computer algorithms. Berger (1989) observed that some researchers (e.g., Teng and Zadock, 1980) blended these two approaches, starting with analytic models and gradually increasing the degree of realism and the representativeness of the real world until each model was no longer amenable to an analytical solution.

In rice blast forecasting, Japanese research primarily considered inoculum intensity as determined by spore traps and plant predisposition (Yamaguchi, 1970). Predisposition to infection related to biological and ecological characteristics of plants for disease progression and degree of occurrence. In Thailand, spore trapping was established in blast-prone sites using trap plants instead of spore samplers. Disease severity was assessed on susceptible cultivars used as trap plants and effects of environment on variations in severity were evaluated. However, in the Philippines Pinnschmidt et al., (1993) reported variations in the conidium numbers trapped by trap plants, compared to electronic and conventional spore trapping devices, due to weather effects. Similarly, viability of *P. oryzae* conidia from a spore trap differed from plant exposure because of environmental variations where spores were exposed prior to sampling (Bonman et al., 1987; Pinnschmidt et al., 1993).

Another approach was used for forecasting rice blast in India. Researchers had used information derived from planting susceptible cultivars at different times for several years (Chaudhary and Vishwadhar, 1988; Padhi and Chakrabarti, 1981). Similarly, Manibhushanrao et al. (1989) further studied effects of continuous planting of susceptible cultivars and weather on population structure of *P. oryzae*, to improve existing forecasting methodologies in that country.

The relationships of weather to above-canopy conidium numbers and plant predisposition to infection has been explored with the aid of computer modeling. Several statistical techniques have been used to develop reliable predictions. Models developed in Japan (Chiba, 1988; Uehara et al., 1988; Ishiguro and Hashimoto, 1988, 1989; Ishiguro, 1991) were considered as extensive rice blast forecasting packages. Deterministic mathematical functions that relate weather conditions to leaf blast development via regression analysis, and stochastic probability models for panicle blast, were used to improve understanding of pathosystem dynamics. Regression analysis provided an excellent way of characterizing the environment as a few meaningful factors (Campbell and Madden, 1990). In Korea, computerized blast forecasting systems had also been implemented based on the relationship between aerial numbers of conidia, leaf blast infection, and meteorological variables as revealed by regression analysis (Kim, 1987; Kim et al., 1987; Kim et al., 1988; Lee et al., 1989; Kim and Kim, 1991). Regression analysis had also been applied to derive forecasting models in Iran (Izadyar and Baradaran, 1990), the Philippines (El-Refaei, 1977), India (Manibhushanrao et al., 1989; Tilak, 1990), China (Zhejiang Research Group, 1986), and Taiwan (Tsai, 1986).

Path coefficient analysis is a technique in multivariate regression technique that is potentially useful in choosing which weather variable is the best disease predictor. This approach could identify direct and indirect effects of factors on disease without the confounding influences caused by multicollinearity. The analysis had two major components: the path diagram, and the decomposition of observed correlations into a sum of path coefficient terms representing simple and compound paths (Johnson and Wichern, 1992). These features enabled measurement of the direct and indirect influences of one variable upon another. Mohanty et al. (1983), using path coefficient analysis, positively correlated leaf angle, leaf pubescence, epicuticular wax and quantity of deposition of conidia with disease incidence. Torres and Teng (1993), similarly using path analysis, positively correlated leaf and neck blast with plant height and percentage of unfilled grains, while a significant effect of both symptoms was reported on plant yield reduction. Furthermore, they concluded that under field conditions, yield losses to rice blast could be estimated with more than 70% confidence through knowledge of the disease leaf area at the end of tillering stage and neck blast at harvesting.
Most rice blast forecasting models related weather variables to the occurrence and the development of disease, using statistical procedures. The choice of weather variables was mainly influenced by epidemic development. This is essential for successful application of forecasting schemes to wide-scale production areas. Table 2 presents an overview of forecasting models, which are categorized by weather variable inputs and the prediction type outputs.

Brief descriptions of the published models are presented in the next three sections, which represent the three forecasting category types: leaf blast, leaf and neck blast, and neck blast. Each section indexes the models according to prediction type, in chronological order of publication.

### Leaf blast forecasting models

Leaf blast is the first major symptom that occurs following *P. oryzae* invasion. Forecasting favourable conditions for leaf blast is critical for early control and management of the disease. Thus, most published models aim to forecast leaf blast.

#### Decade 1970

In the 1970s and 1980s in Japan, researchers taking advantage of developments in computer hardware and software programming reported the development of computer simulation models to forecast rice blast (Fukuoka Agricultural Experiment Station, 1975; Hashimoto *et al.*, 1982, 1984; Oota, 1982; Takai *et al.*, 1985; Ishiguro 1986). However, these models were insufficient for quantifying the dispersion and the deposition of *P. oryzae* conidia within rice canopies, which is an important stage for the disease development (Koizumi and Kato, 1991). Limited information could be retrieved from the literature, since these studies were published in Japanese and the original papers were difficult to locate.

El Refaei (1977), in the Philippines, used data from blast nursery trials to develop several linear regression equations. He separately related numbers of lesions per seedling to weather variables, such as dew period, mean day or night temperatures, mean day or night RHs, and rainfall, along with airborne inoculum density. When conidia were incubated in water, an increase in germination was observed at optimum temperatures between 20 and 25°C. The model could forecast leaf blast 5 d in advance. The set of equations showed exponential relationships between the disease, dew duration in hours and aerial conidium concentrations. However, this work was limited to nursery experiments. Furthermore, negative coefficients in the equations could not be biologically interpreted, and plant growth and ontogenetic changes in susceptibility were neglected.

An approach was developed by Yoshino (1979) in Japan, that has continued to be used. This determined *P. oryzae* infection periods, evaluating weather conditions every hour, and produced hourly results that indicated if the conditions would result in successful infections. The model was in two parts. The first contained three favourable conditions for successful conidium penetration and therefore successful infections:

1) the moving average of air temperature during past 5 d is 20-25°C
2) the rainfall to be below 4 mm h⁻¹, and
3) the continuous wet period >4 h than the base wet hours, calculated by the equation below:

\[
\text{Base wet hours} = 60.09 - 4.216 \times \text{temp}_{\text{wet}} + 0.08858 \times \text{temp}_{\text{wet}}^2,
\]

(\text{where temp}_{\text{wet}} is the air temperature when the leaves are wet)

The second part estimated the number of “infection hours”, the hours where the three conditions of the first part are true. The infection hours for each day determined by the model were accumulated for 1 d, in order to calculate the daily infection warning hours (DIWH). The DIWH was categorized into four risk levels: 1) Zero Risk, DIWH = 0 h; 2) Low Risk, 1 h ≤ DIWH < 3 h; 3) Intermediate Risk, 3 h ≤ DIWH < 6 h; and 4) High Risk, DIWH ≥ 6 h. The Yoshino model is still used as part of three forecasting systems: a commercial system developed in Austria (http://www.fieldclimate.com), and in the models published by Kang *et al.*, (2010) and Kim *et al.*, (2015). Yoshiro’s approach has also been adopted in five other published models, including those of Koshimizu (1983; 1988) and Hayashi and Koshimizu (1988); Tastra *et al.* (1987); Kim *et al.* (1987; 1988); Lee *et al.* (1989); and Ishiguro and Hashimoto (1988; 1989; 1991) and Ishiguro (1991).

#### Decade 1980

Hashimoto *et al.* (1982; 1984) developed BLASTL, using published data in combination with their own,
Table 2. Characteristics of 52 reviewed rice blast forecasting models, including their input variables, outputs and current usage.

<table>
<thead>
<tr>
<th>Model references (alphabetic order)</th>
<th>Inputs</th>
<th>Outputs</th>
<th>Currently in use/Operational</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spore release</td>
<td>Sporulation</td>
<td>Spore penetration</td>
</tr>
<tr>
<td>Ashizawa et al., 2001</td>
<td>X X X X X</td>
<td>X X X X</td>
<td>X X X X</td>
</tr>
<tr>
<td>Ashizawa et al., 2005</td>
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<tr>
<td>Billoni et al., 2006</td>
<td>X X X X X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Bregaglio et al., 2016</td>
<td>X X X X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Calvero and Teng, 1991, 1992</td>
<td>X X X X</td>
<td></td>
<td></td>
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<tr>
<td>Calvero et al., 1996a</td>
<td>X X X X X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Calvero et al., 1996b</td>
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### Model references (alphabetical order)

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**Note:** Currently in use / Operational.
conducted in simulation units. This was probably the first simulation leaf blast model developed. Life cycle stages of *P. oryzae*, sporulation, conidium discharge, dissemination and deposition, blast infection and lesion development were simulated in relation to weather conditions, plant growth, leaf position, and host susceptibility as affected by weather, fertilizer application, plant or leaf age and leaf position. The dynamics of leaf blast were calculated as temporal changes in the number of lesions. Air temperature, rainfall, wind, sunlight duration and wetness period were used to feed the model, and additionally meteorological data were collected from the Automated Meteorological Data Acquisition System (AMeDAS). Time was advanced every 3 h. Leaf blast infection was measured by the number of lesions, while leaf area was assessed in field surveys. The model also included other variables, such as susceptibility index of leaves and initial inoculum dynamics, which were determined by observing the disease epidemics. This model was developed to assist farmers in applying control measures, and the model could predict leaf blast outbreaks in 7 d short-term forecasts. The model was tested in prefectures of Japan for several years, and was useful and practical. Since it contained a fungicide sub-model, it was also a practical tool for determining the timing and the efficiency of fungicide applications (Takai et al., 1985; Ishiguro et al., 1988). However, Ishiguro and Hashimoto (1990) concluded that BLASTL required further improvements, to estimate the parameters which were first determined by trial and error procedures. Furthermore, the model could be improved through integration of a module that included the initial prediction of leaf blast epidemics.

The rice blast simulation model BLASTCAST was developed by Ohta et al. (1982; 1987) in Japan, which was a plant disease simulator similar to that of Hashimoto et al. (1982; 1984). BLASTCAST involved variables such as conidium production, dissemination, attachment, penetration and blast severity. Additionally, it collected daily data on host variables, such as lesion formation, variability of resistance to leaf blast and lesion incubation period. Hourly recorded field meteorological data were also collected, including humidity, wind speed, precipitation and LW. The model gave satisfactory results in the years 1973-1976 and 1979-1981. The authors concluded that increasing the amount of input data and including rice varietal resistance would improve the model, although these developments have not been reported.

Koshimizu (1983; 1988) and Hayashi and Koshimizu (1988) developed BLASTAM as a software tool to predict rice leaf blast epidemics in Japan. This relied on hourly weather data collected from 840 sites from throughout the country using AMeDAS. The meteorological variables used were: air temperature, precipitation, (> 1 mm h$^{-1}$), sunshine duration and wind force. The model also used variables of LW period, mean temperature during LW and mean temperature of the five preceding days, along with other secondary weather variables, which met certain model criteria. The model first estimated LW conditions using AMeDAS, and subsequently determined the infection potential through relationships between the estimated LW condition and the surface air temperature. When evaluating the effects of climate change on LW, BLASTAM encountered many of the aforementioned difficulties that are typical of empirical models. The model classified favourable to unfavourable weather for infection, 7 d after the onset of the conditions. The BLASTAM approach was similar to that of the Yoshino (1979) model. The model is currently reported by the http://www.reigai.affrc.go.jp as operational for leaf blast prediction, using data from AMeDAs.

A forecasting model was also developed in Taiwan by Tsai and Su (1984) and Tsai (1986). This used multiple regression equations to analyze relationships between meteorological variables and the percentage of leaf area infected by *P. oryzae*, developing an early disease warning system. The equations contained three to four meteorological variables, such as average RH hours when RH was over 90 %, rainfall and number of rainy days. Model operation required that average RH, hours of RH over 90% and rainfall were the most influential factors for predicting blast severity. However, the model’s equations have not been further validated or used in rice fields.

The model PYRICULARIA described by Gunther (1986) was a systematic theoretical approach written in a Continuous System Modelling Program (1972). It was a polycyclic leaf blast simulation model developed using information available from the literature, and it derived structural data from experiments carried out in temperate ecosystems. The model simulated phases of the *P. oryzae* life cycle, including conidium formation, free and resident conidia, conidium deposition and germination, appressorium
formation and penetration, latent lesions, infectious lesions, and ageing of lesions. PYRNICULARIA accounted for plant growth, but neglected host susceptibility to the blast fungus, while the weather effects were simplified. Specific features depended on the chronological order of sequential events, and these were handled using "boxcar trains." The model could predict leaf blast until the end of active host tillering. However, the model was not validated against field data.

In China, 40-50% yield losses were observed from severe rice blast infections, and in some cases, 100% yield losses were found in severely infected fields (Wang et al., 2014). Although rice blast impacts are severe, few published prediction models have come from that country. The Institute of Plant Protection, Zhejiang Academy of Science, developed a computerized rice blast forecasting system (Zhejiang Research Group, 1986). Meteorological and biological factors affecting the P. oryzae and rice blast severity were related to field management, growing area, and cultivars, to establish a database. Stepwise regression analysis was used to predict disease indices based on 20 meteorological, biological and cultural factors.

Torres (1986) developed a leaf blast simulation model in the Philippines, by adding increasing complexity to a logistic growth function. The P. oryzae life cycle components used in the model were sporulation, conidium dispersal, landing and infection. Number of lesions per 100 cm² was used as the major component of host resistance, which was affected by plant age. Varietal differences in the number of developed lesions were observed for each leaf, but varietal ranking varied between the leaf assessments. Torres (1986) concluded that the factors which affected epidemic development were: plant age, which affected host susceptibility, and conidium deposition, temperature, dew period, crop row spacing and nitrogen fertilization. The model considered latent period and host area to be constant. Preliminary validation results revealed inconsistent prediction of rice blast epidemics. Torres (1986) identified the need to test varieties for both leaf and neck blast to evaluate their resistance patterns, and noted the need for further refining and validation at the International Rice Research Institute. No further improvements have been published.

Tastra et al. (1987), adopting and modifying the PYRNICULARIA model (Gunther, 1986), developed PYRNEW, dedicated to the upland rice farming systems of Indonesia. New variables were incorporated, including the effects of nitrogen fertilization and varietal resistance derived from field experiments. The preliminary results of model validation suggested the need for further development on structure and the stimulus-response relationships.

Kim et al. (1987; 1988), in Korea, developed a computer-based program for predicting rice blast occurrence, based on microclimatic events. It was tested as an on-site microcomputer in upland and flooded field plots. The battery-operated computer continuously monitored mean air temperature, hours of LW and hours of RH greater than 90%, and then interpreted the microclimate information in relation to rice blast development and displayed daily values using the scale 0-8 called Blast Units of Severity (BUS). Mean temperatures outside the range of 15 to 38°C were considered unsuitable for blast development. Temperatures of 19 to 29°C for a period more than 16 h were considered as highly favourable for blast development. The most favourable conditions (BUS = 8) were mean temperature between 23 and 26°C, with 24 h of LW and 24 h of RH greater than 90%. BUS values were calculated using algorithms employing logical functions that correlated disease to meteorological variables. Accumulated daily BUS values were highly correlated to blast development on the two rice cultivars grown in upland conditions, and were then used to predict disease progression. The model approach was similar to that of Yoshino (1979). The authors considered that accuracy improvement could be the inclusion of soil moisture for blast epidemics in upland conditions. This could also enable adaptation of PYRNEW for flooded conditions. Once effects of the soil moisture on blast development could be quantified, the microcomputer units could be retrofitted with soil moisture probes and the algorithm for BUS could be adjusted.

LEAFBLST, a computer simulation model (Choi et al., 1988), was developed based on the data derived from growth chamber experiments with one rice cultivar, and from previously reported data. The model consisted of modules that computed conidium germination, infection, latent period, lesion growth, and conidium production, dispersal, and deposition, as affected by weather factors. Input variables of the model were daily air temperature, relative humidity, rainfall, wind speed and LW. LEAFBLST was written
in FORTRAN IV, and included six input subroutines. These were: 1) conidium germination, 2) infection, 3) latent period, 4) lesion expansion, 5) conidium production, 6) dissemination. Another four output subroutines were also used, including: 1) for initialization, 2) leaf area calculation, 3) numerical and 4) graphic outputs. The results were tested on two rice nursery plots. Leaf blast progress was computed in terms of lesion number and disease severity. The model was successfully validated on two rice nursery datasets and in crops for only one rice cultivar. Ontogenetic and environmentally-associated changes in host susceptibility were not considered. Choi et al. (1988) concluded that LEAFBLST should be modified to accommodate incoming inoculum dispersed from surrounding infected fields, and to include temporal changes in host plant susceptibility. However, no further development of this model has been reported.

Decade 1990

A dynamic simulation model was developed by Koizumi and Kato (1991) at the National Agriculture Research Center in Tsukuba, Japan. This quantified dispersal and deposition of conidia over rice canopies. Microclimates inside rice cropping systems were considered. The simulation was based on data derived from the distribution of conidia from leaf lesions through sporulation and release. Wind velocity and turbulent diffusion coefficients were estimated at the canopy level. Conidium deposition and washing off during rain for every hour from 13:00 to 12:00 the next day were included. The model consisted of six subroutines, written in Microsoft FORTRAN, including: 1) weather, 2) canopy structure, 3) wind velocity and turbulence, 4) conidiophores and conidium formation, 5) conidium discharge and 6) residual conidium concentration. Experimental data were integrated using equations derived by previous publications (Uchijima, 1962; Inoue, 1963; Horie, 1981). Dispersal and deposition of conidia within or above rice canopies were simulated by modifying a model developed for barley (Legg and Powell, 1979). Suzuki (1969a) studied the effects of windspeed on the liberation, dispersion, and deposition of \textit{P. oryzae} conidia in a rice crop. Koizumi and Kato (1991) suggested that windspeed and leaf area indices could affect conidium production, and, consequently, conidium concentration in the air. These factors could influence the number of conidia attached on the leaves of susceptible rice plants.

Izadyar and Baradaran (1990) studied rice blast on five local cultivars transplanted four times with 6–7 d intervals, for 6 years in Iran. At every sowing date, minimum temperature and the number of days after transplanting (NDAT) were recorded until the appearance of leaf blast lesions. Regression models were generated to establish relationships between NDAT and both maximum leaf blast severity and minimum temperature. Model predictions showed increases in leaf blast severity due to decreases in the NDAT and increases in minimum temperature. There was a negative correlation between days after transplanting to appearance of leaf blast symptoms in the field and the average of minimum temperature during the same period.

An empirical forecasting model was developed in Thailand by Surin et al. (1991). Microscope slides from spore traps placed 80 cm above ground in several fields, were used to collect \textit{P. oryzae} conidia at each growth stage of the crops. The number of conidia was correlated with disease severity, in combination with the weather conditions. When conidia numbered more than five per slide, blast occurred in that field after a period ranging from 7 to 15 d. The model correlated rice varieties with climatic conditions, such as temperature, RH, rainfall, and the number of conidia and blast occurrence. Optimum conditions for rice blast development were considered to be RH of 90% or greater and temperature between 25 and 28°C. A method of estimating blast severity was developed by measuring blast on the top four plant leaves. The close relationship between severity on the 3rd leaf and the average severity on all leaves indicated that samples taken from the 3rd leaf could be used as the basis for fungicide application decisions, and for crop loss assessments. Direct guidelines were developed to assist the farmers to control the disease.

EPIBLA (EPIdemiology of BLAst) simulated incidence of blast in India, and made 7-d forecasts of disease progression in tropical rice cultivation areas of that country (Manibhushanrao and Krishnan, 1991). This model was developed using multiple regression equations. Daily values of maximum temperature and maximum RH were used as predictors of numbers of conidia in the air. The predicted conidium amounts, the minimum temperature and the amount of dew, summed and averaged over the 7-d period.
preceding disease onset, were used to estimate disease incidence. Three equations were proposed: one for predicting the number of airborne spores, and the other two for predicting disease progress. It was confirmed that disease susceptibility was related to plant age. Positive correlation was found between the amount of dew and minimum temperature. However, the model was developed using only two rice varieties, IR50 and IR20. Improvement of the accuracy of prediction required further reformulation using feedback from at least two growing seasons combining data derived from the field and from growth chambers.

BLASTSIM.2 was developed in the Philippines by Calvero and Teng (1991; 1992). This simulated leaf blast monocyte epidemics based on crop growth and weather conditions in different tropical rice management systems. The model had two main components: the blast simulation, in which the state values were computed, and the dew period simulation component, which predicted dew periods and the amount per day using the program DEWFOR (Luo and Goudriaan, 1991). BLASTSIM.2 followed the leaf blast factors such as, conidium production, release, deposition, and latency, pathogen penetration and colonization, and lesion production and development. Other included data were derived from interactive climatic, edaphic and agronomic factors considered to affect rice blast. The model was successfully validated in 1989 to accurately simulate leaf blast progressions in nursery trials with high correlation co-efficients. One limitation was that the model did not include a crop growth subroutine. After the trials, the authors concluded that BLASTSIM.2 could mimic the rice leaf blast pathosystem. However, further validation was needed in various locations, because data collections were derived only from nursery trials. Consequently, Luo et al. (1993) carried out blast surveys to determine the intensity of disease at specific locations, and assess whether models accurately estimated the disease. They included BLASTSIM.2 in their surveys. Also, GIS was used to superimpose the effect of UV-B radiation on BLASTSIM.2-generated blast progressions, converted into area under disease progress curve units. The GIS-generated raster maps of several Asian countries revealed possible blast prone areas. Their results were compared with actual blast incidence at those sites. The results confirmed that BLASTSIM.2 correctly simulated the expected blast-prone locations in tropical and temperate Asian countries. However, there are no reports of further development or use of this model.

EPIBLAST was published by Kim and Kim (1993) in Korea. The model was developed by collecting field rice blast epidemiological and meteorological data. The model comprised three groups of input variables: 1) meteorological (temperature, RH, rainfall, dew period and wind velocity); 2) plant physiological state (healthy, diseased and dead leaf area); and 3) epidemiological processes (inoculum potential, sporulation, conidium release and dispersal, penetration and incubation period). Validation tests of EPIBLAST during the 1990 crop season indicated that the model needed corrections for sporulation potential under natural conditions, to improve predictions to better fit actual leaf blast outbreaks. The accuracy of EPIBLAST was validated during 1991, and the model predicted field leaf blast epidemics. However, some fluctuations were observed, particularly when weather was changing rapidly, and Kim and Kim (1993) stated that further revision of the model was required.

A combined model simulation that studied effects of leaf blast epidemics on yield loses was developed by Luo et al. (1997). Historical daily weather data were collected from 53 locations in Japan, Korea, China, Thailand and the Philippines. Two simulation models were used: CERES-Rice, a growth simulation model, and BLASTSIM (Calvero and Teng, 1992). These were combined by linking the effects of leaf blast on rice leaf photosynthesis and biomass production. BLASTSIM was modified by adding new subroutines or modifying the existing ones. Two weather generators, derived from the Decision Support System for Agro-technology Transfer, were utilized to produce estimated daily weather data to run in the combined model. The two weather generators and the estimation methods were applied to produce a complete set of estimated weather data required by the combined model, including temperature, solar radiation, humidity, windspeed, rainfall, dew period, cloudiness and soil temperature. The combined model also simulated daily incidence and severity of leaf blast and crop growth parameters such as root weight, green leaf area, dead leaf biomass and grain weight. Thirty years of historic daily weather data were used as inputs to simulate blast epidemics for each temperature change based on the Monte Carlo method, for each of the generators for
every location. The outputs included disease severity, the area under disease pressure and yield loss. Temperature was the most sensitive variable in the model, while precipitation was insensitive. However, the ability to simulate rainfall effects to estimate dew formation and rice blast epidemics was limited. Luo et al. (1997) concluded that elevated temperature increased maximum blast severity and epidemics in cool subtropical zones, but inhibited disease development in warm humid subtropics. GIS graphics showing scenarios of blast epidemics for each country were produced from the simulated information for several locations for each country, using spatial interpolated methods. The model could not produce accurate yield loss forecasts because it failed to predict collar and panicle blast. No further development of this model has been published.

Decade 2000

In 2001 a simulation model was developed for forecasting leaf blast epidemics in rice multi-lines by Ashizawa et al. (2001). Very little information on this model can be retrieved as it was published in Japanese and is not available from the Web.

Lanoiselet et al. (2002) developed a different model approach to evaluate the risks of rice blast in Australia. Two climate simulation software programs, DYMEX and CLIMEX, were used to investigate risk of potential infection and sporulation of the rice P. oryzae. An area with typical climate for Australian rice cultivation was chosen for comparison to other foreign locations where rice blast occurs. Comparisons were carried out using temperature, RH and rainfall data. Additionally, a rice blast model was developed using the software DYMEX to predict the behavior of the pathogen in the rice-growing area of the country. The model was operated for the period 1988 to 1999, using the meteorological data of four representative Australian rice-growing locations. CLIMEX results were confirmed as the most suitable, and these highlighted the hypothetical threat of rice blast in Australia. This approach theoretical, while some validations were achieved for simulated data with real rice blast records in certain areas. However, the model needed datasets from real canopy conditions to give improved disease predictions.

Holcombe et al. (2003) specified the individuality of the P. oryzae pathosystem, considering the way the fungus invades host plants and propagates. They developed a simple model by applying hybrid computational techniques, using computer simulation and automated analysis to understand the behaviour of this complex biological system. They concluded that a fundamental problem was the understanding of the complex interactions between the different sub-systems. They have expressed doubts about capability of understanding and analyses of the model, even when it was correctly constructed. They also stated that long term research covering 5 to 10 years was required to build realistic models.

Ashizawa et al. (2005) developed BLASTMUL in Japan. This model modified BLASTL (Hashimoto et al., 1984). The model mimicked leaf blast epidemics in “Sasanishiki” and “Koshihikari” rice multilines, giving a very specific orientation. BLASTL was considered reliable. They stated that rice blast resistance was low in Japan, and that chemical control was the major disease management practice in Japan. For this reason, mixtures of near-isogenic lines (NILs) with different complete resistance (multilines) had been released. For the modification, new variables such as conidium dispersion and deposition were added to the model developed by Ashizawa et al. (2001). The new model calculated the numbers of lesions per crop subunit, for mixtures of susceptible and resistant NILs in given proportions, under various weather conditions. BLASTMUL was appropriate for evaluating rice mixtures for blast control in different locations and cultivars. The model could contribute to clarifying the stable use of blast resistance. However, the accumulated epidemiological data revealed the need to integrate more reliable variables in the model.

Kaundal et al. (2006) developed a model based on machine learning techniques for rice blast forecasting in India. They selected six significant weather variables, temperature (minimum and maximum), RH (minimum and maximum), rainfall and rainy days per week. They introduced a new forecasting method based on the powerful machine learning technique Support Vector Machines (SVM). This had been developed by Vapnik and coworkers, and was considered effective for general purpose supervised predictions (Cortes and Vapnik, 1995). Among the weather variables, rainfall was shown to be the best predictor, followed by relative humidity and rainy days per week. Temperature was found to have the least effect on disease development. This disagreed with most published models, where tem-
perature, especially low temperature, was indicated as one of the most critical variables for the disease development. Kaundal et al. (2006) concluded that the developed SVM was better for forecasting plant diseases than other existing machine learning techniques and conventional REG approaches. They have also developed an SVM-based web server for rice blast forecasting, the first of its kind, which can assist decision making. The server is available online at http://www.imtech.res.in/raghava/rb-pred/submit.html. The web-based model can predict leaf blast severity as percentage. Users input temperature, RH (minimum and maximum), rainfall and number of rainy days per week. However, percentage leaf blast severity output can be difficult to interpret where no limits and threshold information are provided.

Decade 2010

A forecasting model was published by Kang et al. (2010) describing an online information system for plant diseases based on weather data. This was developed for rice farmers in Gyeonggi-do in Korea, and is available at http://www.epilove.com. The information delivery system was based on a Linux server, using MySQL database, PHP and Java. Weather data are derived from a network of 82 synoptic and 627 automatic weather stations in Korea, collecting data at 1 h intervals. The input data are air temperature, RH and rainfall. The system generates hourly or daily warnings at the spatial resolution of 240 x 240 m. Interpolation of the weather data at this resolution was performed after evaluation. The leaf blast forecasting model was based on that of Yoshino (1979). Kang et al. (2010) concluded that the interpolation of rainfall and LW required improvement. They also highlighted that failure to estimate LW events based on the interpolated weather data was the main reason for low accuracy in the disease forecasting.

EPIRICE, a generic model for plant diseases, was developed by Savary et al. (2012) in Korea. This was coupled with GIS to map simulated potential epidemics of five major rice diseases globally, including leaf blast, brown spot, bacterial blight, sheath blight and rice turgo disease. The model used for the development of EPIRICE was based on that developed by Zadoks (1971), which forecast cereal rusts epidemics. The Zadoks model was modified by the addition of elements of plant growth, plant senescence and spatial disease aggregation. EPIRICE encompassed different hierarchy levels of a growing crop canopy, including disease sites on a leaf, whole leaves, tillers, plants, crop stands, world regions, and the world. The model was parameterized using reported data for each of the five diseases, and was combined with a few simplified growth stage characteristics. The model was linked to GIS, and crop establishment and daily historic climate data over a 2 year period. The data included temperature, precipitation, RH, dew point, solar radiation and wind speed. Other variables used were: sites, crop growth, epidemic onset, residence times, infection rate, age effect, temperature effect, wetness effect and aggregation. After the model’s successful simulations of epidemics, the authors used the rice crop as a model system. They showed that the same model could be used at different levels of the crop hierarchy to simulate and map potential plant disease epidemics at the global level. They also suggested improvements in three specific areas: 1) the treatment of spatial structure of disease epidemics, 2) the handling of epidemiological processes in vector-borne diseases, and 3) the limited published disease progress curves and basic information.

In India, the Central Road Research Institute (CRRI) operated a simple leaf blast forecasting system based on empirical predisposed factors, which interacted with rice varieties. Seedling, rapid tillering after transplanting, and flower emergence were identified as the plant stages most susceptible to rice blast. It was also concluded that leaf age influenced the host susceptibility; plants with old leaves were less susceptible to blast than those with young leaves. The critical range of temperature for conidium penetration and infection was in the range of 25 to 26°C. Conidium germination appressorium formation occurred within 6–10 hours at 20–30°C in the presence of LW. The formation of dew, light rainfall or the occurrence of fog provided the necessary water required for germination of conidia. Analysis of the intensity of infection included records from experiments over several years. Infection had occurred under natural conditions when the minimum temperature during the night was 26°C and below, with the concomitant occurrence of 90% RH and greater. These conclusions were verified by experiments leading to the development of a forecasting system to assist rice farmers.
Kim et al. (2015), in Korea, published a novel model approach, which modified EPIRICE (Savary et al., 2012). Their study involved two components: the modified EPIRICE and linkage to climatic change data, aiming to generate disease risk maps. Historical disease data and 1 km scale weather data were acquired for South Korea for 2002 to 2010. Additionally, the Yoshino model (1979) was used as a temperature effect module. Likely changes in the national disease probabilities were assessed under climatic change scenarios, to allow robust planning, while EPIRICE was calibrated and validated against the observed leaf blast incidence. They predicted daily climatic data based on the Intergovernmental Panel 4.5 on Climatic Change and Representative Concentration Pathways 8.5, while the outputs were displayed using GIS. The simulation predicted rice blast incidence epidemics until 2100. The authors concluded that likely magnitude of changes in disease risk in South Korea could be predicted. The model also estimated climate change impacts on crop losses from the disease and on disease control. Since this model was recently released, the authors suggested that more testing was required to validate the accuracy and integrity of the predictions.

Leaf and neck blast forecasting models

Japanese researchers were pioneers in the development of rice blast models due to the importance of the disease and the large quantities of agrochemicals used for the disease control in their country. Japan required elaborate forecasting to precisely determine the optimum time for applying fungicides to maximize profitable returns. The most original study on forecasting models was published by Kuribayashi and Ichikawa (1952). They studied the time relation between the number of conidia deposited on spore trap slides and severities of neck and nodal blast outbreaks for several rice varieties. An average of eight conidia was recorded for mild outbreaks, 24 for moderate outbreaks, and 175 for severe outbreaks. Many conidia were trapped in a region with severe blast outbreaks, while few or no conidia were trapped in a region with mild outbreaks. Data sets from 1934 to 1949 were used, and numbers of trapped conidia were correlated with blast severity for data derived from eight observatory stations at 5 d intervals. There were close correlations between conidium numbers and disease severity from July to September. It was concluded that spore trapping could provide reliable information for disease forecasting. Although questions were raised concerning calculations based on conidium trapping data at each station, combined data from eight stations could be used to forecast areas within a Nagano Prefecture. Similar forecasting attempts were made at many other prefectural experimental stations in Japan, and it was concluded that a developed formula for one region did not always fit another. This research was considered of great importance for Japanese rice growing. Many rice blast forecasting studies have since been published in Japan, based upon further knowledge of P. oryzae, the rice hosts and the environment.

Decade 1960

Ono (1965), also in Japan, developed a leaf and neck blast prediction model. This involved air-borne conidia in combination with sums of sunshine and a fertilizer index, using mean percent of sunshine, and temperature or precipitation, for forecasting leaf and neck blast outbreaks.

In India, Padmanabhan (1965) developed a model to formulate several forecasting rules. These were: 1) seedbed infection occurred when minimum temperature was 24 to 26°C for 4–7 d; 2) leaf blast occurred when minimum temperature was below 24°C for 4-5 days after transplanting and during tillering, and RH ≥ 90%; and 3) neck blast occurred when September-October conditions favoured leaf infection and temperatures were 20–24°C for a number of days coinciding with RH ≥ 90%. Severe leaf blast was necessary for neck blast occurrence.

Chiba et al. (1966) outlined a method for forecasting rice blast using field sheath inoculation. Variables of temperature, rainfall, sunlight and crop growth stage were correlated with disease severity, which was assessed each week by measuring the mycelium growth in rice sheath cells. A linear relationship was found between mycelium growth and disease severity, and a formula was proposed for the calculation of standard mycelium growth values. After testing predictions in the field, it was concluded that the standard value was related more to leaf blast than neck blast.

Suzuki (1969b, 1974) devised a rotary spore trap and determined that blast incidence was correlated with the number of spores collected. In earlier studies, Suzuki (1969c) found that when dry conidia ab-
sorbed water, they germinated within 2 h at temperatures above 16°C. The maximum number of conidia dispersed was detected in the middle of each night. Once conidia were discharged from conidiophores, they moved with the air flow. The number of conidia dispersing were indicated by an exponential formula, showing that the stronger the wind, the greater was conidium dispersal. For horizontal dispersion, the number of conidia dispersed in different wind velocities followed a log linear relationship with distance from an inoculum source. Almost all conidia were deposited near the source. Forecasting precision was improved by correcting for average wind velocity at the time of sampling.

Uehara (1985) in Japan used multivariate analysis techniques to classify regions according to occurrence of leaf blast in late July and neck blast from mid-September to early October. Seventeen years of data derived from 120 stations within paddy fields were used to correlate disease distribution with altitude. Leaf and panicle blast were shown to have similar distribution patterns, and panicle blast occurred in areas with mild leaf blast infections, when weather conditions were favourable after heading. This approach resembles the “pest zoning” concept proposed by Teng (1990).

Decade 1980

Uehara et al. (1988) tested BLASTAM (Koshimizu, 1983, 1988; Hayashi and Koshimizu, 1988) for forecasting leaf and panicle blast. Leaf blast occurrence was well-predicted, but not panicle blast. This indicated that hourly weather records should be used for disease forecasting. The model used daily weather data inputs supplied by AMeDAS. This system automatically recorded weather conditions, including wind direction and speed, types and amounts of precipitation, types and base heights of clouds, visibility, air temperature, humidity, sunshine duration and atmospheric pressure. BLASTAM could identify when and where favourable infection conditions occurred on a meso-scale. This extension service aimed to provide current and projected situations of local epidemics, and to recommend topical disease management advice for local rice growers. BLASTAM predictions were found to be reasonably accurate for leaf blast, but not panicle blast, so further improvements were needed. Although BLASTAM did not provide quantitative information on the disease progress besides predictions of disease outbreaks, it was useful in several prefectures of Japan. The theory was adopted that leaf blast epidemics start approx. 10 d after the first appearance of conditions favourable for infection. BLASTSAM predictions gave farmers enough time for disease management decision-making. Hourly weather recordings were also used as the basis for the forecasting. Nemoto and Ishiguro (2004) tested BLASTAM and BLASTL models (Hashimoto et al. 1982, 1984) in combination with AMeDAS, as a decision tool to identify rice blast favourable conditions in Japan. Their predictions were freely displayed on the Web.

The forecasting system of Ishiguro and Hashimoto (1988, 1989, 1991) and Ishiguro (1991) in Japan operated using stochastic functions to accurately predict leaf and panicle blast epidemics. In 17 cases, the leaf blast pathosystem was mostly described by deterministic equations generated from empirical data from previous laboratory and field studies. The framework of the model was very similar to BLASTL (Hashimoto et al., 1982, 1984), except that the panicle blast model was stochastic, while BLASTL was a deterministic model. This stochastic panicle blast simulation model (PBLAST) used the Monte Carlo method (Hammersley and Handscombe, 1964); conidium deposition and penetration were treated as stochastic processes, and each panicle was subdivided into small infection site units. A probability function was used for conidium deposition, with consideration of wetness duration and wetness-temperature, and the probability of penetration of each deposited conidium into an infection site unit was computed. This pathogen penetration approach was similar to the Yoshino model. Rice heading, fertilization, grain growth, susceptibility of each infection site, appearance and growth of lesions, panicle blast severity and yield loss were calculated daily. Conidium formation, discharge, dispersal, deposition, and pathogen penetration and colonization were calculated every 3 h. AMeDAS weather data, additional wetness duration data, and data of host development, variety and cultivation practices, as well as number of conidia formed on leaf lesions, were used as model inputs. Validation results were inconsistent, while the model required an extensive computer resources. This model was a tool for epidemiological research rather than for practical disease forecasting. Furthermore, the model used some preliminary variables and functions that had not been experimentally verified.
Lee et al. (1989) in South Korea used spore traps to investigate blast outbreaks in experimental fields in Icheon and Suwon, to monitor leaf blast outbreaks. Primary meteorological variables included were temperature, RH, rainfall, sunshine hours and LW duration in the field. The number of conidia trapped in samplers was used to predict leaf blast severity and neck blast incidence. Differences in disease trends were found between the two sites and were attributed to differences in LW periods at each site. Differences were found for LW hours obtained by synoptic meteorological data and micro-meteorological data from within fields. These differences became greater for meteorological observatories distant from the field. This model’s approach was similar to Yoshino’s (1979), but was highly dependent on data derived from specific locations.

Decade 1990

Empirical models to predict rice blast were developed by Calvero et al. (1994) and Calvero et al., (1996a) in the Philippines, using regression equations generated from weather factors highly correlated with disease and the WINDOWS Pane program. Equations were used to predict rice blast on two cultivars cultivated at two testing sites, at Icheon in South Korea and at Cavite in the Philippines. This was an early effort to develop a model to forecast rice blast in two different countries. The input variables were: RH, precipitation (per day and total), mean, maximum and minimum temperatures, solar radiation and wind speed. Weather data acquisitions were from both sites but not from in-field collection points. The important role of saturated air for survival of airborne conidia to initiate infection was validated. However, the negative correlation of RH with neck blast was likely to be due to the lack of direct relationship between leaf and neck blast, because the two diseases require different weather conditions. Validations showed that all models developed for the two sites predicted blast reasonably well, with very few prediction errors. The only exception was for maximum lesion number and panicle blast incidence predicted at Icheon, and panicle blast severity on cultivar IR50 at Cavite. These models were shown to be useful for rice production systems, but further validation was suggested to improve prediction accuracy.

A procedure to assess temporal risk of rice blast was developed by Calvero et al. (1996b). This patterned the relationship between proneness to disease and time of sowing at three sites in the Philippines and Indonesia. The data were analyzed using multivariate statistical procedures. Historical meteorological data were used for the construction of the databases, including parameters of temperature, rainfall, RH, wind speed and solar radiation, and a single year weather database representative of the historical weather patterns. Using simulated weather avoided bias in selecting particular years at a particular site, because rice blast did not occur every year. Patterns were developed by combining predicted diseased leaf area and neck blast severity with hypothetical sowing dates, and they were grouped using cluster analysis. Differences in sowing dates fell into blast proneness groups, and these were difficult to identify from long-term weather patterns at the studied sites. Additionally, from discriminant analysis, various weather factors were shown to influence the classification of sowing dates into blast proneness groups. The discriminant empirical equations generated were therefore cultivar- and site-specific.

An information delivery system for the implementation of rice blast forecasting was developed in Korea by Park et al. (1998), based on real-time weather data. This system was composed of four Linux OS servers for: 1) the weather data management; 2) the database; 3) the program; and 4) a web server. The system collected hourly weather data through telephone modems from eight automatic weather stations installed in paddy fields in eight provincial rural development administrations. The input variables were: conidium release, solar radiation, wetness period, conidium deposition, air temperature, wind speed, infection, air temperature and rainfall. The program server ran the BLAST model to predict leaf blast severity (infected leaf area) and neck blast incidence. Accuracy of the forecasting information could be increased using weather data measured within rice paddy fields rather than that measured on macro or meso scales. This model might cause inaccurate forecasting due to its limited validity. Furthermore, the BLAST model had forecasting accuracy limitations especially when disease development was at low levels.

Decade 2000

Kapoor et al. (2004) reported a 50% reduction in rice blast in experimental plots managed using a
forecasting model developed for the Kangra district of Himachal Pradesh in India. Meteorological data were collected from farmer fields and experimental plots, while analyses of 13 years’ data (1984–1996) was used to define critical periods of particular weather conditions, for comparisons with rice blast epidemics. In the 3 years of experimentation, optimum requirements for disease development during a crop season were: temperature 18–28°C and RH to remain greater than 90% for more than 9 h. Leaf blast rules for moderate to high severity were identified, along with neck blast predictions. Data on blast and on meteorological variables, including temperature, RH, rainfall, sunshine hours, wetness durations and wind velocity, were subjected to linear regression analysis. The requirements were RH greater than 80%, prevailing low temperature from 16-19°C with maximum limit of 28°C, 6–8 d of cloudy weather (low solar radiation) and 5–6 rainy days in 7 d. Further studies on rice blast and critical weather factors, such as LW period and distribution of rainfall, were required in the model to refine the predictions.

In Europe, development of rice blast forecasting models has been much less extensive than in Asia. Billoni et al. (2006) developed SIRBInt (Simulation of Rice-Blast Interaction), by monitoring airborne P. oryzae conidia with volumetric spore traps, and measuring temperature, RH, LW and rainfall. All input data were correlated to visual estimation of necrotic lesions on leaves, culms and panicle necks. The model consisted of Rice and Blast interacting sub-models. The Rice sub-model was derived from Oryza-1, while the Blast sub-model was newly developed. Oryza-1 was originally written in Fortran, and was modified for Italian rice characteristics and growing conditions. It was written for Visual Basic in an MS Excel environment, since it had already been used as the modelling environment in another study (Bocchi et al., 1997). The model simulated rice blast interactions and development, including weather dependent crop and pathogen growth patterns. During four trial years the model simulated blast appearance in the field, and could be used as an advisory tool for fungicide applications. The SIRBInt model consisted of many data, while the achieved approximation was not uniform. However, after an uncertainty analysis, it was shown that the more simulated processes were used within the model, the greater became the errors, since every simulation had its own uncertainty. The model could be improved with further research to reduce the uncertainty risk, with more calibration and validation processes, and collecting data for more growing seasons. However, no further development of this model has been reported.

Mousanejad et al. (2009), developed a leaf blast and neck blast severity prediction model in Iran. This was based on data collected by weather stations 5 km from experimental rice paddies, and using simple spore traps in the Guilan province. The leaf and neck blast model was similar to that of Calvero et al. (1994). The collected weather data were: precipitation, daily minimum and maximum temperature, daily minimum and maximum RH and sunshine hours. Two quantitative models were developed for the prediction of leaf blast and neck blast indices. These parameters were also related to N fertilization and plant population density. Precipitation, RH, decreased temperature and sunny hours were shown to be the most important weather predictors for rice blast, since the correlations were high. Also, N fertilization was highly correlated with final leaf blast incidence. This research was a starting point for a comprehensive study on blast forecasting in Guilan province. The model is well-organized regarding input variables, but large distance of 5 km from experimental plots may have affected prediction accuracy.

**Decade 2010**

An early warning system for cool weather conditions was developed and operated by the Japan Meteorological Agency and the National Agriculture and Food Research/Tohoku Agricultural Research Center (Kanda, 2012). This was developed for the Tohoku District (Northern Japan). The model indicates high rice blast risk, as the disease is most serious when summer temperatures are low. The system estimates rice growth stage, abnormal weather damage, and occurrence of rice diseases, based on weekly weather forecasting data, and is presented on the Google Maps API. The current version provides 2-week temperature forecasts so farmers can make timely disease management decisions. Each user can choose an individual rice field. If a warning situation occurs, the users immediately receive notification by email or mobile phone, so control measures can be implemented before disease occurs. The system is available at http://www.reigai.affrc.go.jp.

Liang et al. (2013) developed a forecasting system that processed data collected from agricultural envi-
environments through Wireless Sensor Network (WSN) technologies. The system aimed to provide a precise decision-making system for farmers. The sensor data stream was different from traditional streams characterized by real-time, sequential, missing data and lack of precision. The new system, used a sliding window to model the sensor data. Fuzzy rules were constructed based on expert knowledge, and fuzzy inference was used to collect different environmental data streams. This provided intelligent services to guide disease management or other applications. A simple disease outbreak prediction system was developed for rice blast, using Java and MATLAB. Environmental variables used for disease prediction, include temperatures for *P. oryzae* hyphal growth and conidium development, humidity and time. The fuzzy system gave probabilities of rice blast, classified into three risk levels, as 0-50% (low), 50-80% (moderate), and 80–100% (high). The models needed to enrich the database to make diagnoses versatile. The confidence factors of all the fuzzy rules and the each environmental variable affected the accuracy of the results. Increasing the number of environmental variables made definition of the rules very complicated, and the number of rules would increase exponentially.

In a more recent model approach in India, CLIMARICE II was developed by Rafoss *et al.* (2013). This exploited the potential for climate adaptation and mitigation through online dissemination of pest and disease forecasts to rice farmers. The system was based on the reasoning that farmer’s daily adaptation to the day-to-day variability in weather is a short-term analogy to the need for adaptation to long term climatic changes. Weather-driven mathematical models incorporating scientific insights on the biological responses of plant pests to climate were linked to automatic weather station networks, to provide pest risk forecasting/forewarning/early warning to rice farmers. The model used 224 automatic weather stations operated by the Tamil Nadu Agricultural Weather Network. The stations automatically transmitted weather variables implicated in the disease development process, including air temperature, wind speed, rainfall, solar radiation, soil temperature and moisture, LW and air humidity. The data were combined with disease epidemiology knowledge, and were formulated mathematically and stored in a MSQL database. The model followed rice blast, with assessments of leaf and neck blast used by Tamil Nadu Agricultural University (India). No information was provided on the efficiency or current status of the model.

The most recently developed model was published in Italy by Bregaglio *et al.* (2016). The WARM model (Confalonieri *et al.*, 2009) was used as a coupling generic model to simulate leaf and panicle blast impacts in a temperate climate. The hypothesis was that rice blast symptoms occurred in Northern Italy around the mid July. Weather and disease data derived from field trials under flooding irrigation were collected from 1996 to 2012. Variables used in the first coupling point were: air temperature, RH, LW, wind speed and precipitation. The simulation evaluated disease impacts on leaf area index and aboveground plant biomass. The second coupling point between the crop and the disease models reproduced the impacts of panicle blast on final yield by simulating reduced photosynthate accumulation in kernels. Good correlation between yield and disease assessments was achieved. This approach allowed exploration of blast-associated yield losses in relation to climate change or optimized fungicide strategies. The main limitations identified were: the lack of dedicated field experiments for collection of micro-meteorological data, the use of single values for the two blast symptoms and the lack of important pathogenesis information, including LW and conidium dispersal. Correcting these limitations would improve correlations, allowing the model to precisely predict real disease occurrence.

### Neck blast models

A statistical method for forecasting neck blast was developed by Sasaki and Kato (1972), using data from 1962 to 1967. Cumulative numbers of diseased spikelets were plotted against time, forming sigmoid relationships for all cultivars grown under different conditions for all six years. Based on 112 sets of readings, each linear equation related the logit of the percentage of diseased spikelets 12 d after the crop stage of 50% heading and the rate of increase during the following 6 d. The numbers of diseased spikelets in the next 6 d were predicted by extrapolation. The regressions and correlations were shown to be valid only if the data were acquired during the same stage of development and within similar environmental conditions. Modifications were suggested to allow specific inhibitory or stimulatory effects on rates of infection development.
The first neck blast simulation model was developed by Takasaki (1982) in Japan. Conidium deposition and penetration were treated as stochastic processes, and individual panicles were treated as infection site units. Infection was computed according to a probability function, and affected panicles were classified into several types. The model’s main limitation was that it did not account for secondary neck blast infections.

**Rice blast forecasting models currently in use**

Few rice blast forecasting models are currently in use for rice growers. Of the 52 published models, three operate inside the processes of other models or systems as modules or subroutines. These are those outlined by Yoshino (1979), Hashimoto et al. (1984) and Gunther (1986). Furthermore, four models are currently in use with the derived information available on the Web. Three of these were developed by Kaundal et al. (2006), Kang et al. (2010) and Kanda (2012). The fourth is currently available in Europe as a module implemented in the EU service “Monitoring Agricultural ResourceS” (MARS), operated by the Joint Research Center at Ispra (Italy). The system incorporates data from 1450 European weather stations and satellites. MARS issues bulletins on rice yield predictions every year, which include rice blast forecasting. Bulletins are available at [http://mars.jrc.ec.europa.eu/mars](http://mars.jrc.ec.europa.eu/mars). MARS uses the subsystem Water Accounting Rice Model (WARM) (Confalonieri et al., 2010), which is an object-oriented simulation tool. The structure of WARM allows development of separate class modules for each aspect, and testing in an independent environment. Crop damage from rice blast is simulated within the processes, using variables of temperature, humidity and dew.

Examination of currently used rice blast forecasting systems has shown that they all require inputs from extended and systematic datasets, so that the forecasts cover large areas of rice cultivation. They require powerful computers, and advanced networks and servers with extensive database capabilities. Moreover, Yoshino’s approach to LW operates through Kang et al. (2010) models, and the Japanese service based on Kanda’s (2012) low temperature approach along with BLASTAM. The approach of Kaundal et al. (2006) to rainfall is closely connected to increased RH and moisture saturation, which leads to elevated LW. The WARM model in MARS interpolates LW with a temperature and RH general approach, giving emphasis to *P. oryzae* penetration from germinating conidia.

**Discussion**

We have carried out an analysis of several factors to provide a deeper understanding of the model reviewed in the present study, to facilitate more accumulated knowledge, and to analyse information provided by each model.

**Type of forecasting and input variables**

**Output type**

The majority (60%) of the published rice blast models were developed to forecast leaf blast. This is the first symptom of *P. oryzae* infection that appears, so prediction of leaf blast is critical for early blast control, particularly in countries where the disease occurs early in the growing season. Just over a third (37%) of the blast models could forecast both leaf and neck blast. These models are likely to be more suitable for practical decision-making, since they can assist farmers throughout the crop growing period. In contrast, few of the models (4%) can forecast neck blast. Furthermore, neck blast prediction accuracy is reported to be low.

**Input variables**

The frequency of different input variables used in rice blast prediction models is presented in Figure 1. “Air Temperature” (67% of the models), “Relative Humidity” (58%) and “Rainfall” (56%) are the predominating weather variables used. Also, in more than the 30% of the models, variables regarding either *P. oryzae* or plant biology were included. These were “Spore Dissemination” (37% of the models), “Leaf Wetness (LW)” and “Plant Stage” (35%), “Sunlight” and “Wind Speed” (31%). Although variables such as “Air temperature”, RH and conidium related inputs (“Spore Dissemination”, “Spore Penetration”, “Spore Disposition”) are known to be critical factors affecting pathogenesis and disease development, these parameters have not been included in all models.

The infrequent integration of LW in the models (used in 35% of the models) may account for the general lack of prediction certainty, because LW is considered in the literature to be among the most
critical factors for the rice blast pathogenesis, and for connecting forecasting with rice canopy microclimate (Greer and Webster, 2001; Lanoiselet et al., 2002; Yoshida et al., 2015). Field measurements of LW require in-field devices, increasing the need of human interaction or automatic transmission systems. However, of the models with LW inputs, only 33% acquired real canopy data, and the others interpolated these parameters. Lanoiselet et al. (2002) suggested that data loggers should be placed in rice fields to assess microclimates of waterlogged fields to record realistic meteorological data needed to run the models. Significant differences occur between the RH values recorded outside field compared with those from rice canopies. Fluctuations in RH can reach an average of at least 20% greater inside canopies than above canopies or outside rice fields. Also, RH ≥ 95%, equivalent to saturation, is assumed to indicate LW or moisture on leaf surfaces sufficient for sporulation and infection initiation on leaf tissues (Abrol, 2013). Trials carried out in three Mediterranean countries (Italy, Greece and Portugal) in 2015 and 2016 (RICE-GUARD FP7 project, unpublished data), where commercial mini-weather stations were installed inside rice paddies for monitoring canopy air temperature, RH and LW, allowed useful conclusions or hypothesis development relating to different published results. For example, the high correlation of the LW with RH ≥ 95% reported by Albröl (2013) could not be validated as a narrow principal, because high LW values (> 65% coverage) occurred where RH was less than 95%, when rice blast risk could still be great. Nevertheless, interpolations with other variables may produce errors affecting the accuracy of the predictions. For example, linear regression analyses of variables “Air temperature” and RH, derived from these recent trials, resulted in $R^2$ values ranging from 0.203 to 0.683. Although adding more variables in the regression analyses, such as “Wind speed” and “Solar radiation”, improved the $R^2$ values, but these were still not satisfactory, ranging from 0.750 to 0.762 (RICE-GUARD FP7 project, unpublished data). This level of relationship, although acceptable for field experiments, may still produce uncertainties in interpolations at a minimum of 24%. These findings agree with those of Kang et al. (2010), who concluded that inaccuracies in predictions from rice blast models are due to failures to interpolate LW with other weather variables.

Less frequently incorporated variables were “Spore release” (12% of the models), “Dew Point” (15%) and “Spore Penetration” (17%), while important parameters such as “Nitrogen Fertilization” and “Varieties” (host resistance) were infrequently used

![Figure 1. Frequency of different meteorological variables used in 52 rice blast forecasting models.](image-url)
(19% of models) (Ou, 1985; Freitas et al., 2010). This limited integration could lead to anomalies, because both factors play important roles in *P. oryzae* pathogenesis and blast progress. For example, excessive nitrogen fertilization can increase disease severity by altering host susceptibility, even in highly resistant varieties. These varieties could escape disease even under favourable conditions for the pathogen, because of strong field resistance. The main reasons for limited integration of these variables may be that they require direct user interactions for inputs, or development of extended databases with frequent update requirements. However, recent technology improvements allow these features to be easily adopted, to improve future forecasting systems.

**Input variable combinations**

Combinations of variables were used in 54% of the models (Figure 2). “Air temperature + RH” and “Air temperature + Rainfall” were most commonly used (50%), followed by “LW + Air temperature” (29%) and “LW + Air temperature + RH” (27%). Less used combinations were “LW + Air temperature + RH + Rainfall” (23%) and “LW + Wind speed” (19%). Combinations with the least integration were “Air temperature + RH + Nitrogen fertilization” and “LW + Air temperature + RH + Rainfall + Spore dissemination” (7.7%).

**Geographical distribution**

The geographical distribution of the 52 forecasting models is presented in Figure 3. Most models originated from Japan (38%), while 13% came from Korea, 11% from India and 10% from the Philippines. Despite the magnitude of rice production in China, only 4% of the models originated from that country.

**Timeline for model publication**

The greatest numbers of model publications were from the 1980s (31%) or the 1990s (21%). Publications from the decades of 2000 and 2010 were less (15%), and frequency of publication since then remains at a stable rate. Introduction of advanced software engineering and new computer and sensor technologies has not recently increased the numbers of models developed, with relatively few models published after 2000.

**Model modifications**

In more than 30% of the publications, further revisions/development/modifications were suggested to be required by their authors to improve the efficiency and accuracy of disease predictions. Nevertheless, no evidence was presented for im-
Figure 3. Country distribution of published rice blast prediction models.

Figure 4. Publication date decades for rice blast prediction models.
Implementing these improvements or that the models were further developed. Only four of the 52 models (8%) were modified after their original publication. These were: BLASTL (Hashimoto et al., 1984), modified by Ashizawa et al. (2005); the model of Gunther (1986), modified by Tastra et al. (1987); BLASTSIM.2 (Calvero and Teng, 1991; 1992), modified by Luo et al. (1977); and EPIRICE (Savary et al., 2012), modified by Kim et al. (2015).

**Reference area**

Most of the models, including those not based only on field data, reference areas were either small or limited, with reference to the magnitude of rice cultivation, the destructiveness of disease caused by *P. oryzae*, and the high annual crop losses. Even where an application or a tool is still in use, the forecasting is restricted to specific areas. There is also little or no evidence that the published models were evaluated or validated in geographical areas other than those where they were developed, including regions with similar environments. The only exceptions were: the model published by Luo et al. (1997), which was tested in five Asian countries; BLASTAM (Koshimizu, 1983; 1988; Uehara et al. 1988), tested in several prefectures of Japan; and BLASTSIM.2 (Calvero and Teng, 1991, 1992), which was refined and validated at IRRI in 1992.

**Spatial distance scenarios**

Reliability of forecasting type is affected by the source of weather data and whether data logging systems are located near or away from rice crops. Only 12 of the models (23%) used in-field weather data collection, and there is little evidence that this was from within rice canopies. Park et al. (1998) concluded that the absence of rice crop microclimatic conditions could lead to unreliable model predictions. Moreover, some theoretical approaches have developed forecasting models that are based only on historic data derived from study areas or countries.

**Recommendations**

Future attempts to develop rice blast prediction systems should consider the recommendations outlined below. The integrity of weather data collected from different points (in-field, outside the field or large distances from rice fields) should also be considered.

1) Model integration of modules or routines with two-way interactions should be used, giving the ability for end-users to input or parametrize variables. These can affect rice blast incidence or severity, and could include sowing dates, variety resistance and rates of nitrogen fertilization.

2) Canopy recordings should be made of the most critical variables (e.g. LW, air temperature and RH).

3) LW interpolation errors can be reduced by adding variables that can greatly affect dryness (wind speed and solar radiation). Interpolation of LW should be eliminated.

4) The number of data collection points should be large, utilizing and integrating modern technologies (smartphones, GSM networks) for in-field recording and data transmission.

5) Conidium trapping methods, which require specialized in-field expertise, should not considered to be an essential model component. Automatic systems should be used to improve widespread monitoring of rice cultivation areas.

**Conclusions**

Analysis of published rice blast prediction models has provided comprehensive knowledge on rice blast forecasting. Weather variables, such as “Air temperature”, “Relative Humidity”, “Spore Dissemination” and “Leaf Wetness” are among the most critical model inputs, since these play important roles in *P. oryzae* pathogenesis and rice blast development. However, the present review has shown that most studies have not included the combinations of inputs of these variables. Nevertheless, interpolations were often attempted, to calculate weather variables, an approach likely to lead to uncertainties. Difficulties in retrieving canopy monitored microclimate data is another limitation. In-field conditions differ substantially compared with the parameters recorded in weather stations located above, or well-separated from, rice crops or cultivation areas.

This review has also shown that very few published rice blast prediction models can be used for long periods (years) or in different geographical regions. Study of errors, uncertainties, improvements and modifications will assist development of more reliable forecasting systems. New remote sensing
technological innovations will assist canopy data collection.

The contributions of information derived from rice blast prediction models towards improvement of disease management has been limited through the decades. Prediction of initial \( P. oryzae \) infection and the patterns of rice blast development are the most important factors for forecasting this disease. Despite the development of 52 published rice blast prediction models in the last 67 years, the majority of these are research oriented. The question of Gold (1988) is still very relevant: “How useful is the information provided by the model relative to its intended purpose?”

Acknowledgments

Research leading to this review was funded by the European Union’s Seventh Framework Programme managed by Research Executive Agency (REA) http://ec.europa.eu/research/rea(FP7/2007-2013), under grant agreement n° 606583. We are grateful to Dr Richard Fallon for his insightful suggestions and revisions of the present paper.

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Accepted for publication: May 21, 2017
RESEARCH PAPERS

Turkish barley landraces resistant to net and spot forms of Pyrenophora teres

Arzu Çelik Öğuz¹, Aziz Karakaya¹, Namuk Ergün² and İsmail Sayım²

¹ Ankara University, Faculty of Agriculture, Department of Plant Protection, Dişkapi, Ankara, Turkey
² Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey

Summary. Pyrenophora teres is an important pathogen of barley. The pathogen has two biotypes: Pyrenophora teres f. teres, which causes the net type of net blotch, and P. teres f. maculata causing the spot type of net blotch. Turkey is an important gene centre of barley and has a rich barley landrace population. Finding disease resistant barley germplasm has potential for world agriculture. Three virulent Pyrenophora teres f. maculata (Ptm) isolates and three virulent Pyrenophora teres f. teres (Ptt) isolates were tested for their pathogenicity to 198 barley landraces, and landraces resistant to both forms of the pathogen were identified. Thirteen landraces (numbered 17, 40, 71, 98, 101, 103, 104, 143, 162, 167, 171, 183 and 185) were resistant to the Ptm isolates and seven (numbered 18, 21, 22, 24, 40, 71 and 153) were resistant to the Ptt isolates. Two landraces (40 and 71) were resistant to all six P. teres isolates. In addition, several of the landraces exhibited reactions to one or two isolates of Ptt or Ptm, in the resistant to moderately resistant range. Using disease resistant host genotypes will help to reduce the use of disease control chemicals, and with development of efficient host resistance strategies to combat net blotch diseases. These landraces could be used as sources of resistance for barley breeding.

Key words: Drechslera teres, Hordeum vulgare, Disease resistance.

Introduction

Barley is one of the oldest cultivated plants in the world, which has been cultivated for thousands of years (Kün, 1996). The net blotch fungus Pyrenophora teres (anamorph: Drechslera teres) belongs to the phylum Ascomycota, and has two biotypes. Pyrenophora teres Drechs. f. teres Smedeg. (Ptt) causes net type leaf symptoms, and P. teres f. maculata Smedeg. (Ptm) causes spot type symptoms on the barley leaves. These are among the most important barley diseases, which occur in many countries and causes significant economic losses (Shipton et al., 1973; Mathre, 1982; Karakaya et al., 2014). Losses due to the pathogen range between 10–40% (Mathre, 1982). Fungicide applications, cultural practices and the use of resistant cultivars are the recommended disease management methods (McLean et al., 2012). The prevalence of net blotch is closely related to the susceptibility of barley cultivars grown in specific areas. The most eco-friendly, practical and profitable method for net blotch control is the use of resistant barley cultivars.

Barley landraces are important sources of genetic variation (Yitbarek et al., 1998; Ellis et al., 2000). Landraces can be successfully cultivated even in unfavourable conditions, owing to their adaptability to changing environmental conditions (Allard and Bradshaw, 1964). Turkey ranks very highly regarding the abundance of landraces. Anatolian landraces and hulless barleys have been shown to be far superior to other cultivars in terms of efficiency and endurance against drought (Gökgöl, 1969). Landraces are still planted in Turkey.

Plant breeders need sustainable new resources of resistance against diseases. Efficient use of the rich genetic resources in Turkey is believed to be one of
the best ways to combat the diseases caused by \textit{P. teres}. Recently in Turkey, approx. 3,500 barley landraces obtained from Turkey and different parts of the world, and maintained at Anatolian Agricultural Research Institute, were renewed by the Central Research Institute for Field Crops located in Ankara, Turkey. Agromorphological, biochemical and molecular characterization of these landraces was also carried out by this Institute. Two hundred winter type landraces originating from Turkey were selected. These were obtained with single spike selection. In the study reported here, three single conidium isolates of \textit{P. teres f. maculata} and three single conidium isolates of \textit{P. teres f. teres}, the most virulent isolates identified in a previous study (Çelik Oğuz, 2015) were tested on these 198 landraces, to determine their resistance status against both forms of net blotch. The resistance status of these landraces to net blotch diseases has not been previously assessed.

**Materials and methods**

**Plant material**

Two hundred barley landraces were used. These were collected from various parts of Turkey and conserved by the Field Crops Central Research Institute (Ankara, Turkey). Agromorphological, biochemical and molecular characterization of these landraces was performed previously by the Field Crops Central Research Institute, and landraces suitable for winter type sowing were selected. Seed of each landrace was multiplied from a single spike. Almost all (198) of these landraces provided sufficient seeds, and these were included in the present study. Insufficient seeds were obtained from landraces Nos 43 and 116. The reactions of the landraces to virulent \textit{Ptm} and \textit{Ptt} isolates were determined for the first time with this study.

**Pyrenophora teres isolates**

In a previous study, 425 single conidium isolates of both forms of the net blotch pathogen were obtained from different regions of Turkey, and 50 isolates of \textit{Ptm} and 40 isolates of \textit{Ptt} were tested on a barley differential set that consisted of 25 genotypes (Wu \textit{et al.}, 2003). This determined the pathotypes of both biotypes of \textit{P. teres} in Turkey (Çelik Oğuz, 2015). Three isolates of \textit{Ptm} and three isolates of \textit{Ptt} that were found to be the most virulent were used to determine seedling stage resistance of the 198 barley landraces, under controlled conditions in a greenhouse. \textit{Ptm} isolate GPS263PTM was obtained from the Ankara-Bala region of Turkey, isolate 13-179PTM from the Kahramanmaraş-Pazarlık region, and isolate 13-167PTM from the Diyarbakır-Central region. \textit{Ptm} isolates GPS263PTM, 13-179PTM and 13-167PTM were the most virulent \textit{Ptm} isolates, their mean virulence values over 25 differential set genotypes (Wu \textit{et al.}, 2003) were 7.36, 7.04 and 6.84, according to the Tekauz (1985) scale (Çelik Oğuz, 2015). The response of susceptible local barley cultivar Bülbül 89 to these three isolates was, respectively, 9, 8 and 8 according to the Tekauz (1985) scale (Çelik Oğuz, 2015).

\textit{Ptt} isolate GPS18PTT was obtained from the Sivas-Yıldızeli region, isolate UHK77PTTM from the Kilis region, and isolate 13-130PTT from the Şanlıurfa-Ceylanpınar region. \textit{Ptt} isolates GPS18PTT, UHK77PTT and 13-130PTT were also the most virulent \textit{Ptt} isolates, and their mean virulence values over 25 differential set genotypes were, respectively, 5.84, 5.80 and 5.64 according to the Tekauz (1985) scale (Çelik Oğuz, 2015). The response of susceptible local barley cultivar Bülbül 89 to these three isolates was, respectively, 9, 7 and 6, according to the Tekauz (1985) scale (Çelik Oğuz, 2015).

**Preparation of inoculum, inoculation and incubation**

Sterile mixtures of soil, sand and organic substances (60:20:20, v:v:v) were placed in plastic pots (7 cm diam.), and (depending on the quantity of available seeds of landraces) five to ten seeds were placed into each pots. The pots were maintained under greenhouse conditions before and after inoculation. Resulting plants were inoculated at growth stages 12-13 (Zadoks \textit{et al.}, 1974). Single conidia were isolated using blotter method. Diseased leaves were cut in 2–3 cm lengths, and after surface sterilization with 1% NaOCl for 1 min, they were placed into sterile Petri dishes containing wet filter paper. The Petri dishes were incubated under room conditions. Three days later, single conidia were taken using a stereomicroscope. Inoculum was prepared from cultures grown in potato dextrose agar (PDA). For inoculum production, mycelia were scraped from Petri plates using a paintbrush. Inoculum concentration was adjusted to $15\text{–}20 \times 10^4$ mycelial fragments mL$^{-1}$. 

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(Douiyssi et al., 1998). One drop of Tween 20 was added to every 100 mL of inoculum (Aktaş, 1995). The temperature of the greenhouse was 18±1°C night and 23±1°C day with a 14h/10h light/dark regime. Following inoculation, the plants were kept covered with nylon in transparent boxes with lids for 76 h. Then, they were kept in high humidity for another 48 h after which they were uncovered and ventilated. Three replicate pots of each landrace were used in the experiment.

Evaluation of disease

After 7 d, the plants were evaluated for disease using the severity scales developed for net and spot forms of net blotch by Tekauz (1985). The scales use lesion morphology. Scale values of 1, 2 and 3 were considered as resistant. In the scale for the spot form of net blotch, seven numerical classes were recognized (1 = R: resistant, 2 = R: resistant to MR: moderately resistant, 3 = MR: moderately resistant, 5 = MR: moderately resistant to MS: moderately susceptible, 7 = MS: moderately susceptible, 8 = MS: moderately susceptible to S: susceptible, and 9 = S: susceptible). In net form scale ten numerical classes were recognized (1 = R: resistant, 2 = R: resistant to MR: moderately resistant, 3 = MR: moderately resistant, 4 = MR: moderately resistant to MS: moderately susceptible, 5 = MR: moderately resistant to MS: moderately susceptible, 6 = MR: moderately resistant to MS: moderately susceptible, 7 = MS: moderately susceptible, 8 = MS: moderately susceptible to S: susceptible, and 9 = S: susceptible). Net blotch lesions classified as resistant or moderately resistant are small and remain restricted in size. These lesions are primarily composed of necrotic tissue, and leaf tissue surrounding each lesion appears normal green in color. Lesions classified moderately susceptible or susceptible have chlorotic surrounding zones. These zones enlarge with time and may coalesce and result in the death of entire leaves (Tekauz, 1985).

Agronomic evaluation of the barley landraces

Under field conditions, agronomic evaluations of landraces were carried out. These included: days to heading, days to maturity (d), plant height (cm), numbers of fertile heads per m², 1,000 kernel weights (g), grain yields (kg ha⁻¹) and cold tolerance (0–5 scale) during 2012/2013 cropping year at İkizce (Gölbaşı/Ankara) location, Turkey. These evaluations were carried out in an experiment using Augmented Experimental Design (Peterson, 1994). Planting date was 23 October, 2012, and harvesting date was 10 July, 2013. Agronomic traits were evaluated according to Ergün and Geçit (2008). Only the results of the disease resistant landraces are presented in the present paper.

Results

Three isolates of Ptmt and three isolates of Ptt that were previously shown to be virulent (Çelik Oğuz, 2015) were tested on 200 barley landraces. Thirty of these landraces were six-rowed barleys and 170 2-rowed. Insufficient seeds were obtained from landraces 43 (six-rowed) and 116 (two-rowed).

Novel resistance sources to both forms of P. teres were identified (Table 1). Forty-eight barley landraces were moderately resistant, six were resistant-moderately resistant and one was resistant to Ptmt isolate GPS263PTM. Seventy-four landraces were moderately resistant to the Ptmt isolate 13-179PTM, and 74 landraces were moderately resistant, and eight landraces were resistant-moderately resistant to Ptmt isolate 13-167PTM. Thirteen landraces were resistant to all three isolates of Ptmt (landraces 17, 40, 71, 98, 101, 103, 104, 143, 162, 167, 171, 183 and 185). Of the resistant landraces, 23% were six-row barleys and 77% were two-rowed.

In addition, 46 landraces (landraces 1, 13, 16, 31, 41, 44, 49, 51, 54, 61, 62, 69, 85, 93, 97, 99, 100, 106, 113, 114, 115, 118, 119, 120, 121, 123, 124, 125, 126, 128, 129, 132, 136, 137, 140, 144, 145, 159, 163, 165, 172, 176, 180, 181, 182 and 187) exhibited resistance to two virulent isolates of Ptmt, and 75 landraces showed resistance to one virulent Ptmt isolate.

Eight landraces were moderately resistant and one was resistant-moderately resistant to Ptt isolate GPS18PTT. Thirteen landraces were moderately resistant and one was resistant-moderately resistant to Ptt isolate UHK77PTT. Sixty three landraces were moderately resistant and three were resistant-moderately resistant to Ptt isolate 13-130PTT. Seven landraces were resistant to all three Ptt isolates (landraces 18, 21, 22, 24, 40, 71, 153). Of the landraces resistant to Ptt, 29% were six-row barleys, and 71% were two-rowed. In addition, eight landraces (landraces 32, 79, 80, 81, 84, 127, 132, 200) were resistant to two virulent isolates of Ptt, and 51 landraces were resistant to one Ptt isolate.
Table 1. Seedling reactions on some resistant barley landraces to six virulent isolates of *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, based on the Tekauz (1985) scale (see text). Some agronomic parameters measured for the landraces are also presented.

<table>
<thead>
<tr>
<th>Landrace No</th>
<th>Accession No.</th>
<th>Row Type</th>
<th>Days to heading</th>
<th>Days to maturity (d)</th>
<th>Plant height (cm)</th>
<th>Number of fertile heads/ m²</th>
<th>1,000 kernel weight (g)</th>
<th>Grain yield (kg ha⁻¹)</th>
<th>Cold tolerance (0-5 scale)</th>
<th>Cold tolerance (0-5 scale)</th>
<th>P. teres f. teres isolates</th>
<th>P. teres f. maculata isolates</th>
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</table>
Landraces 40 and 71 were resistant to the three *Ptm* and the three *Ptt* isolates used in this study. Both of these resistant landraces were six-row barleys. Several of the landraces exhibited resistant to moderately resistant reactions to one or two isolates of *Ptt* or *Ptm*. Three of the landraces that exhibited resistant reactions (R-MR to MR) to *Ptm* were six-row landraces and the remaining ten were two-rowed. Four of the landraces that exhibited resistant R-MR to MR reactions to *Ptt* were six-row landraces, and three were two-rowed. Resistance in Turkish six- and two-row barley germplasm has been reported previously (Karakaya and Akyol, 2006; Taşkoparan and Karakaya, 2009; Aktaşdoğan et al., 2013; Gerlegiz et al., 2014; Usta et al., 2014; Yazıcı et al., 2015).

Resistant landraces exhibited considerable variation in days to heading (174–193 d), days to maturity (221–237 d), plant height (86–106 cm), numbers of fertile heads (240–758 m⁻²), 1,000 kernel weight (31.5–48.7 g), grain yield (1,880–7,426 kg ha⁻¹) and cold tolerance (scale values 2–4) (Table 1).

**Discussion**

Turkey is at the crossroads of the main barley gene centres, so this country has a rich barley landrace potential (Vavilov, 1951; Kün, 1996). Presence of genetic resources and their utilization, transfer of superior quality traits of wild relatives to cultivars via gene transfer, and reduction of the use of chemicals during crop production are important for barley (Laurei et al., 1992).

Frankel and Hawkes (1975) indicated the importance of plant genetic resources and emphasized the importance of wild relatives. These resources should be collected from their natural habitats and protected in stock cultures. Many resistant barley genotypes were present in centres of barley evolution areas (Afanasenko et al., 2000). McLean et al. (2009) determined resistance among the barley genotypes from Middle East.

Turkey has important barley genetic resources (Kün, 1996). Chakrabarti (1968) tested 6,246 barley varieties in the World Barley Collection for reaction to net blotch disease, and 417 varieties were found to be resistant to the disease, and 30 were highly resistant. The majority of resistant varieties were from Turkey. Khan (1969) tested 8,756 barley varieties in the World Barley Collection, which originated from Turkey, and six were highly resistant. Studies conducted in Turkey also revealed diversity of resistance and susceptibility among barley cultivars and genotypes (Karakaya and Akyol, 2006; Taşkoparan and Karakaya, 2009; Aktaşdoğan et al., 2013; Gerlegiz et al., 2014; Usta et al., 2014; Yazıcı et al., 2015).

New pathotypes of fungi can be more virulent than the established pathotypes. Resistance studies should be continuous and a wide range of resistance sources should be available. There are numerous studies of the resistance of barley landraces to *P. teres*. Legge et al. (1996) tested 176 Turkish barley lines for reaction to *P. teres*. More resistant lines were found to the spot form of net blotch compared to the net form, and similar results occurred in the present study. Lakev et al. (1995) evaluated Ethiopian landraces for disease resistance and agronomic traits, and Yitbarek et al. (1998) evaluated Ethiopian landraces for disease resistance. Considerable variation was found among these landraces for reaction to *P. teres* and for agronomic traits, such as days to heading, days to maturity and plant height. Also in our study, considerable variation was evident among the Turkish barley landraces for disease resistance and agronomic traits. Endresen et al. (2011), under field conditions, evaluated 2,786 barley landraces to an isolate of *Ptt* at four different research stations during 8 years. A majority of the landraces were resistant or moderately resistant to the pathogen. In the present study, performed under greenhouse conditions, out of 198 landraces, seven were resistant (Tekauz scale ≤3) to three virulent isolates of *Ptt*.

Limited resistance to *P. teres* was found among the some landraces used in different studies. Silvar et al. (2010) evaluated the reactions of 159 barley landraces and 16 cultivars obtained from the Spanish Barley Core Collection to three *Ptt* isolates. The overall resistance against net blotch in the Spanish landraces was low. Most of the accessions were classified as susceptible or moderately susceptible to each of the isolates. Only one accession was resistant to all three isolates, and one was classified as moderately resistant to one isolate and resistant to two other isolates. Similarly, the cultivars also displayed low resistance levels. Neupane et al. (2015) tested 2,062 barley accessions obtained from the World Barley Core Collection to four *Ptm* isolates obtained from United States, Australia, New Zealand and Denmark. Only fifteen accessions were resistant to all four isolates. In Ethiopia, 900 landrace lines, from 45 populations representing three locations, tested and four lines
were resistant to net blotch (Semeane, 1995). Greater levels of resistance were found in the present study. Jana and Bailey (1995) assessed resistance to Canadian isolates of three foliar pathogens (Cochliobolus sativus, Ptt and Ptm) in wild and cultivated landrace barley (Hordeum vulgare subsp. spontaneum and H. vulgare subsp. vulgare) from Turkey and Jordan. Seedlings were inoculated separately with the pathogens in growth cabinet tests. More wild than cultivated barley accessions were resistant to C. sativus (4.5% of wild accessions vs. 0.3% cultivated) and Ptt (21.8% vs. 0.5%). Equal numbers of wild and cultivated accessions were resistant to Ptm. A larger proportion of wild barley accessions (10.5%) had at least moderate resistance to all three leaf diseases compared to only 1.3% of cultivated accessions. The average disease rating on these accessions was less for wild barley (65%), but not significantly different from cultivated barley (73%). Resistance in wild barleys is, therefore, more common, and future studies to identify resistance should utilize more wild barley genotypes. In our study, we also observed a similar pattern related to resistance of P. teres biotypes, where more barley landraces showed resistant reactions to virulent Ptm isolates.

Several studies of host resistance to net blotch have been carried out under controlled environmental conditions. Gupta et al. (2003) reported that resistance to Ptt expressed in seedlings was frequently expressed in adult plants in the field. Similarly, Düşünceli et al. (2008) found a significant correlation between the seedling resistance and adult plant resistance (r = 0.53) to another important barley pathogen, Rynchosporium secalis. On the other hand, Douiyssi et al. (1998) reported that seedling and adult plants often differed in responses to an isolate of P. teres. Resistant barley landraces identified in the present study as resistant to both forms of the net blotch pathogen should also be tested under field conditions to provide more reliable results.

In the present study, 13 barley landraces were found to be resistant to all three virulent isolates of Ptm, and seven landraces were resistant to all three virulent Ptt isolates. Two landraces were resistant to all six virulent isolates. In addition, several landraces exhibited resistant to moderately resistant reactions to one or two of the virulent isolates of Ptt or Ptm. More landraces were resistant to Ptm than to Ptt. This is particularly promising, since Ptm is more common in Turkey than Ptt (Karakaya et al., 2014).

Barley landraces are good sources of plant resistance to biotic and abiotic stresses. In order to control new pathotypes, resistance studies should be continuous, and large genetic source is necessary for identification of rare resistance traits. Genetic host resistance is a desirable disease control strategy, because of environmental concerns. Disease resistant barley landraces could be used efficiently in developing disease resistant barley cultivars.

Acknowledgement

This study is supported by The Scientific and Technological Research Council of Turkey (Project No: 111O644).

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Endresen D.T.F., K. Street, M. Mackay, A. Bari and D.E. Pauw,


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**Barley landraces resistant to *Pyrenophora teres***

**Accepted for publication: June 18, 2017**
RESEARCH PAPERS

Pathotypes of *Pyrenophora teres* on barley in Turkey

**Aruz Çelik Oğuz** and **Aziz Karakaya**

Ankara University, Faculty of Agriculture, Department of Plant Protection, Dışkapı, 06110, Ankara, Turkey

**Summary.** Net blotch foliar diseases of barley are important in Turkey, lowering grain yields and quality. There are two forms, the spot form (caused by *Pyrenophora teres* f. *maculata* (*Ptm*)) and the net form (caused by *P. teres* f. *teres* (*Ptt*)). To determine the pathotypes of *Ptt* and *Ptm* in Turkey, surveys were carried out during 2012, 2013 and 2015. *Pyrenophora teres* samples were collected from 34 provinces of Turkey. From these samples, 258 *Ptm* and 167 *Ptt* single conidium isolates were obtained. Pathotypes of 50 *P. teres* f. *maculata* and 40 *P. teres* f. *teres* isolates were assessed by inoculating onto a differential set of 25 barley genotypes. Twenty six *Ptm* pathotypes and 24 *Ptt* pathotypes were identified, and significant pathogenic variation was found among the isolates. Barley breeding programmes in Turkey should consider the pathotypes identified for incorporation of net blotch resistance. Continuous virulence monitoring for the *P. teres* population should be carried out to inform resistance breeding priorities.

**Key words:** Barley, *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*.

**Introduction**

Barley (*Hordeum vulgare* L.) is an important cereal crop in Turkey, being the second most planted cereal after wheat (Tuik, 2016). Barley is cultivated in 2.598 million ha, producing 6.31 million tonnes of grain, at an average of 2,450 kg ha\(^{-1}\) (Tuik, 2016). Net blotch diseases, caused by the fungus *Pyrenophora teres* (anamorph: *Drechslera teres*) are important foliar diseases of barley, which limit barley production by reducing grain yield and quality (Matthre, 1982; McLean *et al*., 2009; Liu *et al*., 2011). There are two main net blotch diseases: the spot form caused by *Pyrenophora teres* f. *maculata* (*Ptm*), and the net form caused by *Pyrenophora teres* f. *teres* (*Ptt*) (Smedegard-Petersen, 1971). Symptoms of the spot form consist of necrotic spots surrounded by chlorosis (McLean *et al*., 2009; Liu *et al*., 2011). The net form symptoms consist of thin, dark brown, longitudinal streaks on leaves which merge to create irregular streaks on leaves (Liu *et al*., 2011).

These diseases can cause significant grain yield and quality losses (Matthre, 1982; Aktaş, 1997; Karakaya *et al*., 2014). Yield losses can reach up to 100% in severely affected fields where very susceptible cultivars are grown, but generally losses are between 10-40% (Matthre, 1982).

Planting resistant barley cultivars is an effective way of controlling the net blotch diseases. However, both *Ptm* and *Ptt* show pathogenic variation and have the potential to overcome host resistance. Pathogenic variation needs to be considered in plant breeding programmes (Tekauz, 1990; Liu *et al*., 2011; Çelik Oğuz and Karakaya, 2015; Akhavan *et al*., 2017). The pathogenic variation in *P. teres* has been known since 1949 (Pon, 1949). Khan and Boyd (1969) used differential barley lines to determine the physiological races of *D. teres*. Later studies reported pathogenic variation in both forms of *P. teres* populations from different parts of the world. These studies utilized different lines for variation studies, and large variation among the *P. teres* populations were reported (Khan and Tekauz, 1982; Harrabi and Kamel, 1990; Steffenson and Webster, 1992b; Sato and Takeda, 1993; Jonsson *et al*., 1997; Platz *et al*., 2000; Arabi *et al*., 2003; Cromey and Parkes, 2003; Wu *et al*., 2003;
Tuohy et al., 2006; Afanasenko et al., 2009; Lehmensiek et al., 2010; McLean et al., 2011; Boungab et al., 2012; McLean et al., 2014; Leisová-Svobodová et al., 2014; Akhavan et al., 2017).

In the present study, 50 *Ptm* and 40 *Ptt* isolates were tested on 25 differential barley test cultivars and genotypes under greenhouse conditions, to determine the pathotypes of these fungi in Turkey.

**Materials and methods**

**Survey and collection of *Pyrenophora teres* isolates**

Two hundred and seventy nine barley fields in 2012, 105 in 2013 and 71 in 2015, were surveyed in 34 provinces of Turkey. Fields were sampled at distances of approx. 30 km, within different regions of the country (Aktaş, 2001). Leaves with spot form and net form symptoms were sampled in each field.

**Single conidium isolates, isolate selection and verification of isolates**

Leaves containing net or spot form symptoms were cut into small pieces, 2–5 cm in length, and surface sterilized by placing in 1% sodium hypochloride solution for 1 min. Leaf pieces were then placed onto Petri dishes containing sterile moistened filter paper and incubated for 3 d for conidium production. Single conidia were individually placed onto water agar. Hyphal tips from germinating conidia were transferred to potato dextrose agar (PDA) to develop cultures. Two hundred and fifty eight *Ptm* and 167 *Ptt* single conidium isolates were obtained from different regions of Turkey. From these, 90 isolates (50 *Ptm* and 40 *Ptt*) were selected. These isolates were obtained from 23 provinces of Turkey, including: Edirne, Denizli, Afyon, Eskişehir, Ankara, Konya, Çankırı,Kirikkale, Aksaray, Kırşehir, Mersin, Kaysere, Kilis, Kahramanmaraş, Sivas, Gaziantep, Diyarbakir, Şanlıurfa, Mardin, Şırnak, Siirt, Batman and Adıyaman. Isolates were chosen based on their geographic separation, size of barley cultivation area in respective provinces, and isolate morphological characteristics (growth rate, colour, growing habit) in agar cultures. The identities of the isolates were verified for their net and spot form status by inoculating cultures onto local barley cv. Bülbül 89, which is susceptible to net and spot forms of the pathogen (Karakaya et al., 2014; Usta et al., 2014; Yazıcı et al., 2015).

**Differential host set**

The differential set outlined by Wu et al. (2003) was used for pathotype determination of both forms of *P. teres*. This set consisted of 25 barley genotypes. Twenty two of these were used by Steffenson and Webster (1992b) in an earlier study.

**Inoculation, incubation and disease assessments**

Five to ten seeds of each differential set genotype were planted in 7 cm diam. plastic pots containing a mixture of top soil, sand and organic matter (60:20:20, v:v:v). Plants were maintained in greenhouse conditions before and after inoculation. Three replicates of each genotype were sown to pots. They were arranged in a randomized fashion. Inoculum of each single conidium isolate was obtained from a 10-d-old culture grown on PDA, by scraping the culture with a paintbrush and washing through cheesecloth with water. Inoculum density, consisting of mycelium pieces, was adjusted to 1.5–2.0 × 10^5 mycelium parts per mL. One drop of Tween 20 was added to each 100 mL of inoculum suspension (Aktaş, 1995). Seedlings were inoculated at the two to three leaf stage (Z12-13; Zadoks et al., 1974). Mycelium suspensions were sprayed individually onto sets of seedlings, and the inoculated plants were kept at high humidity in closed transparent lid boxes for 76 h in a greenhouse. The temperature of the greenhouse was 18–23±1°C with a 14h/10h light/dark regime. After this period, the box lids were opened for 48 h under the same conditions. After 7 d, the seedlings were assessed for disease severity using the net and spot form scales described by Tekauz (1985).

**Pathotype determination**

The differential set of barley genotypes were numbered from 1 to 25, as follows: 1 = Tifang, 2 = Canadian L. Shore, 3 = Atlas, 4 = Rojo, 5 = Coast, 6 = Manchurian, 7 = Ming, 8 = CI 9819, 9 = Algerian, 10 = Kombar, 11 = CI 11458, 12 = CI 5791, 13 = Harbin, 14 = CI 7584, 15 = Prato, 16 = Manchuria, 17 = CI 5822, 18 = CI 4922, 19 = Hazera, 20 = Cape, 21 = Beecher, 22 = Rika, 23 = NDB 112, 24 = FR 926-17, and 25 = Hector.

The genotypes that scored as 1, 2, 3, 4, 5 according to Tekauz (1985) scale were evaluated as resistant (R); whereas those that scored as 6, 7, 8, 9, 10 were evaluated as susceptible (S).

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The pathotype terminology described by Steffenson and Webster (1992b) and Wu et al. (2003) was used. Each number in a pathotype assay corresponds to the numbered virulence type of the isolate, which is virulent (severity scale values 6–10). The isolates that were not virulent (scale values 1–5) to all the differential set genotypes were identified as Pathotype 0 (Wu et al., 2003).

**Results**

**Pathotypes**

From 50 Ptm isolates and 40 Ptt isolates, 26 Ptm and 24 Ptt pathotypes were determined on the 25 differential barley genotypes (Tables 1 and 2).

The most common pathotype among the Ptm isolates was Pathotype 6-18, represented by 12 isolates (Figure 1). The other common pathotypes were Pathotype 0 and Pathotype 18, which consisted of seven isolates, corresponding to 14% of total isolates each. The most complex pathotype, Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25 (isolate Gps 263) was virulent to all 25 of the tested differential barley genotypes.

The most common pathotype among Ptt isolates was Pathotype 0 which was represented by seven isolates (Figure 1). The most complex pathotype, Pathotype 3-4-6-7-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25 (isolate Gps 263) was virulent to all 25 of the tested differential barley genotypes.

**Differential set**

Differential genotype CI 4922 was susceptible to 34 Ptm isolates (68% of total Ptm isolates). Cultivar Manchurian gave susceptible reactions to 25 Ptm isolates (50% of Ptm isolates) and cv. Kombar was susceptible to 21 Ptm isolates (42% of Ptm isolates). No genotype was resistant to all Ptm isolates, although genotype NDB 112 was resistant to 48 Ptm isolates and susceptible to only two Ptm isolates. Cultivar Prato and genotype FR 926-17 were resistant to 45 Ptm isolates and susceptible to five of these isolates.

Cultivar Kombar was susceptible to 29 Ptt isolates (73% of the Ptt isolates). Genotype CI 4922 was susceptible to 26 Ptt isolates (65% of Ptt isolates) and cv. Manchurian was susceptible to 22 Ptt isolates (55% of Ptt isolates). Cultivar Tifang and genotypes NDB 112 and FR 926-17 were resistant to all of the Ptt isolates. Also, cvs. Ming, Harbin, Manchuria and CI 5791 genotype were susceptible to only one Ptt isolate, and resistant to 39 of these isolates.

**Discussion**

This is the first detailed study of virulence of *Pyrenophora teres f. teres* and *P. teres f. maculata* populations in Turkey. The populations were pathogenically diverse, with 26 pathotypes identified for Ptm, and 24 identified for Ptt.

In previous studies, researchers identified numerous pathotype/isolate ratios of Ptt. Pathotype/isolate ratios varied between 0.14 and 1 (Tekauz, 1990; Steffenson and Webster, 1992b; Jonsson et al., 1997; Douiyssi et al., 1998; Cromey and Parkes, 2003; Wu et al., 2003; Bouajila et al., 2011; Fowler and Platz, 2011; Bourgab et al., 2012, Liu et al., 2012; Akhavan et al., 2016). In our study, the pathotype/isolate ratio for Ptt was 0.6. This pathogenic variation was less than reported by Douiyssi et al. (1998), Wu et al. (2003) and Liu et al. (2012), but greater than reported for the other studies mentioned above.

In previous Ptm pathotype determination studies, Karki and Sharp (1986) recognized six groups, and Gupta et al. (2012) recognized seven groups. In other studies, pathotype/isolate ratios varied between 0.47 and 0.55 (Tekauz, 1990; Wu et al., 2003; McLean et al., 2014; Akhavan et al., 2016). In our study, the pathotype/isolate ratio of Ptm was 0.52. This variation was less than that of McLean et al. (2014), but greater than in the other studies mentioned above.

Serenius et al. (2007) reported that pathogenic and genetic structures of Ptm populations could be different in every continent. According to McLean et al. (2011), there were different reactions of different host genotypes to isolates from Australia and Canada, and even for pathogen isolates from the same continent. Other studies showed that the resistance to both net and spot pathogen forms can change when alternating barley cultivars are planted (Khan, 1982; Gupta and Loughman, 2001; Cromey and Parkes, 2003).

Although there are several studies for the spot form of this pathogen, studies on the net form have been more common, since the net form is more prevalent globally (Louw et al., 1996; McLean et al., 2009; Liu and Friesen, 2010). In our survey, net and spot forms of *P. teres* were found, but the spot form was more common (Karakaya et al., 2014). Several
Table 1. Twenty six pathotypes of *Pyrenophora teres f. maculata* determined in Turkey.

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Location</th>
<th>Susceptible genotypes No./ Pathotype No.</th>
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</thead>
<tbody>
<tr>
<td>13-181</td>
<td>K.Maraş Pazarcık</td>
<td>Pathotype 0</td>
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<tr>
<td>13-157</td>
<td>Diyarbakır</td>
<td></td>
</tr>
<tr>
<td><em>H. spontaneum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gps 49</td>
<td>Kayseri Tomarza</td>
<td></td>
</tr>
<tr>
<td>13-177</td>
<td>Adıyaman Gölbashi</td>
<td></td>
</tr>
<tr>
<td>Gps 68</td>
<td>Kırşehir Central District</td>
<td></td>
</tr>
<tr>
<td>13-167</td>
<td>Diyarbakır</td>
<td></td>
</tr>
<tr>
<td><em>H. spontaneum</em></td>
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<td></td>
</tr>
<tr>
<td>Gps 265</td>
<td>Ankara</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ş.Koçhisar</td>
<td></td>
</tr>
<tr>
<td>Gps 116</td>
<td>Konya Bozköy</td>
<td>Pathotype 18</td>
</tr>
<tr>
<td>Gps 3</td>
<td>Ankara Elmadağ</td>
<td></td>
</tr>
<tr>
<td>Gps 81</td>
<td>Çankırı Ilgaz</td>
<td></td>
</tr>
<tr>
<td>13-116</td>
<td>Niğde Ulukuşla</td>
<td></td>
</tr>
<tr>
<td>Gps 79</td>
<td>Çankırı Central District</td>
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<tr>
<td>Gps 270</td>
<td>Konya Ereğli</td>
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<td>Gps 129</td>
<td>Konya Cihanbeyli</td>
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<td>Gps 90</td>
<td>Ankara Haymana</td>
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<td>Konya Karatay</td>
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<tr>
<td>Gps 101</td>
<td>Konya Akşehir</td>
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</tr>
<tr>
<td>Gps 122</td>
<td>Konya Çumra</td>
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</tr>
<tr>
<td>Gps 272</td>
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<tr>
<td>13-194</td>
<td>Kayseri İncesu</td>
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(Continued)
### Table 1. (Continued).

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<th>Isolate No.</th>
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<tr>
<td>13-149</td>
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<td>Gps 50</td>
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<tr>
<td></td>
<td>Tomarza</td>
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</tr>
<tr>
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<td>Eskişehir</td>
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</tr>
<tr>
<td></td>
<td>Beylikova</td>
<td></td>
</tr>
<tr>
<td>Gps 158</td>
<td>Eskişehir</td>
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<td>Odunpazari</td>
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<td>Gps 227</td>
<td>Eskişehir</td>
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<td>Sivrihisar</td>
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<td>Kırşehir</td>
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<td>Kaman</td>
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<td>13-139</td>
<td>Mardin</td>
<td>Pathotype 3-10</td>
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<tr>
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<td>Central District</td>
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<td>Gps 119</td>
<td>Konya</td>
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<td>Gps 162</td>
<td>Eskişehir</td>
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<td>Alpu</td>
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<td>Ankara</td>
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<td>Uhk 74</td>
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<td>Kargamuş</td>
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<td>Konya</td>
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<td>Yunak</td>
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<td>Ömerli</td>
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<td>Bünyan</td>
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<td>Edirne</td>
<td>Edirne</td>
<td>Pathotype 2-5-7-9-10-13-18-21</td>
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<td>Gps 27</td>
<td>Sivas</td>
<td>Pathotype 2-4-5-6-10-11-12-13-14-18</td>
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<td>Şarkışla</td>
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<td>13-168</td>
<td>Diyarbakır</td>
<td>Pathotype 5-8-10-11-12-14-19-20-21-22</td>
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<td>Gps 155</td>
<td>Afyon</td>
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<td>Emirdağ</td>
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<tr>
<td>13-136</td>
<td>Mardin</td>
<td>Pathotype 3-5-6-7-9-10-11-14-19-20-21-22</td>
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<tr>
<td></td>
<td>Nusaybin</td>
<td></td>
</tr>
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<tr>
<td></td>
<td>Central District</td>
<td></td>
</tr>
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</table>

(Continued)
researchers have used different differential host sets for this type of study. Some of these sets included the same barley cultivars for spot and net form of the disease. In these studies, several common differential lines were used (Karki and Sharp, 1986; Tekauz, 1990; Gupta and Loughmann, 2001; Wu et al., 2003), and comparisons of global virulence variations have been made (Afanasenko et al., 2009). In the present study, we employed the differential set used by Wu et al. (2003), and this was useful for revealing the pathotypes of both forms of *P. teres*.

Cultivar Kombar was used as a susceptible control cultivar in previous studies (Steffenson and Webster, 1992a; Steffenson and Webster, 1992b; Cromey and Parkes, 2003). This cultivar was susceptible to more than half of the isolates tested in the present study.

Cromey and Parkes (2003) found the barley genotype CI 4922 to be resistant to all isolates tested, whereas Steffenson and Webster (1992a) and Wu et al. (2003) reported this genotype to be susceptible to some pathotypes. In our study, genotype CI 4922 was susceptible to 68% of *Ptm* and 65% of *Ptt* isolates tested.

Cultivar Tifang and genotypes NDB 112 and FR 926-17 were resistant to all *Ptt* isolates tested in the present study. Genotype CI 5791 was resistant to all except one isolate, namely isolate Gps 18. A similar result was reported by Akhavan et al. (2016), where genotype CI 5791 was resistant to all but one isolate tested. Furthermore, Afanasenko et al. (2009) and Fowler et al. (2014) emphasised that genotype CI 5791 was highly resistant. Cultivar Tifang was a resistant control cultivar in the Cromey and Parkes (2003) study, and exhibited a resistant reaction. Also, Steffenson and Webster (1992b) reported that cv. Tifang was resistant to all Californian *P. teres* pathotypes.

In the case of our *Ptm* isolates, host genotype NDB 112 was susceptible to two isolates (4%) and resistant to 48 isolates. Genotype FR 926-17 was susceptible to five isolates and resistant to 45 isolates (10%), whereas cv. Tifang and genotype CI 5791 were susceptible to nine (18%) isolates and resistant to 41 isolates. Tekauz and Mills (1974) indicated that genotype CI 5791 was less resistant to the spot form of barley net blotch disease.

Wu et al. (2003) reported that cvs. Rojo and Coast, and genotypes CI 9819, CI 5791, CI 7584, CI 5822, NDB 112, FR 926-77 were resistant to all *Ptt* and *Ptm* isolates they tested. In our study, from 50 *Ptm* isolates; two isolates (4%) were virulent on genotype

Table 1. (Continued).

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Location</th>
<th>Susceptible genotypes No./ Pathotype No.</th>
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<tbody>
<tr>
<td>13-163</td>
<td>Diyarbakır Central District</td>
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<td>Gps 276</td>
<td>Kilis Central District</td>
<td>Pathotype 1-2-3-4-5-7-8-9-10-11-12-13-14-18-19-20-22-24-25</td>
</tr>
<tr>
<td>13-127</td>
<td>Şanlıurfa Ceylanpınar</td>
<td>Pathotype 1-2-3-4-5-6-7-8-9-10-11-13-14-15-16-17-20-21-22-25</td>
</tr>
<tr>
<td>Gps 76</td>
<td>Ankara Kalecik</td>
<td>Pathotype 1-2-3-4-5-6-8-9-10-11-12-14-15-16-17-18-19-20-21-22-23-25</td>
</tr>
<tr>
<td>13-167</td>
<td>Diyarbakır Central District</td>
<td>Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-16-18-19-20-21-22-24</td>
</tr>
<tr>
<td>13-179</td>
<td>Kahramanmaraş Pazarcık</td>
<td>Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-17-18-19-20-21-22-24-25</td>
</tr>
<tr>
<td>Gps 263</td>
<td>Ankara Bala</td>
<td>Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25</td>
</tr>
</tbody>
</table>
Table 2. Twenty four pathotypes of *Pyrenophora teres f. teres* determined in Turkey

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Location</th>
<th>Susceptible genotypes No./ Pathotype No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gps 134</td>
<td>Eskişehir</td>
<td>Pathotype 0</td>
</tr>
<tr>
<td>15-61</td>
<td>Gaziantep Şahinbey</td>
<td></td>
</tr>
<tr>
<td>15-41</td>
<td>Siirt Central District</td>
<td></td>
</tr>
<tr>
<td>13-134</td>
<td>Mardin Kızıltepe</td>
<td></td>
</tr>
<tr>
<td>Denizli</td>
<td>Denizli</td>
<td></td>
</tr>
<tr>
<td>Gps 271</td>
<td>Mersin Central District</td>
<td></td>
</tr>
<tr>
<td>13-172</td>
<td>Diyarbakır Central District</td>
<td></td>
</tr>
<tr>
<td>13-174</td>
<td>Adıyaman Central District</td>
<td>Pathotype 22</td>
</tr>
<tr>
<td>13-111</td>
<td>Ankara Ş.Koçhisar</td>
<td>Pathotype 18</td>
</tr>
<tr>
<td>13-123</td>
<td>Şanlıurfa Central District</td>
<td>Pathotype 2-10</td>
</tr>
<tr>
<td>15-66</td>
<td>Kilis Central District</td>
<td>Pathotype 6-22-25</td>
</tr>
<tr>
<td>Gps 167</td>
<td>Eskişehir Seyitgazi</td>
<td>Pathotype 6-10-18</td>
</tr>
<tr>
<td>15-48</td>
<td>Batman Central District</td>
<td></td>
</tr>
<tr>
<td>Gps 205</td>
<td>Eskişehir Sivrihisar</td>
<td></td>
</tr>
<tr>
<td>Gps 33</td>
<td>Sivas Gemerek</td>
<td></td>
</tr>
<tr>
<td>15-60</td>
<td>Gaziantep Şahinbey</td>
<td></td>
</tr>
<tr>
<td>Gps 145</td>
<td>Eskişehir İnönü</td>
<td></td>
</tr>
<tr>
<td>Gps 198</td>
<td>Eskişehir Mahmudiye</td>
<td>Pathotype 2-6-10-18</td>
</tr>
<tr>
<td>Gps 213</td>
<td>Eskişehir Çifteler</td>
<td>Pathotype 6-10-18-25</td>
</tr>
<tr>
<td>Gps 53</td>
<td>Kayseri Kocasinan</td>
<td></td>
</tr>
<tr>
<td>Gps 243</td>
<td>Eskişehir Sivrihisar</td>
<td>Pathotype 6-10-18-20</td>
</tr>
</tbody>
</table>
Pathotypes of *Pyrenophora teres* on barley in Turkey

NDB 112, five isolates (10%) on genotype FR 926-17, six isolates (12%) on genotype CI 5822, nine (18%) on genotype CI 5791 and cv. Rojo, ten (20%) on genotype CI 9819, 13 (26%) on genotype CI 7584, and 18 isolates (36%) were virulent on cv. Coast. From 40 *Ptt* isolates; five (12.5%) were virulent on genotype CI 5822, four (10%) on cv. Rojo and genotypes CI 9819 and CI 7584, three (7.5%) on cv. Coast, and one isolate was virulent on genotype CI 5791. The genotypes NDB 112 and FR 926-77 were found resistant to all of the *Ptt* isolates. Tekauz and Mills (1974) reported that resistant hybrid lines CI 5791 and BT 201 were resistant to the net form of *P. teres*, but less resistant to the spot form in production areas. In an-

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Location</th>
<th>Susceptible genotypes No./ Pathotype No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-62 Hordeum spontaneum</td>
<td>Kilis Central District</td>
<td>Pathotype 6-10-18-22-25</td>
</tr>
<tr>
<td>15-39 Hordeum spontaneum</td>
<td>Siirt Tillo</td>
<td>Pathotype 3-6-10-18-20-25</td>
</tr>
<tr>
<td>15-13</td>
<td>Ankara Yenimahalle</td>
<td>Pathotype 3-6-10-18-20-25</td>
</tr>
<tr>
<td>15-65</td>
<td>Kilis Central District</td>
<td>Pathotype 2-6-9-10-18-25</td>
</tr>
<tr>
<td>Uhk 67</td>
<td>Şanlıurfa Birecik</td>
<td>Pathotype 2-6-9-10-18-25</td>
</tr>
<tr>
<td>Gps 110</td>
<td>Konya Meram</td>
<td>Pathotype 2-6-9-10-18-25</td>
</tr>
<tr>
<td>15-37</td>
<td>Şırnak Cizre</td>
<td>Pathotype 3-6-10-18-22-25</td>
</tr>
<tr>
<td>15-26</td>
<td>Şanlıurfa Ceylanpunar</td>
<td>Pathotype 2-6-9-10-18-25</td>
</tr>
<tr>
<td>Gps 201</td>
<td>Eskişehir Mahmudiye</td>
<td>Pathotype 3-5-6-9-10-17-18-25</td>
</tr>
<tr>
<td>Gps 48</td>
<td>Kayseri Tomarza</td>
<td>Pathotype 2-3-5-6-9-18-21-25</td>
</tr>
<tr>
<td>13-126</td>
<td>Şanlıurfa Central District</td>
<td>Pathotype 2-3-8-10-17-18-19-20-21</td>
</tr>
<tr>
<td>15-32</td>
<td>Mardin Central District</td>
<td>Pathotype 2-3-6-9-10-15-18-19-20-21</td>
</tr>
<tr>
<td>13-151</td>
<td>Mardin Midyat</td>
<td>Pathotype 2-3-4-8-9-10-14-18-19-20</td>
</tr>
<tr>
<td>13-175</td>
<td>Adıyaman Besni</td>
<td>Pathotype 3-4-8-9-10-11-15-17-20-21</td>
</tr>
<tr>
<td>13-130</td>
<td>Şanlıurfa Ceylanpunar</td>
<td>Pathotype 2-3-8-9-10-14-15-18-19-20-21</td>
</tr>
<tr>
<td>Uhk 77</td>
<td>Kilis Central District</td>
<td>Pathotype 2-3-4-5-9-10-11-13-14-17-18-19-20-21-22-25</td>
</tr>
<tr>
<td>Gps 18</td>
<td>Sivas Yıldızeli</td>
<td>Pathotype 3-4-6-7-9-10-11-12-14-15-16-17-18-20-21-22-25</td>
</tr>
</tbody>
</table>

Table 2. (Continued).
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other study, 15 *Ptt* isolates were tested on 38 differential barley genotypes including genotype NDB 112. No reaction was the same for 15 isolates, and no barley genotype was completely resistant to all isolates tested (Douiyssi *et al.*, 1998).

A study in New Zealand showed that all *Ptt* isolates tested were virulent to cvs. Herta and Rika, whereas 19 differential other cultivars and lines were resistant to all isolates. More than half of the isolates were virulent to cv. Kombar and genotype CI 11458, and these isolates were less virulent to cvs. Algerian, Atlas, Cape, Harbin, Manchurian, Ming and Prato, and genotype CI 2330 (Cromey and Parkes, 2003). In contrast, the present study showed that only seven of the *Ptt* isolates (17.5%) were virulent to cv. Rika. In our study, of all the isolates tested, ten isolates were virulent on cv. Beecher, ten on cv. Canadian Lake Shore, three on cv. Coast, eight on cv. Hazera, four on cv. Rojo, 26 on genotype CI 4922, four on genotype CI 7584, four on genotype CI 9819, and one isolate was virulent on genotype CI 5791. All isolates were avirulent to cv. Tifang. Cultivars Heartland, Manchu, Norbert, Rabat 071, Steptoe, and genotypes TR 043, CI 1243, CI 9214, CI 9820 were not used in our study.

The studies show that virulence of *Ptm* and *Ptt* varies at the local and global levels. Furthermore, resistance to the diseases caused by these pathogens changes when alternating barley cultivars are planted (Khan, 1982; Gupta and Loughman, 2001; Cromey and Parkes, 2003).

This study has demonstrated the high level of pathogenic variation among the *Ptt* and *Ptm* populations in Turkey. Recombination, gene flow and mutation can induce variation in fungi (Burdon and Silk, 1997). Pathotypes with increased virulence could appear as a result of these mechanisms. These new pathotypes could cause increased disease and render resistant plant genotypes susceptible. This creates challenges for plant breeders. In order to breed disease resistant plants, pathotype composition should be elucidated. For deployment of successful and durable plant resistance, dominant and virulent pathotypes should be considered in breeding studies. Continuous monitoring of the virulence of *P. teres* enhances the study of resistance to this pathogen and helps to develop appropriate resistance strategies for barley breeding programmes.

**Acknowledgement**

This study is supported by The Scientific and Technological Research Council of Turkey (Project No. 111O644).

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Accepted for publication: May 29, 2017
RESEARCH PAPERS

Evaluating severity of leaf spot of lettuce, caused by *Allophoma tropica*, under a climate change scenario

**MARIA LODOVICA GULLINO**1,2, **GIOVANNA GILARDI**1 and **ANGELO GARIBALDI**1

1 Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy
2 Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy

**Summary.** Climate changes, particularly increases in temperature and CO$_2$, are seriously challenging agriculture, and are one of the main factors that should be considered in the emergence of new diseases and their potential spread. Six trials were carried out to evaluate the effects of increased temperature and CO$_2$ on the severity of leaf spot of lettuce, caused by *Allophoma tropica* (syn. *Phoma tropica*), a pathogen that was first observed on lettuce in northern Italy in 2011. Temperature, CO$_2$ and their interactions were significant factors ($P<0.0001$) influencing incidence and severity of leaf spot on lettuce. Temperatures between 22 and 26°C were the most favourable to the pathogen, and increased disease incidence and severity. Reductions in disease incidence and severity were observed at lower (18–22°C) and higher (26–30°C) temperatures. Concentrations of CO$_2$ ranging from 800 to 850 ppm increased disease incidence and severity at all the temperature ranges tested, and these effects were greatest at 22-26°C. Analysis of these results could be useful for mid-term agricultural planning at a regional scale, so that crops and their varieties can be adapted to anticipated future climate trends.

**Key words:** phytotron, leafy vegetable, CO$_2$, temperature, *Lactuca sativa*, Phoma leaf spot.

Introduction

Climate changes, and increases in temperature and CO$_2$ in particular, are seriously challenging agriculture and affecting pathosystems, together with other global change components, such as air, water and soil pollution, the long-distance introduction of exotic host and pest/pathogen species and globalization of markets (Pautasso et al., 2012). Southern Europe is expected to be seriously affected by these climate changes, with consequent extensification of its cropping systems and abandonment of some crops and cultivation areas (Bindi and Olesen, 2011). Climate changes have recently been indicated as one of the main factors that should be taken assessed when considering the emergence of new diseases and their potential spread (Garibaldi and Gullino, 2010; Garibaldi et al., 2014).

Such a perspective has stimulated the search for solutions, through different approaches, to mitigate the negative effects of climate changes. Phytotrons have proved to be useful tools to simulate climate change scenarios, as the most important environmental parameters, i.e. temperature and atmospheric CO$_2$ concentrations, can be varied, and the effect of such changes on plants and their pathogens can be evaluated. Although the first research on effects of climate changes on plant pathogens was concentrated on field crops, such as cereals and grapevine (Coakley et al., 1999; von Tiedemann and Firsching 2000; Garrett et al., 2006; Salinari et al., 2006; White et al., 2006; Bregaglio et al., 2013), more recent research has evaluated climate change effects on foliar and soil-borne pathogens of vegetable and ornamental...
crops (Pugliese et al., 2010, 2012a, 2012b; Ferrocino et al., 2013; Chitarra et al., 2015; Gilardi et al., 2016a, 2016b).

Many Mediterranean countries, including Italy, have long been areas appropriate for production of leafy vegetables, including lettuce (*Lactuca sativa* L.), in greenhouses and in open fields. Lettuce is a popular crop throughout the world, and its consumption has been increasing as a consequence of the attention being paid to healthy eating habits and to the availability of convenience foods. Lettuce is also a crop that has been frequently studied to evaluate the effects of climate changes, and because it is susceptible to several pathogens (Mortensen 1985; Wheeler et al., 1993; Ferrocino et al., 2013). Lettuce is susceptible to many fungal diseases, caused by soilborne and foliar pathogens (Davis et al., 1997; Blancard and Maissoneuve 2003). Recently, many new diseases have been reported on lettuce, most of which have been linked to the intensification of the production systems, to the use of new varieties corresponding with new consumer preferences (i.e. ready-to-eat salads), and to the quick and simultaneous spread of some pathogens through the use of infested/infected seeds in globalized markets (Gullino et al., 2014).

The present study was undertaken to evaluate the effects of increased temperature and atmospheric CO$_2$ concentrations, which were simulated by working in phytotron conditions, on the severity of the leaf spot of lettuce, caused by *Allophoma tropica* (syn. *Phoma tropica*). This pathogen was first observed on lettuce in northern Italy in 2011 (Garibaldi et al., 2012), and was renamed by Chen et al. (2015).

### Material and methods

#### Experimental layout

Six trials were carried out in 2016 at the Center for Innovation in the Agro-environmental Sector (AGROINNOVA), at the University of Torino, in Grugliasco (Italy), in six physically and electronically separated phytotrons (each with internal dimensions of 2 m wide × 2 m long × 2.5 m high). These were operated with a 14 h light/10 h dark photoperiod, provided by two lighting systems (master-colour CDM-TD metallic iodine discharge lamps and TLD 18-830 Philips neon lamps) (Gullino et al., 2011). Eight environmental combinations were tested under completely controlled conditions. The following temperature and CO$_2$ concentration combinations were tested in the first set of trials: 1) 400–450 ppm CO$_2$, 14–18°C; 2) 800–850 ppm CO$_2$, 14–18°C; 3) 400–450 ppm CO$_2$, 18–22°C; 4) 800–850 ppm CO$_2$, 18–22°C; 5) 400–450 ppm CO$_2$, 22–26°C, and 6) 800–850 ppm CO$_2$, 22–26°C.

In the second set of trials (Table 1), since it had been observed that leaf spot caused by *A. tropica* was...

| Table 1. Treatments and experimental details for six trials carried out on *Allophoma tropica* on lettuce (cv. Elisa). |
|--------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                   | First set of trials |                             | Second set of trials |                             |
|                   | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 6 |
| CO$_2$ × Temperature | 400-450 ppm CO$_2$, 14-18 °C | 800-850 ppm CO$_2$, 14-18 °C | 400-450 ppm CO$_2$, 18-22 °C | 800-850 ppm CO$_2$, 18-22 °C | 400-450 ppm CO$_2$, 22-26 °C | 800-850 ppm CO$_2$, 22-26 °C |
| Sowing date | 22/03/16 | 12/04/16 | 29/04/16 | 11/08/2016 | 26/09/16 | 10/10/16 |
| Transfer of plants to phytotrons | 1/04/16 | 3/05/16 | 20/05/16 | 01/09/16 | 7/10/16 | 31/10/16 |
| Inoculations with *Allophoma tropica* | 11/04/16 | 11/05/16 | 27/05/16 | 8/09/16 | 19/10/16 | 08/11/16 |
| First symptoms | 13/04/16 | 16/05/16 | 31/05/16 | 12/09/16 | 21/10/16 | 14/11/16 |
| Final disease assessment and end of the trial | 18/04/16 | 19/05/16 | 3/06/16 | 16/09/16 | 26/10/16 | 17/11/16 |
significantly reduced at the lowest temperature, the temperature range of 14–18°C was replaced with a higher temperature range, 26–30 °C, which was combined with CO₂ concentration at a standard 400–450 ppm and double concentration, 800–850 ppm.

A total of five to six pots (one pot = one experimental unit per phytotron per pathosystem per trial) were examined.

The environmental parameters (light, temperature, humidity and atmospheric CO₂) inside the phytotrons were continuously monitored and kept constant. The light intensity regime, which resulted from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to 1,200 μmol m⁻² s⁻¹, and relative humidity (RH), were regulated in the same way in all the phytotrons. The phytotrons were randomized by changing the environmental conditions and combinations during the first and second set of trials (Table 1).

**Plant material and artificial inoculation**

Lettuce plants (cv. Elisa; T&T) were grown in 2 L capacity plastic pots, filled with a steamed (90°C for 30 min) white pea:perlite (80:20 v:v) mix (Turco Silvestro). At least 20 plants were present in each pot. The plants were kept in a nursery compartment in a glasshouse at 22–23°C before being transferred to the phytotrons.

The PHT30 coded strain of Allophoma tropica, isolated from infected plants, was used for the inoculation of the lettuce plants. The pathogen was grown on potato dextrose agar (PDA; Merck) amended with streptomycin sulphate, for 7–10 d at 20–23 °C, with a 12 h photoperiod. Suspensions containing $5 \times 10^5$ conidia mL⁻¹ of the pathogen were used to inoculate 20 to 25-d-old plants. The inoculum suspensions were applied 7 d after the plants had been transferred to the phytotrons. After inoculation, the pots were placed under a plastic support (100 × 100 × 50 cm) and covered with a transparent polyethylene film (50 mm thick) for 24h, to maintain relative humidity at 95–100%.

**Disease assessments and statistical analysis**

The inoculated plants were observed for disease development. Disease incidence (DI, expressed as percent of infected leaves) was evaluated using a rating scale of 0 to 5 (0 = no symptoms; 1 = up to 5% of leaf area affected; 2 = 6 to 10% affected; 3 = 11 to 25% affected; 4 = 26 to 50% affected; and 5 = 51 to 100% of leaf area affected). DI was calculated using the formula:

$$DI = \frac{\sum(\text{n° leaves} \times x_{0-5})}{\text{total number of leaves recorded}}$$

where $x_{0-5} = (x_0 = 0; x_1 = 5%; x_2 = 10%; x_3 = 25%; x_4 = 50%$ or $x_5 = 75%$).

Two-way analysis of variance confirmed that the temperature, CO₂ and their interactions were statistically significant factors ($P<0.0001$) of influence on disease incidence and severity of A. phoma on lettuce.

Temperatures between 22 and 26°C are the most favourable for the pathogen, and caused significantly more disease (incidence and severity) than the other two temperature regimes. Significant reductions in disease incidence and severity were observed at lower (18–22°C) and higher temperatures (26–30°C) (Figures 1 and 3). High atmospheric CO₂ concentrations significantly increased disease incidence and severity for all three temperature regimes. This effect was amplified for DS at 22–26°C, the most favourable for disease development (Figure 2).
Discussion

The pathogen *A. tropica*, with temperate temperature requirements, is ideal for studies to evaluate effects of climate changes on plant diseases. Recent epidemiological studies have shown that this pathogen is more aggressive at temperatures of 20 and 25°C, which are typical under tunnel house conditions in northern Italy during spring. At these temperatures,
short periods of high relative humidity (1–6 h) are sufficient to cause significant crop losses (Gilardi et al., 2017).

Studies carried out to understand effects of climate changes on pathogens, have shown that effects of increased temperatures and CO₂ concentrations vary according to the pathosystem. In some cases, increased temperature is the most important factor, while in others increased CO₂ is more relevant. In still other cases, increased temperature and CO₂ interact to affect disease severity. As underlined by Chakraborty (2013), to improve confidence in future predictions, a broad range of scenarios and case studies need to be evaluated. Empirical research, using factorial combinations of CO₂ and temperature, is useful to develop future prediction models, since the current models are not based on findings from multifactorial studies. A study carried out in phytotrons with the for the lettuce/Fusarium oxysporum f. sp. lactucae pathosystem showed that severity of Fusarium wilt increased with elevated temperature, while elevated CO₂ did not affect severity of this disease (Ferrocino et al., 2013).

In the present study, which was carried out under controlled conditions in phytotrons in short duration experiments, the elevated temperatures alone did not result in increased disease incidence or severity, caused by A. tropica on lettuce. Instead, there was clear effect of elevated atmospheric CO₂ on leaf spot of lettuce for the 22 to 26°C temperature regime.

Analysis of these results could be useful for midterm agricultural planning at a regional scale, to adapt crops and their varieties to anticipated future climate trends. This is particularly important for agricultural systems in the Mediterranean and South European regions, which are likely to be more vulnerable to climate changes than other European regions (Bindi and Olesen, 2011).

Increases in temperature and CO₂ concentrations are among the main factors that should be assessed when considering the emergence of new diseases and their potential spread. Here we have demonstrated that temperature, CO₂ concentrations and their interactions were significant factors influencing on disease incidence and severity for A. phoma leaf spot on lettuce. Temperatures of 22 to 26°C were the most favourable to the pathogen, and CO₂ concentrations of 800 to 850 ppm increased disease incidence and severity at this temperature range in comparison to the other climate regimes assessed.

These results provide new information on the effect of elevated temperature and CO₂ concentrations for a new disease of lettuce, highlighting the presence

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**Figure 3.** Effects of different CO₂ and temperature (18–22, 22–26 and 26–30°C) combinations on leaf spot caused by Allophoma tropica on lettuce (cv. Elisa). A) Mean disease incidence, % of affected leaves. B) disease severity, % of leaf area affected. Mean values for trials 4–6. Data accompanied by the same letter are not significantly different, according to Tukey’s Test (P<0.05). The boxes represent the interquartile ranges (IQR) between the first and third quartiles, and the lines inside the boxes represent the median (2nd quartile). The whiskers denote the least and the greatest values within 1.56 IQR from, respectively, the first and third quartiles.
of different responses to climate change in different pathosystems. More research in this field is needed, at a regional scale, to provide plant breeders with greater insights supporting development of crop varieties that could adapt to future weather conditions, and which could show resistance to pathogens likely to become predominant in the future, as a result of forecasted climate changes.

Acknowledgements

The research was supported by the European Union Horizon 2020 research and innovation programme, under grant agreement No. 634179 “Effective Management of Pests and Harmful Alien Species - Integrated Solutions” (EMPHASIS). The authors thank Federico Berta and Andrea China Gallo for technical support, and Marguerite Jones for language revision.

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Phytotron study of Phoma leaf spot of lettuce

Accepted for publication: June 10, 2107
Proficiency of real-time PCR detection of latent Monilinia spp. infection in nectarine flowers and fruit

CARLOS GARCIA-BENITEZ, PALOMA MELGAREJO, ARUNAS BENIUSIS, CÉCILE GUINET, SÜREYYA ÖZBEN, KEMAL DEĞIRMENCİ, MARIA TERESA VALENTE, LUCA RICCIONI and ANTONIETA DE CAL

1 Department of Plant Protection, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de La Coruña km 7, 28040 Madrid, Spain
2 Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di Ricerca per la Patologia Vegetale (CREA-PAV), Via G. Bertero, 22, 00156 Rome, Italy
3 General Directorate of Agricultural Research (GDAR) Plant Protection Central Research Institute, Gayret Mah. Fatih Sultan Mehmet Bulv. No: 66 Yenimahalle, Ankara, Turkey
4 ANSES-Plant Health Laboratory, Domaine de Pixerecourt, Bat E, CS40009, F-54220 Malzeville, France
5 The State Plant Service under the Ministry of Agriculture (SPS-MoA), Sukileliu str. 9A, LT-11352, Vilnius, Lithuania
6 Sentromer Building, Construction and Agricultural Research Limited Company, Ankara, Turkey

Summary. Rapid and reliable detection of Monilinia latent infections is needed to prevent and control dispersion of Monilinia spp. in infected localities and non-infected countries. A fast multiplex quantitative real-time PCR method (qPCR) for the detection and identification of Monilinia spp. latent infections in blossoms and fruit of nectarine trees (Prunus persica var. nucipersica) was tested in an inter-laboratory trial. The test performance study involving five laboratories was conducted to validate the sensitivity and specificity of several real-time PCR platforms for the detection of low amounts of Monilinia DNA (latent infections), using a common protocol, and to identify possible difficulties when these tests were implemented by diagnostic laboratories or national reference centres. The method has two hydrolysis probes distinguishing between Monilinia fructicola and M. fructigena/M. laxa. Validation included test performance accuracy, analytical specificity and sensitivity, repeatability, and reproducibility, as defined by standard PM7/98 of the European Plant Protection Organization (EPPO). All qPCR platforms detected Monilinia latent infections and mycelium samples with both hydrolysis probes, and healthy flowers and fruit samples gave negative results. The method was consistent between different laboratories, despite different equipment used, and there were no laboratories with z-scores in the unacceptable region. Monilinia fructicola latent infection samples were correctly detected by all laboratories, but some M. laxa samples were cross-detected as if they were M. fructicola. Monilinia laxa cross-detection could be compensated by including the allelic discrimination step in qPCR runs, which permitted differentiating between M. fructicola and M. laxa samples. The inter-laboratory comparison demonstrated the robustness of the developed method and confirmed in-house validation data. This method could be used to detect latent infections of Monilinia in asymptomatic nectarine fruit and flowers.

Key words: brown rot, qPCR, inter-laboratory validation, performance assessment, sensitivity, specificity.

Introduction

Brown rot is an economically important disease of stone fruit, responsible for substantial pre-harvest and post-harvest losses caused by the fungi Monilinia fructicola, M. fructigena, and M. laxa (Byrde and Willetts, 1977). Monilinia fructicola is not considered a quarantine pathogen in the European Union since 2014 (European Commission, 2014), but is still considered as an A2 quarantine pest by the rest of the European and Mediterranean Plant Protection Organization (EPPO, 2009). Detection of the different Monilinia spp. is important for eradication and sur-
Monilinia spp. can produce visible infections when ambient conditions are favourable for the infection, or the fungi remain latent until favourable conditions are present (Byrde and Willetts, 1977). Monilinia fructicola and M. laxa have been detected as latent infections in fruit of peach, nectarine and plum (Northover and Cerkaukas, 1994; Emery et al., 2000; Fourie and Holz, 2003a, 2003b; Gell et al., 2008). Tissues of mature nectarine fruit with latent M. fructicola infections are characterized by the presence of subcuticular intercellular hyphae (Garcia-Benitez et al., 2016). The incidence of latent Monilinia infections in harvested fruit ranges from 0 to 30%, and may be as high as 50% (Emery et al., 2000; Luo et al., 2001; Luo and Michailides, 2001, 2003). Most stone fruit with latent Monilinia infections do not develop visible signs of disease until they arrive at consumer markets or after sale. Hence, latent infections constitute a source for the diffusion of brown rot (Gell et al., 2008, 2009; Villarino et al., 2013), and detecting a latent Monilinia infections in stone fruit is crucial for preventing spread of these pathogens to countries and/or regions.

There are several PCR-based methods for diagnosis of brown rot and/or detecting the different Monilinia species, but these methods require the macroscopic presence of fungal structures (Fulton and Brown, 1997; Förster and Adaskaveg, 2000; Hughes et al., 2000; Ioos and Frey, 2000; Boehm et al., 2001; Ma et al., 2003; Côté et al., 2004; Gell et al., 2007). There are three real-time PCR based methods used for symptomatic samples. One uses the SYBR™ green intercalation agent and is only validated for M. fructicola (Luo et al., 2007), the second uses hydrolysis probes in a duplex detection to differentiate between M. fructicola and other Monilinia species (van Brouwershaven et al., 2010), and the third uses hydrolysis probes in a tetraplex assay that enables the simultaneous detection of M. fructicola, M. fructigena, and M. laxa (Guinet et al., 2016). In the EPPO Bulletin (2009), the use of the PCR method developed by Ioos and Frey (2000) or the real-time PCR method developed by van Brouwershaven et al. (2010) are suggested for distinguishing Monilinia spp. However, neither the PCR nor the real-time PCR-based methods have been used to detect latent Monilinia infections.

Recently, a qPCR-based method has been reported (Garcia-Benitez et al., 2017) that is able to detect latent Monilinia infections in the blossoms and fruits of nectarine (Prunus persica var. nucipersica), and to distinguish among the Monilinia spp. in the infections. The method was based on the real-time PCR method previously described and tested by van Brouwershaven et al. (2010) for Monilinia identification on fruit with visible infections, and was adapted for detecting latent infections. Artificial latent infections were established in nectarine flowers and fruit for development of this method. The frequencies of latent Monilinia infections in the flowers and fruit detected using qPCR, and using the overnight freezing-incubation technique (ONFIT), were compared. The qPCR-based method was more sensitive, reliable and rapid than ONFIT for detecting latent Monilinia infections (Garcia-Benitez et al., 2017).

The aim of the present study was to compare the sensitivity and specificity of several real-time PCR platforms for the detection of low concentrations of Monilinia DNA (latent infections), on diverse plant material, using a common protocol. This is crucial for determining the transferability of a real-time PCR protocol for detection of Monilinia latent infections, based on the research of van Brouwershaven et al. (2010) and modified by Garcia-Benitez et al. (2017), as a tool for Monilinia latent infection risk quantification on imported or/and exported fruit. This evaluation was done through international inter-laboratory evaluation.

Materials and methods

Design of the study

The qPCR-based method proposed by Garcia-Benitez et al. (2017) for the detection of Monilinia spp. latent infections in fruit and flowers was tested across five different laboratories in five countries (France, Italy, Lithuania, Spain, and Turkey). Each laboratory analysed ten identical blind samples following pre-specified working protocols and data collection procedures. This “ring test” was carried between September 2015 and September 2016, from sample preparation to data statistical analysis and final report.

To ensure homogeneity and avoid quarantine organism manipulation, sample preparation and DNA extraction was carried out according to the following procedure:

1. Extraction:
   - A total of 100 mg of nectarine tissue was homogenized with 1 mL of alkaline lysis buffer (100 mM Tris (pH 7.5), 2 M NaCl, 1% (v/v) Tween 20, 1% (w/v) SDS, and 0.1% (w/v) b-mercaptoethanol). 100 μL of homogenate was used for DNA extraction.
   - DNA extraction was carried out in the scheme organizer (Qiagen RNeasy Midi Kit).
   - DNA yield and purity were determined as described previously (Verhagen et al., 2017, 2017).

2. qPCR assay:
   - qPCR was performed using the final protocol detailed by Garcia-Benitez et al. (2017). qPCR was carried out in 96-well plates in a 25 μL volume containing the following components: 12.5 μL of qPCR Master Mix (Premier SYBR® Green PCR Master Mix; Bio-Rad Laboratories, Hercules, CA, USA), 0.5 μL of each primer (10 μM), 0.5 μL of both probes (10 μM) and 5 μL of DNA template.
   - The qPCR system was analyzed using a Real-Time PCR system (C1000; Bio-Rad Laboratories, Hercules, CA, USA) with the following thermal cycling protocol: 95°C for 15 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.

The qPCR detection of Monilinia latent infection
laboratory. Samples were then shipped by fast courier with the rest of the required reagents inside boxes with dry ice, to the other participant laboratories. A working-protocol and a data sheet to record results were sent by e-mail to the participant laboratories.

Sample preparation

Shipped samples contained DNA from: uninfected nectarine fruit, uninfected nectarine flower, nectarine fruit with a latent infection by *M. fructicola*, nectarine flower with a latent infection by *M. fructicola*, nectarine fruit with a latent infection by *M. laxa*, nectarine flower with a latent infection by *M. laxa*, *M. fructicola* mycelia, *M. laxa* mycelia, and a mixture of *M. fructicola* and *M. laxa* mycelia. Samples were designed to give low or high quantification cycle values (C_q = 17 or 35, respectively). Latent infections on flowers and fruit were artificially induced with cold storage following a previously described protocol (Garcia-Benitez et al., 2016).

Genomic DNA from whole lyophilised flowers or 20 mg of pooled-lyophilised fruit epidermis (1 mm thick), was extracted using the DNeasy® Plant Mini Kit (Qiagen GmbH) in accordance with the manufacturer’s instructions. Eighteen μL DNA aliquots were prepared and then lyophilized in a laboratory freeze dryer (Cryodos -50, Azbil Telstar Technologies, SLU).

Other items supplied by the organizer laboratory

The reagents needed for the qPCR assay included: nuclease-free water, 2 × GoTaq® probe qPCR Master Mix (Promega Corporation), Mon139F and Mon139R primers, and P_fc and P2_fgn/lx/ps hydrolysis probes (van Brouwershaven et al., 2010) obtained from Integrated DNA Technologies Inc. The hydrolysis probes were labelled with different reporters and quenchers from those used by van Brouwershaven et al. (2010). A FAM reporter and a ZEN / Iowa Black FQ quencher were used for *M. fructicola* probe (P_fc) instead of a FAM-TAMRA, and a HEX reporter with a ZEN / Iowa Black FQ quencher was used instead of a VIC-TAMRA for *M. fructigena*, *M. laxa*, and *M. polystroma* probe (P2_fgn/lx/ps).

qPCR conditions

Genomic DNA from the samples (10 ng) was amplified in 20 μL reaction mixture, which contained 1× GoTaq® probe qPCR Master Mix, 200 nM of each of the primers (Mon139F and Mon139R), and 200 nM of each of the probes (P_fc and P2_fgn/lx/ps). Thermal cycling was carried out using the real-time PCR platform of each laboratory. These platforms were: Applied Biosystems® 7500 Fast Real-Time PCR (Thermo Fisher Scientific); CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.); LightCycler® 480 Real-Time PCR System (F. Hoffmann-La Roche AG); Mastercycler® RealPlex² (Eppendorf AG); and Rotor-Gene Q (Qiagen GmbH). The cycle conditions conditions were: polymerase activation at 95°C for 10 min, followed by 40 amplification cycles at 95°C for 15 s and 60°C for 1 min. Emission was measured at the annealing-extension step. The threshold value was set at a fluorescence (ΔRn) of 23,000 or automatically for those qPCR platforms for which that value was too high. A quantification threshold (C_q) value below 40 was scored as a positive detection. Additionally, due to cross-detection of *M. laxa* when using the P_fc probe detected in initial testing, an allelic discrimination step was added when the real-time PCR platform permitted, to distinguish between *M. fructicola* and *M. laxa* isolates, and to identify mixtures of the *Monilia* isolates.

Data collection and analyses

Collaborating laboratories were asked to record the C_q value and the standard deviation of each sample with each hydrolysis probe in the results data-sheets and send these with the raw data to the organizer laboratory. For validation of the qPCR assay, the following conditions had to be met: the negative control (DNase- and RNase-free water) yielded no target signal and the *M. fructicola* and *M. laxa* mycelium samples yielded positive signals with their corresponding probes. The results were transformed into qualitative results, detection (C_q < 40) and negative detection (C_q undetermined) to compare between laboratories and techniques.

Results of the assay specificity were calculated as a percentage of false positive and false negative results for each of the hydrolysis probes (P_fc and P2_fgn/lx/ps) (Broeders et al., 2014). The false positive and negative rates are calculated as follows:

- False positive rate is the number of misclassified known positive samples divided by the total number of known positive samples.
False negative rate is the number of misclassified known negative samples divided by the total number of known negative samples.

The qualitative results of the qPCR-method were compared against those obtained with the ONFIT-method, following EPPO bulletin (2014). This method is normally used to compare a new method against a validated one. Even though the ONFIT method in not validated, it is routinely used in the laboratories for latent infection detection. The comparison of both methods is based on the positive agreement \( P_a \), negative agreement \( N_d \), positive deviation \( P_d \), and negative deviation \( N_d \) between results. The studied parameters were as follows:

- Relative accuracy \( A \) of the method, which represents the correlation between the results obtained with ONFIT and those obtained with qPCR. This was calculated by using \( A = (P_a + N_d) / (P_a + P_d + N_d + N_d) \).
- Diagnostic specificity \( S_p \) of the method, which provides an estimation of the ability of the qPCR to not detect the target when it is not detected by ONFIT. This was calculated by \( S_p = N_d / (N_d + P_d) \).
- Diagnostic sensitivity \( S_\text{e} \) of the method, which provides an estimation of the ability of the qPCR to detect the target when it is detected by ONFIT. This was calculated by \( S_\text{e} = P_a / (P_a + N_d) \).

To assess the proficiency of the method, “The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories” (IUPAC Technical Report) (Thompson et al., 2006) was followed. The use of the z-scores was limited to identify those laboratories producing results out of line. The z-scores are calculated to assess the results of each sample for each participant. The z-score was calculated by \( z = (x - x_s) / \sigma_p \), where \( x \) is the result obtained by the participant, \( x_s \) is the “assigned value” for that sample and \( \sigma_p \) is the fitness-for-purpose basis “standard deviation for proficiency assessment”.

The assigned value for each analysed sample was determined by the consensus of the participants using the Hubert robust mean, and the robust standard deviation of the participants’ results were used as \( \sigma_p \).

**Results**

All participating laboratories results were transformed into qualitative results (Table 1). All qPCR platforms detected *Monilinia* in latent infection and mycelium samples with both hydrolysis probes, but not on healthy flowers and fruit (Table 1).

*Monilinia fructicola* samples were correctly detected by all five laboratories and qPCR platforms with hydrolysis probe P_fc (Table 1). However, the *M. fructicola* samples were also cross-detected by the hydrolysis probe P2_fgn/lx/ps, as if they were *M. laxa*, when using the Rotor-Gene Q platform (Table 1). *Monilinia laxa* samples were detected with the P2_fgn/lx/ps probe by all qPCR platforms, except for the low-concentration *M. laxa* latently infected flower sample that was not detected by the LightCycler® 480 Real-Time PCR System (Table 1).

With the exception of the LightCycler® 480 Real-Time PCR System, the other qPCR platforms cross-detected *M. laxa* samples with the probe P_fc, as if they were *M. fructicola* (Table 1). Two of the four platforms, Applied Biosystems® 7500 Fast Real-Time PCR and CFX96 Touch™ Real-Time PCR Detection System, cross-detecting *M. laxa* as *M. fructicola* could add an allelic discrimination step to the qPCR assay, differentiating between *M. fructicola* and *M. laxa* samples (Table 1).

The specificity of the qPCR results was tested using the qualitative data, calculating the percentages of false positive and false negative results. Neither the P_fc probe, nor the P2_fgn/lx/ps probe were specific, since the false positive rates (respectively 0% and 5%), and/or the false negative rates (respectively, 20% and 10%) were greater than 0%. The greatest bias came from the Rotor-Gene Q platform, which was not able to differentiate between *M. fructicola* and *M. laxa* with either hydrolysis probe adding 10% to each false negative rate. The P_fc probe was specific for LightCycler® 480 Real-Time PCR System and for Applied Biosystems® 7500 Fast Real-Time PCR and CFX96 Touch™ Real-Time PCR Detection System, when the allelic discrimination step was incorporated into the qPCR assay. However, the LightCycler® 480 Real-Time PCR System was not able to detect the *M. laxa* latently infected flower sample with low concentration of *M. laxa* DNA, adding a 5% false positive rate for the P2_fgn/lx/ps probe.

A comparison between qPCR detection and ONFIT detection was made with the data provided by the laboratories, obtaining the computed values for diagnostic sensitivity (100±2%), diagnostic specificity (72±13%), and relative accuracy (82±17%). The qPCR method was as sensitive as the ONFIT method, since there was no negative deviation between meth-
ods. However, because the qPCR method detected 15 more positive samples than the ONFIT method, both the relative accuracy and diagnostic specificity of the qPCR-method were less than 100%.

Z-scores were calculated from the data to determine the qPCR platforms producing out of line results with respect to the rest (Figure 1). Only the LightCycler® 480 Real-Time PCR System produced results with z-scores between 2 and 3, and therefore subject to revision (Figure 1). This slight deviation of the z-scores for the LightCycler® 480 system was because the sample C\textsubscript{q} values were consistently greater than those obtained with the rest of the platforms. This also explains why the M. laxa low DNA concentration sample (latently infected flower) was not detected, because this C\textsubscript{q} was out of range. The rest of the platforms scored z-scores in the acceptable region between -2 and 2 (Figure 1). There were no laboratories with z-scores in the unacceptable region.

**Discussion**

A real-time PCR method for detection of *Monilinia* latent infections was tested through an international inter-laboratory trial, where all qPCR platforms detected *Monilinia* latent infections and mycelia on nectarine flower and fruit samples. The qPCR method performed as expected by in house validation. The assay was sensitive, and results were consistent, even when tested under different conditions (time, equipment, location, analyst), and was therefore reproducible between different laboratories. No qualitative results and data interpretation differences were observed between five different qPCR platforms used, even though C\textsubscript{q} values differed. In addition, the assay was simple to use and can be performed by any plant pathology laboratory equipped with a real-time PCR platform.

The qPCR method was at least as sensitive as the ONFIT. It detected all the latent infections detected
qPCR detection of *Monilinia* latent infection

by ONFIT and 15 additional infections that remained undetected by ONFIT. The same was observed in the previous study of Garcia-Benitez et al. (2017), where qPCR detected 67% more latent infections than ONFIT. Garcia-Benitez et al. (2017) also showed that the latent infection detection qPCR-based method is more consistent than ONFIT since the number of replicates scoring positive detections was greater, especially when it was used for detecting latent infections caused by *M. fructigena* and *M. laxa*. This makes qPCR a good method to detect latent infections and/or low DNA concentrations of *Monilinia* spp. Furthermore, the time required to detect the fungal pathogens in latently infected flowers and fruit using this qPCR-based method is between 24 and 48 h, whereas the ONFIT method required 7 to 9 d of sample preparation and incubation, plus additional time to identify the specific *Monilinia* spp. using PCR or another molecular method (Garcia-Benitez et al., 2017). The rapid detection of latent fungal infections is very important for predicting outbreaks of brown rot in fruit after harvest and/or after storage (Thomidis and Michailides, 2010).

No unacceptable z-scores for any of the qPCR platforms were obtained, but not all the tested qPCR platforms performed as expected. Cross-detection of *M. fructicola* occurred in the Rotor-Gene Q system, while cross-detection of *M. laxa* appeared in

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**Figure 1.** Z-scores for the detection of different *Monilinia fructicola* (A) and *M. laxa* (B) samples with P_fc and P2_fgn/lx/ps hydrolysis probes, for each of five laboratories. Z-scores between 2 and -2 are acceptable (dotted line), those greater than 3 or lower than -3 are unacceptable (solid line) and those between the lines need to be reviewed.
four of the five qPCR platforms, the exception being the LightCycler® 480 Real-Time PCR System. Other studies have reported some differences between qPCR platforms while studying virus or bacterial infections (Kamihira et al., 2010; Ebentier et al., 2013). However, it is more common to observe the same detection results in different platforms. For example, Agren et al. (2013) tested five methods for detection of Bacillus anthracis in five qPCR platforms without finding differences between the results of ABI 7500 Fast Real-Time PCR and LightCycler® 480 Real-Time PCR systems. Little variability between results obtained with those two systems was also observed by Braun-Kiewnick et al. (2016) while testing a Meloidogyne enterolobii qPCR detection method across seven laboratories. These results indicate that the influence of the qPCR platforms depends on: (i) the region of amplification, primer, and probe DNA sequences; (ii) extraction matrices; (iii) organism; or other factors. This highlights the need to test qPCR methods in several platforms to determine their reproducibility. Monilinia laxa cross-detection was compensated with an allelic discrimination step. However, allelic discrimination is not a feature common to all qPCR platforms, and is normally used for other purposes. Thus, we consider that the P_{fc} hydrolysis probe should be modified, to avoid this cross-detection.

Several inter-laboratory trials evaluating detection and diagnostic methods for plant pathogens using molecular methods, such as conventional PCR, qPCR, or LAMP, have been carried out or are on-going inside the Euphresco initiative, which encourages cooperation among European diagnostic laboratories for method testing (EPPO, 2016). Inter-laboratory trials are considered essential across several biological and chemical scientific disciplines for method validation, and determination of reproducibility and robustness (ISO, 2005; Thompson et al., 2006; European Network of GMO Laboratories, 2011; AOAC INTERNATIONAL, 2012; Broeders et al., 2014; Magnusson and Örnemark, 2014). To facilitate this validation, the present study only tested one DNA extraction method and one qPCR method, and all reagents for the qPCR were provided by the organizer laboratory to the rest of the participants. In addition, a previous test performance study on M. fructicola and M. laxa mycelium detection by real-time PCR was carried out to provide the range of Cq values for the qPCR method and the limit of detection.

In conclusion, this inter-laboratory comparison confirmed the in-house validation of the qPCR-method developed by van Brouwershaven et al. (2010) and modified by Garcia-Benitez et al. (2017) for detection of Monilinia spp. latent infections in asymptomatic nectarine fruit and flowers. This method could be used as a tool for quantifying Monilinia spp. latent infection risk on imported and/or exported fruit, implementation of phytosanitary measures, and surveys or monitoring studies for brown rot pathogen distribution, spread, and survival. Additional investigation of new primers and probes for Monilinia species identification should be conducted to make identification more transferable among qPCR platforms and laboratories.

Acknowledgements

This research was supported by EUPHRESCO [266505 FP7-ERANET EUPHRESCO II DIMO (ERA37-DIMO-INIA)] and by the Ministry of Science and Innovation (Spain) [AGL2014-55287-CO2-01 and BES-2012-053796 to C.G.B.].

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Accepted for publication: August 12, 2017
RESEARCH PAPERS

Genetic diversity among phytopathogenic Sclerotiniaceae, based on retrotransposon molecular markers

GÖKSEL ÖZER¹, MUHAMMAD SAMEEULLAH², HAREM BAYRAKTAR³ and MEHMET ERHAN GÖRE¹

¹ Department of Plant Protection, Faculty of Agricultural and Natural Sciences, Abant Izzet Baysal University, 14030, Bolu, Turkey
² Department of Horticulture, Faculty of Agricultural and Natural Sciences, Abant Izzet Baysal University, 14030, Bolu, Turkey
³ Department of Plant Protection, Faculty of Agriculture, Ankara University, 06110, Ankara, Turkey

Summary. Molecular marker systems have been widely used for determination, discrimination and population structure analysis in the Sclerotiniaceae. The usefulness of a new marker system iPBS, based on the sequences of reverse transcriptase primer binding sites in long terminal repeats retrotransposons, was investigated with 34 isolates of six species confirmed by species-specific markers. Six of the iPBS primers were found to produce highly polymorphic (98%) and very distinct species-specific band patterns. Each primer amplified, on average, 33.5 polymorphic bands that was sufficient for species differentiation. The polymorphism information content was 0.896, indicating better discriminating power of markers, the Shannon’s information index was 0.438, and the genetic distance was 0.707 as average values. UPGMA cluster analysis based on retrotransposons divided all the isolates into three cluster and six sub-clusters in accordance with their species. Principal co-ordinate analysis also strongly confirmed this cluster pattern. The iPBS marker system was therefore a useful tool for evaluation of genetic variation at intra- and inter-species, and at the population levels for members of the Sclerotiniaceae. Furthermore, the iPBS markers could provide easy discrimination of Botrytis cinerea from Botrytis pseudocinerea.

Key words: iPBS, retrotransposons.

Introduction

Species of the Sclerotiniaceae (Ascomycota: Helotiales) are characterized by stalked apothecia with inoperculate asci produced on the living plant tissues or saprotrophically on dead tissues. Plant pathogenic members of this family, such as Sclerotinia spp., Botrytis spp. and Monilinia spp., cause economically important diseases with wide host ranges. Three white mold pathogens in the Sclerotinia genus infect many plant hosts; these are Sclerotinia trifoliorum Eriks, Sclerotinia sclerotiorum Lib. de Bary and Sclerotinia minor Jagger (Kohn et al., 1988). Botrytis cinerea, the causal agent of gray mold, has more than 200 host plant species, while Botrytis pseudocinerea, newly separated from B. cinerea and identified as a new species, causes losses in several fruit and vegetable crops (Walker et al., 2011). Monilinia fructigena causes severe brown rot on fruits (Côté et al., 2004). These fungi have been studied intensively because of their economic importance. However, their classification at species level based on morphological characters poses difficulties (Ekins et al., 2005; Hirschhäuser and Fröhlich, 2007; Plesken et al., 2015).

Molecular techniques have been widely used in recent decades to overcome bottlenecks and reveal the genetic variation at intra- and interspecies levels. Internal transcribed spacer (ITS) region or 18S rDNA sequence analyses are most commonly used for identification of filamentous fungi. However, these sequences cannot be used to evaluate genetic diversity in Sclerotiniaceae due to high similarity (Freeman et al., 2002). Therefore, researchers have focused on
different molecular marker techniques. Walker et al. (2011) described a method for discrimination of *B. cinerea* from *B. pseudocinerea* using the PCR-RFLP technique relying on the Bc-hch locus. Plesken et al. (2015) differentiated *Botrytis* species by sequence analysis of the g3pdh, tubA, and ms547 genes. Hirschhäuser and Fröhlich (2007) showed that analysis based on sequences of the laccase 2 gene (lec2) situated in the genome of Sclerotiniaceae genomes provided useful information for detection of *S. sclerotiorum* and *S. minor*. Vleugels et al. (2012) discriminated *S. trifoliorum* from *S. sclerotiorum* and *S. minor* in a study by using the AFLP technique to reveal genetic diversity. Andrew and Kohn (2009) identified *S. minor, S. sclerotiorum* and *S. trifoliorum* by using a single nucleotide polymorphism. Abd-Elmagid et al. (2013) developed a multiplex assay to differentiate four *Sclerotinia* species in a single PCR reaction, with species-specific primers for the Aspr gene of *S. sclerotiorum*, the Cad gene of *S. trifoliorum*, the Ef1-α gene of *S. homoeocarpa*, and the lcc2 gene of *S. minor*.

Retrotransposons, which is a main class of transposable elements, have been identified as an excellent source of molecular markers due to their structures and transport mechanisms. These transposons can move from one location to another within a genome, while the original copy remains in its original locus (Muszewska et al., 2011). Many fungal transposable elements (TEs) have been identified, especially in Ascomycota species (Daboussi and Capy, 2003). Based on TEs, various PCR based marker systems, such as retrotransposon-microsatellite amplified polymorphism (REMAP) and inter-retrotransposon amplified polymorphism (IRAP), have been developed to reveal genetic diversities in fungi (Chadha and Gopalakrishna, 2007; Jawhar and Arabi, 2009). These marker systems have some restrictions, such as the requirement of sequencing data to be universal for each examined organism. Kalendar et al. (2010) demonstrated inter primer binding site (iPBS) retrotransposons as an “Universal Retrotransposon Markers” system for molecular characterization of plants and animals. To date, studies performed in plants revealed that iPBS was a powerful DNA fingerprinting technique not requiring previous knowledge of sequencing of long terminal repeat retrotransposons. Recently, Pourmahdi and Taheri (2015) and Özer et al. (2016) indicated that this marker system also provided useful information for the genetic differentiation of fungi at both intra- and interspecies levels.

The aim of the present study was to evaluate the genetic diversity among members of the Sclerotiniaceae using iPBS markers.

**Materials and methods**

**Fungal material**

Fungal isolates were obtained from infected plants or provided by different researchers (Table 1). The isolates were retrieved from different diseased plants, and isolation of pathogens was carried out according to procedures described previously (Boehm et al. 2001; Fournier et al., 2003; Ekins et al., 2005; Vleugels et al. 2012). Surface sterilized plant tissues were placed in Petri dishes containing potato dextrose agar (PDA), and the plates were incubated at 23°C for 7 d under 12 h light/12 h dark cycle. Cultures were then maintained on PDA at 8°C.

**DNA isolation and molecular identification**

Fungal mycelium of each isolate was recovered by scraping with a sterile scalpel from the surface of medium and grinding in liquid nitrogen. DNeasy Plant Mini Kit (Qiagen, Cat No./ID: 69106) was used for DNA extraction according to manufacturer’s instructions. The quantity of resulting DNA was determined using a DS-11 FX+ spectrophotometer (DeNovix) and diluted to 10 ng μL⁻¹ with ddH₂O. PCR was performed (with species-specific primer sets) to identify *S. minor* (SMLcc2 F-SMLcc2R), *S. trifoliorum*, (STCad F-STCad R), *S. sclerotiorum* (SSaspr F-SSaspr R) and *M. fructigena* (ITS1-Mfg-R2) (Table 2; Hughes et al., 2000; Abd-Elmagid et al., 2013). The identification of *Botrytis* species was confirmed using the PCR-RFLP method based on the Bc-hch locus (Fournier et al., 2003).

**iPBS PCR assays**

All iPBS primers (Kalendar et al., 2010) were screened to evaluate their ability to produce sharp banding profiles among the isolates. The PCR assays were performed using two biological replicates with selected iPBS primers (Table 3) in 25 μL reaction volume containing 25-50 ng template DNA, 1× Dream Taq Buffer, 0.6 μM for 18 nt primers or 1 μM primer for 12-13 nt primers, 0.2 mM dNTPs, and 1.5 units of Dream Taq DNA polymerase (Thermo Scientific).
Table 1. Thirty-four fungal isolates, representing six species in Sclerotiniaceae.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Host plant</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
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<td><em>Botrytis cinerea</em></td>
<td>Cornelian cherry</td>
<td>Bolu-Turkey</td>
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<td>Bolu-Turkey</td>
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<td>Hawthorn</td>
<td>Bolu-Turkey</td>
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<td>Artichoke</td>
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<td>France</td>
</tr>
<tr>
<td>Sc_10</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_11</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_12</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_13</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_14</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_15</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_16</td>
<td><em>Sclerotinia minor</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
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<tr>
<td>Sc_17</td>
<td><em>Sclerotinia minor</em></td>
<td>Lettuce</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_18</td>
<td><em>Sclerotinia trifoliorum</em></td>
<td>Red Clover</td>
<td>Belgium</td>
</tr>
<tr>
<td>Sc_19</td>
<td><em>Sclerotinia trifoliorum</em></td>
<td>Red Clover</td>
<td>Belgium</td>
</tr>
<tr>
<td>Sc_20</td>
<td><em>Sclerotinia trifoliorum</em></td>
<td>Red Clover</td>
<td>Belgium</td>
</tr>
<tr>
<td>Sc_21</td>
<td><em>Sclerotinia trifoliorum</em></td>
<td>Red Clover</td>
<td>Belgium</td>
</tr>
<tr>
<td>Sc_22</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_23</td>
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<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_24</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_25</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_26</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_27</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_28</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_29</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Jerusalem artichoke</td>
<td>Ankara-Turkey</td>
</tr>
<tr>
<td>Sc_30</td>
<td><em>Monilinia fructigena</em></td>
<td>Quince</td>
<td>Bolu-Turkey</td>
</tr>
<tr>
<td>Sc_31</td>
<td><em>Monilinia fructigena</em></td>
<td>Quince</td>
<td>Bolu-Turkey</td>
</tr>
<tr>
<td>Sc_32</td>
<td><em>Monilinia fructigena</em></td>
<td>Quince</td>
<td>Bolu-Turkey</td>
</tr>
<tr>
<td>Sc_33</td>
<td><em>Monilinia fructigena</em></td>
<td>Quince</td>
<td>Bolu-Turkey</td>
</tr>
<tr>
<td>Sc_34</td>
<td><em>Monilinia fructigena</em></td>
<td>Quince</td>
<td>Bolu-Turkey</td>
</tr>
</tbody>
</table>
The amplifications were conducted with a Bio-Rad T100™ thermocycler under the following temperature profiles: one cycle initial denaturation at 95°C 3 min, 30 cycles of 95°C 15 sec, 50–65°C 1 min annealing (depending upon primers), and 68°C 1 min; and then at 72°C 5 min (Baloch et al., 2015). The amplified products were separated on a 1.5% (w/v) agarose gel with 1× TAE buffer for 2.5 h and visualized on using an Imager Gel Doc XR+ system (Bio-Rad), after staining with ethidium bromide.

### iPBS data analyses

To construct a binary matrix, reproducible fragments were scored as presence (1) or absence (0). Genetic distance among the isolates was evaluated us-

<table>
<thead>
<tr>
<th>Species</th>
<th>Primer code</th>
<th>Primer sequence 5′–3′</th>
<th>Product size bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrytis cinerea</td>
<td>262</td>
<td>AAGCCCTTCGATGTCTTGA</td>
<td>with HhaI: 517, 287, 155, 119, 84 and 9 bp</td>
<td>Fournier et al., 2005</td>
</tr>
<tr>
<td>Botrytis pseudocinerea</td>
<td>262</td>
<td>AAGCCCTTCGATGTCTTGA</td>
<td>with HhaI: 601, 287, 155, 119, 84 and 9 bp</td>
<td></td>
</tr>
<tr>
<td>Sclerotinia minor</td>
<td>SMLlc2F</td>
<td>CCCTCCTATCTCTCTTCAACA</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>Sclerotinia trifoliorum</td>
<td>STCadF</td>
<td>TCTTAGATCGACTCTCTCCTTT</td>
<td>97</td>
<td>Ahmed Abd-Elmagid et al., 2013</td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>SSasprF</td>
<td>CATTGGAAGTCTGTCGTCGA</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>Monilinia fructigena</td>
<td>ITS1</td>
<td>TCCTCCGGTATTGATATGC</td>
<td>460</td>
<td>Hughes et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Mfg-R2</td>
<td>GGTCGACCATAGAATTTTGGT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Details of the species-specific primers used in this study.

| Table 3. Primer names, their sequences, annealing temperatures, numbers of amplified and polymorphic bands, and some diversity parameters of iPBS retrotransposons primers, used in the study. |

<table>
<thead>
<tr>
<th>iPBS primers</th>
<th>Sequences (5′–3′)</th>
<th>Tm (°C)</th>
<th>Number of bands</th>
<th>Diversity parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPBS2221</td>
<td>ACCTAGCTACGATGCA</td>
<td>57</td>
<td>41</td>
<td>100</td>
</tr>
<tr>
<td>iPBS2237</td>
<td>CCCCCTACCGGATGCA</td>
<td>55</td>
<td>28</td>
<td>96.43</td>
</tr>
<tr>
<td>iPBS2239</td>
<td>ACCTAGGCTCGATGCA</td>
<td>55</td>
<td>53</td>
<td>100</td>
</tr>
<tr>
<td>iPBS2242</td>
<td>GCCCCATGCTGGCAGCA</td>
<td>57</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>iPBS2390</td>
<td>GCAACACCCCA</td>
<td>57</td>
<td>30</td>
<td>93.33</td>
</tr>
<tr>
<td>iPBS2395</td>
<td>TCCCCAGGGGATCGCA</td>
<td>53</td>
<td>29</td>
<td>96.55</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>205</td>
<td>98.04</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>34.17</td>
<td>33.50</td>
</tr>
</tbody>
</table>

Tm, annealing temperatures; P%, polymorphism percentage; Ne, number of effective alleles; h, Nei’s (1973) gene diversity; I, Shannon’s Information index; PIC, polymorphism information contents.
ing R version 3.3.1 according to the Jaccard’s index. Shannon’s information index and gene diversity for each primer were calculated using POPGENE ver. 1.32 software (Yeh et al., 1999). Principal coordinate analysis (PCoA) and the unweighted pair-group method with arithmetic mean (UPGMA) phenogram were carried out using the VEGAN package in R version 3.3.1 and MEGA ver.7 software (Kumar et al., 2016). The mean polymorphism information content (PIC) of each primer were estimated using the following formula: PIC = Σ (1-pi^2)/n, where “pi” is the frequency of presence 1 for each band, and “n” is the number of bands for each primer (Weir, 1990).

Results
Molecular identification of fungal isolates

Specific primer pairs amplified the predicted size DNA fragments, and the classification of 34 fungal isolates, representing six species from two genera, was verified. SMLcc2F/R amplified a 264 bp fragment for S. minor isolates, STCadF/R amplified a 97 bp fragment for S. trifoliorum isolates, and SSasprF/R amplified a 171-bp fragment for S. sclerotiorum isolates. The isolates of M. fructigena produced the expected 460 bp DNA fragment with the primer set ITS1/Mfg-R2. The 262/520L primer pair amplified a 1171 bp DNA fragment corresponding to the region of the Bc-hch gene for all Botrytis spp. isolates. After digestion of this PCR product with the HhaI restriction enzyme, two polymorphic restriction patterns were generated, allowing separation of the isolates (Table 2).

Genetic diversity among members of the Sclerotiniaceae

Six iPBS primers, iPBS2221, iPBS2237, iPBS2239, iPBS2242, iPBS2390, and iPBS2395, were determined to amplify distinct species-specific band patterns among the 34 fungal isolates examined. These markers produced 205 bands, of which 201 were polymorphic with 98% polymorphism among all isolates (Table 3). The number of amplified fragments with iPBS

Figure 1. A representative gel of reproducible profiles with iPBS2239 primer. Sample order (from left to right) is as listed in Table 1. The DNA marker (M) is GeneRuler 100 bp plus (Thermo Scientific).
primers varied from 24 (iPBS2242) to 53 (iPBS2239), with an average of 34.2 bands per primer (Figure 1). The number of polymorphic bands ranged from 24 to 53, while the average number of polymorphic bands being 33.5.

PIC values for each primer ranged from 0.853 to 0.918 with an average of 0.896, and these were less than 0.9 for two primers including iPBS2390 and iPBS2395. The Nei gene diversity was the least (0.217) for iPBS2395 and the greatest (0.312) for iPBS2242. The same trend was observed for the Shannon’s information index which varied from 0.358 (iPBS2395) to 0.478 (iPBS2242), with a mean value of 0.438.

Intraspecies differentiation was observed at different levels for each species (Table 4). The rates of polymorphism were calculated to be 28% for B. cinerea, 26% for S. minor, 58% for S. trifoliorum, 30% for S. sclerotiorum, and 32% for M. fructigena. This analysis could not be performed for B. pseudocinerea because only one isolate was included in the study.

The evaluation of Jaccard distance among all pairs of isolates revealed that the average pairwise genetic distance was 0.707. The greatest genetic distance for isolates was 0.906 between Sc_05 and Sc_21, while the least distance was 0.026 between Sc_12 and Sc_15. The greatest genetic distance among the species was 0.884, between B. cinerea and M. fructigena, while the least genetic distance was 0.533, between B. cinera and B. pseudocinerea (Table 5).

UPGMA cluster analysis grouped all isolates into three major groups (Figure 2). Cluster I included isolates belonging to three Sclerotinia species (S. trifoliorum, S. sclerotiorum, and S. minor), which cause white mold diseases, and subdivided isolates into three distinct subgroups depending on their species. Monilinia fructigena isolates constituted cluster II. Botrytis isolates were grouped in cluster III, which was further subdivided into two subgroups, and the B. pseudocinerea isolate differentiated from eight isolates of B. ci-

Table 4. Intraspecies genetic variation among isolates belonging to five fungal species, based on iPBS retrotransposons data.

<table>
<thead>
<tr>
<th>Species</th>
<th>NI</th>
<th>TAB</th>
<th>NPB</th>
<th>PWS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrytis cinerea</td>
<td>8</td>
<td>67</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>Sclerotinia minor</td>
<td>8</td>
<td>47</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Sclerotinia trifoliorum</td>
<td>4</td>
<td>66</td>
<td>38</td>
<td>58</td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>8</td>
<td>74</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>Monilinia fructigena</td>
<td>5</td>
<td>53</td>
<td>17</td>
<td>32</td>
</tr>
</tbody>
</table>

NI, number of isolates; TAB, total amplified bands; NPB, number of polymorphic bands; PWS%, percent polymorphism within species.

Table 5. Genetic distance matrices among six Sclerotiniaceae species, based on iPBS retrotransposons data.

<table>
<thead>
<tr>
<th></th>
<th>Bc</th>
<th>Bpc</th>
<th>Sm</th>
<th>St</th>
<th>Ss</th>
<th>Mf</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cinerea</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. pseudocinerea</td>
<td>0.533</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. minor</td>
<td>0.868</td>
<td>0.816</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. trifoliorum</td>
<td>0.877</td>
<td>0.853</td>
<td>0.793</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sclerotiorum</td>
<td>0.863</td>
<td>0.870</td>
<td>0.769</td>
<td>0.774</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>M. fructigena</td>
<td>0.884</td>
<td>0.880</td>
<td>0.837</td>
<td>0.819</td>
<td>0.836</td>
<td>***</td>
</tr>
</tbody>
</table>

Figure 2. UPGMA dendrogram of 34 Sclerotiniaceae isolates examined in this study, constructed with retrotransposon data. Isolate identification codes as indicated in Table 1. St = Sclerotinia trifoliorum, SM = Sclerotinia minor, Ss = Sclerotinia sclerotiorum, Mf = Monilinia fructigena, Bc = Botrytis cinerea, Bpc = Botrytis pseudocinerea. The scale bar (0.1) indicates 10% genetic distance.
Genetic diversity among phytopathogenic Sclerotiniaceae

The PCoA analysis strongly confirmed the cluster pattern of UPGMA. The isolates were separated in accordance with their species, and three main groups were formed and plotted (Figure 3).

Discussion and conclusions

Members of the Sclerotiniaceae can infect many plant species, including vegetables, fruits, ornamentals, and field crops, and cause economically important diseases and severe losses, both in the field and postharvest (Agrios, 2005). Rapid and accurate identification and monitoring of population structure of the pathogens are important for achieving effective control strategies for these diseases. Many molecular marker techniques have been developed and widely used in studies conducted for these purposes, because there are limitations in classical diagnostic methods based on morphological characteristics of the fungi. These limitations include that the classical methods are culture-based, labour-intensive, time-consuming and require extensive experience. In the present study, the efficacy of iPBS markers has been investigated for evaluating intra- and interspecies genetic relationships among the Sclerotiniaceae.

Six of 83 iPBS markers provided very distinct species-specific band profiles, allowing unambiguous discrimination of the fungal species. In the reproducible repeated experiments carried out for each primer, there were no changes in the banding patterns obtained for each isolate. A total of 201 polymorphic bands were amplified with an average of 33.5 bands per primer, which is sufficient for species identification. iPBS markers produced a greater proportion of polymorphic bands when compared to results from previous studies of other retrotransposon marker systems for fungi, such as IRAP for Fusarium spp. (Arabi and Jawhar, 2010).

In the present study, the discriminating power of the retrotransposon primers was calculated with PIC analysis, and was 0.86 on average. This is comparable to the results of Andeden et al. (2013) and Yıldız et al. (2015). This value confirmed that the iPBS primers produced highly informative polymorphic loci. The average of gene diversity was 0.28, and the average Shannon’s information index was 0.438. These values are comparable to the results obtained in previous studies (Baloch et al., 2015).

The UPGMA analysis revealed that iPBS markers were very effective for grouping the studied isolates at the species level. The phylogenetic tree divided the 34 isolates into three clusters. The PCoA also distinguished the same clustering.

iPBS retrotransposon markers could be a simpler method for discriminating B. pseudocinerea from B. cinerea than the previously published fenhexamid/fenpropidine sensitivity test and PCR-RFLP with Bc-hch locus (Walker et al., 2011). Sclerotinia species were also differentiated easily with retrotransposons without any species-specific marker. Intraspecies genetic variation was highly polymorphic among S. trifoliorum isolates from Belgium, which can be associated with any sub-species differentiation. This study has demonstrated that this marker system, which provides high polymorphism rate at the intraspecies level, could be used to identify species/sub-species, and could be beneficial for studying populations of fungal isolates. The iPBS technique based on the retrotransposons has considerable potential for studying intra- and interspecies genetic diversity among Sclerotiniaceae members without genome sequencing data.

Acknowledgements

We thank Anne-Sophie Walker, from INRA UR BIOGER-CPP, France, for providing the strains of the various Botrytis spp.; Tim Vleugels, from Eenheid Plant Instituut voor Landbouw- En Visserijonder-
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Abant Izzet Baysal University for financial support (Project Number: 2016.10.06.995).

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*Phytopathologia Mediterranea* 258

Accepte for publication: August 12, 2017


**Xylella fastidiosa** subsp. **pauca** (CoDiRO strain) infection in four olive (**Olea europaea** L.) cultivars: profile of phenolic compounds in leaves and progression of leaf scorch symptoms

**Andrea Luvisi, Alessio Aprile, Erika Sabella, Marzia Vergine, Francesca Nicolì, Eliana Nutricati, Antonio Miceli, Carmine Negro and Luigi De Bellis**

Department of Biological and Environmental Sciences and Technologies, University of Salento, via Provinciale Monteroni, I-73100 Lecce, Italy

**Summary.** *Xylella fastidiosa* subsp. *pauca* (*Xfp*), strain CoDiRO, infects a broad range of olive (*Olea europaea* L.) cultivars. The profile of phenolic compounds, progression of leaf scorch symptoms and population density of *X. fastidiosa* were analyzed in response to *Xfp* infection, in four olive cultivars (Cellina di Nardò, Ogliarola di Lecce, Frantoio and Leccino). Differences in *X. fastidiosa* multiplication in xylem tissues were estimated using qPCR assays, showing that cvs. Cellina di Nardò and Ogliarola di Lecce were characterized by fewer threshold cycles than for cvs. Frantoio and Leccino. Periodical visual inspections of symptomatic plants estimated disease severity and progression using a disease rating scale; cvs. Frantoio and Leccino showed some disease resistance with up to 3-fold severity scores than those for cvs. Cellina di Nardò and Ogliarola. During vegetative growth, Xfp-positive leaf samples were analyzed using HPLC-ESI–TOF–MS. Among quantified phenolic compounds, *Xfp* infection modified hydroxytyrosol glucoside and quinic acid. Constitutive levels of hydroxytyrosol glucoside were greater in cvs. Frantoio and Leccino compared to Cellina di Nardò and Ogliarola di Lecce, while levels were strongly reduced in infected plants (95% reduction in Cellina di Nardò, 94% in Ogliarola di Lecce, 97% in Frantoio and 98% in Leccino). Constitutive levels of quinic acid did not differ among cultivars, but strongly increased in infected Cellina di Nardò and Ogliarola di Lecce (5-fold increases), and to a lesser extent (4-fold increases) in infected Frantoio and Leccino. These results were consistent with the previously reported positive association of quinic acid with *X. fastidiosa* subsp. *fastidiosa* symptoms and titres in grapevine. Differences in the induced responses of these phenolic compounds among cultivars suggest that they play defensive roles in olive tree response to *X. fastidiosa* infection.

**Key words:** Frantoio, Leccino, hydroxytyrosol glucoside, quinic acid, selection marker.

**Introduction**

*Xylella fastidiosa* is the bacterial causal agent of important diseases such as Pierce’s disease (PD) of grapevine (caused by *X. fastidiosa* subsp. *fastidiosa*), citrus variegated chlorosis (CVC) or citrus X disease (subsp. *pauca*), and phony peach disease (subsp. *multiplex*) (Chatterjee *et al.*, 2008; Janse and Obradovic, 2010). *Xylella fastidiosa* is no longer a plant pathogen limited to the Western Hemisphere, and its genetic diversity remains to be fully described (Janse and Obradovic, 2010; Nunney *et al.*, 2014; Almeida and Nunney, 2015). Recently, *X. fastidiosa* was reported under field conditions in Italy (Apulia region), associated with severe cases of an “Olive Quick Decline Syndrome” (OQDS) (Saponari *et al.*, 2013). The strain CoDiRO (Complesso del Disseccamento Rapido dell’Olivo, the Italian name of OQDS), found in infected Apulian olive trees (*Olea europaea* L.) and other plant species, was characterized as a strain clustered...
in one clade with subsp. pauca strains (Cariddi et al., 2014), but was slightly distinct from subsp. pauca (Elbeaino et al., 2014). Olive leaf scorch caused by X. fastidiosa was observed in USA (caused by subsp. multiplex) (Wong et al., 2004; Krugner et al., 2010), Argentina (subsp. pauca) (Haelterman et al., 2015) and Brazil (subsp. pauca) (Coletta-Filho et al., 2016).

The pathogenesis and symptom formation from X. fastidiosa represent research challenges; in grapevine it is clear that the host response is mainly due to the extensive bacterial colonization of xylem tissues (Krivanek and Walker, 2005; Fritschi et al., 2007). Data about colonization behaviour of bacteria within olive xylem are not yet available, even if differences in population size in different olive cultivars were observed, suggesting correlation between bacterial multiplication ability and severity of symptoms (Giampetruzzi et al., 2016). While X. fastidiosa can infect more than 350 plant species, 22 species were found to be infected by the CoDiRO strain (EFSA, 2016). In the OQDS outbreak in Italy, Philaenus spumarius (Hemiptera, Aphrophoridae) was identified as the most important vector for transmission of the CoDiRO strain (EFSA, 2016). In olive trees, while other cultivars represent less than 1% of total plants, cv. Cellina di Nardò (Saponari et al., 2014; Cornara et al., 2016).

Methods for isolation of this pathogen, molecular testing and a pathogenicity test, were recently summarized by the European and Mediterranean Plant Protection Organization (EPPO, 2016). In olive trees, the pathogen is routinely detected using ELISA and PCR assays (Loconsole et al., 2014). Recently, new methods have been introduced for X. fastidiosa detection, including direct tissue blot immunoassay, Loop Mediated Isothermal Amplification (LAMP) and a new Polymerase Chain Reaction (PCR) assay (Djelouah et al., 2014; D’Onghia et al., 2014; Guan et al., 2015).

Because physical or chemical treatments for the control of X. fastidiosa are not (yet) available, particular attention has been given to identification of secondary metabolites that are essential for plant disease resistance and tolerance (Maddox et al., 2010) as possible strategies for disease management. Bacterial growth, aggregation and biofilm formation may be affected by xylem sap components (Cheng et al., 2009; Cruz et al., 2012; Shi et al., 2013). Phenolic acids such as gallic acid, caffeic acid, catechol, rutin and resveratrol have been shown to inhibit the in vitro growth of X. fastidiosa subsp. fastidiosa. Strong inhibitory activities against X. fastidiosa growth were observed with subgroups of flavonoid compounds, such as flavanone (naringenin), flavan-3-ol (catechin), and flavonol aglycone (quercetin) (Maddox et al., 2010). Other phenols such as the limonoid azadirachtin A and the flavanone hesperidin exhibited strong inhibitory effects against Xfp strain CVC (Ribeiro et al., 2008). In grapevine, X. fastidiosa subsp. fastidiosa infection may induce increased levels of catechin, digalloylquinic acid, and astrignin in xylem sap, while multiple catechins, procyanidins, and stilbenoids were found at greater levels in xylem tissues. However, these effects were transient after pathogen infection (Wallis and Cheng, 2012). In grapevine, the potential induction of phenolics in response to X. fastidiosa infection did not affect phenolic levels in xylem sap and tissue as much as pheno- nology or cultivar (Wallis et al., 2013a). It was also shown that the type of grapevine rootstock affected X. fastidiosa subsp. fastidiosa population levels and concentrations of caftaric acid, methyl salicylate (a procyanidin trimer), and quinic acid, which were all at greater levels in infected than non-infected grapevines (Wallis et al., 2013b). However, information on levels of phenolic compounds for olive cultivars infected by Xfp are not yet available. In olive tree, only the phenolic responses due to fungal infections have been investigated. Verbascoside is associated with resistance to Verticillium dahliae pathotypes (Markakis et al., 2010), whereas tyrosol and its derivatives were associated with constitutive resistance against Spilocaea oleagina infections; oleuropein and rutin were associated with induced resistance (Zine El Aabidine et al., 2010).

Under field conditions the most widespread South Apulia traditional olive cultivars Cellina di Nardò and Ogliarola di Lecce show clear symptoms of leaf scorching (Frisullo et al., 2014) when infected (Martelli, 2016), while the cultivar Leccino retains a green canopy with very little leaf scorching (Martelli, 2016). Furthermore, in the cvs. Leccino and Frantoio bacterial colonization and symptom development require longer incubation periods in comparison to cv. Cellina di Nardò (Saponari et al., 2016). It should be noted that in the infected area of South Apulia (Province of Lecce) Cellina di Nardò and Ogliarola di Lecce are the most planted cultivars (which together represent approximately 85% of plants). Leccino represents approximately 2% of planted olive trees, while other cultivars represent less than 1% of plants. Only recently it was proved that X. fastidio-
sa is able to systemically infect olive plants and to induce consistent symptoms of desiccation and leaf scorch 13–14 months post inoculation (Saponari et al., 2016) but a full replication of effects due to natural infection, as observed in mature trees, is not yet fully understood (Baccari and Lindow, 2011; Sun et al., 2011).

The objective of the research described in the present paper was to determine whether OQDS symptom progression, Xfp populations, or amounts of defense-associated phenolic compounds differ between cvs. of several naturally infected mature olive trees. Four olive cultivars, putative susceptible or resistant to the pathogen, were examined to observe if cultivar interferes with OQDS symptoms, and if plant responses to the pathogen are related to particular phenolic compounds.

Materials and methods

Experimental design, collection of samples and disease severity assessments

Trials were carried out in orchards located in the Lecce province of Apulia, Italy, in which OQDS symptomatic and symptomless trees were monitored. For each of four olive cultivars (Cellina di Nardò, Ogliarola di Lecce, Leccino and Frantoio), 12 plants (three groups of four plants per cultivar) were selected in X. fastidiosa-infected areas (in orchards where all plants showed OQDS since 2014, 1 year after pathogen detection), and 12 plants (three groups of four plants per cultivar) in orchards where the pathogen had not yet been detected. Olive trees within groups were selected to be of similar age (25-30 years), and to be growing with similar agronomic practices in the last 5 years and in similar pedoclimatic conditions.

Following management practices were the same for both symptomatic and asymptomatic plants. After winter pruning of trees, the canopy was not pruned during vegetative growth. Phytosanitary treatments were carried out in both plots according to EU Decision 2015/789, including controlling the insect vector (Philaeus spumarius) of strain CoDiRO and removal of wild plant hosts. Control of common insects such as the olive fly (Bactrocera oleae), was also carried out in order to minimize the effects of other pests. Orchards were monitored at weekly intervals to detect eventual insect or other pest outbreaks. No other treatments were carried out in orchards. According to Alagna et al. (2012), to avoid possible effects of different levels of water availability on the phenolic content among the trees, their water status was periodically monitored and, when necessary, irrigation was applied.

Evaluations of disease severity was carried out by visual inspections of the 12 plants every 2 months after pruning (from February 2015 to August 2016). In order to reduce the risk of overestimating disease due to the potential co-infection by other pathogens that can cause olive wilt, the canopies of the trees were each divided into upper and lower portions and subdivided in four sub-portions, according to cardinal directions. The presence of symptoms was recorded and scored using the following severity scale: 0 = symptomless; 1 = leaf scorching on few branches or few desiccated branches affecting the portion of the canopy; 2 = leaf scorching on several branches or desiccation branches affecting a large part of the portion of the canopy; and 3 = canopy with desiccated branches uniformly distributed.

Real-time PCR was used to determine Xfp population levels in the olive tree xylem tissues. Leaf samples were collected from 12 plants per cultivar belonging to infected areas and from 12 plants per cultivar belonging to non-infected areas. In April 2016, leaf samples (25 leaves collected from six branches per plant) were collected to assess X. fastidiosa presence by PCR with primers X.fas-0838-a-S-21-X.fas-1439-a-A-19 and X.Ygyr499-RXYgyr907 (Rodrigues et al., 2003). Samples from infected groups were always collected only from branches showing leaf scorching symptoms, to limit effects due to mixed infections of other pathogens with Xfp. Samples from non-infected groups did not show symptoms due to biotic or abiotic stress. The samples used for Xfp population level determinations were also used for analyses of phenolic compounds.

Relative qPCR assay

To evaluate the presence and the relative population level of Xfp in olive trees, sampling (25 leaves collected from six branches per plant) was carried out 4 months after tree pruning (April 2016). For each sample, leaf petioles and basal portions of leaf blades were cut with a sterile scalpel. Plant tissue from each sample (approx. 1 g of leaf petioles) was transferred into an extraction bag (BIOREBA, Switzerland) and 4
mL of extraction buffer (0.2 M Tris – HCl pH 9, 0.4 M LiCl and 25 mM EDTA) was added. Sample homogenization was performed using a semi-automatic homogenizer (Homex 6, BIONERBA) at 50% maximum speed. DNA extraction was performed following the protocol of Edwards et al. (1991) which described the most commonly used method of purifying and concentrating DNA from samples. In this protocol, the DNA solution is first extracted with a phenol/chloroform/isoamyl alcohol mixture to remove protein contaminants and then precipitated with 100% ethanol.

The TaqMan quantitative PCR protocol with XF-F/R primers and XF-P probe (Harper et al., 2010) was used for analysis of populations of Xfp in samples. The primer pair XF-F/R (Harper et al., 2010) was also used with SYBR Green reagents to run qPCRs. TaqMan and SYBR Green qPCR reactions were performed in a real-time thermal cycler (ABI PRISM 7900HT, Applied Biosystems). Each reaction consisted of 5 μL from a 20 ng μL⁻¹ dilution of DNA extracted from 1 g of leaf petioles, 12.5 μL of SYBR Green PCR Master Mix (Applied Biosystems), 400 nM forward and reverse primers, ultrapure DNase/RNase-free water (Carlo Erba Reagents S.r.l.) in a total volume of 25 μL. The cycling conditions were: an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min, with the final dissociation at 95°C for 15 sec, 60°C for 30 sec and 95°C for 15 sec. Taqman qPCR reactions were performed following the same protocol used for SYBR Green, adding a 200 nM TaqMan probe. The $2^{-\Delta\Delta C_t}$ method of relative quantification (semi-quantitative) was used to determine the fold change of the Xfp populations (Livak and Schmittgen, 2001; Cao and Shockey, 2012). The cycle threshold (Ct) of O. europaea chalcone synthase (Chs, GenBank accession no. KP935223. Forward primer: TCTCTC-CATTTTCATTTTGACATCTATT. Reverse primer: CATCTCCATGCCAACAGTGTA. Taqman Probe: CTTCTCCTCCATTCCCCATTTTGGCTCGT 5’FAM – 3’ TAMRA) was used for calibration. Data analyses and Cc calculations were carried out using SDS 1.2 software (Applied Biosystems). The relative amount of bacteria was determined by amplification of partial rimM open reading frame of Xylella fastidiosa (Harper et al., 2010) relative to the vegetal Chs gene, in the presence of SYBR Green.

qPCR reactions containing variable total DNA concentrations (0.8, 4, 20, 100 or 500 ng) from leaf petioles were used to estimate amplification efficiency based on the equation $E = (10^{1 - \Delta \Delta C_t}) × 100$ (Schneider et al., 2012).

Analysis of phenolic compounds in plant tissues

Leaves used for analyses of phenolic composition were harvested as for the disease severity assessments described above, from plants grown under the described environmental and management conditions.

Leaf samples were collected from branches assayed by qPCR for Verticillium dahliae (Bilodeau et al., 2012), Colletotrichum spp., C. acutatum and C. gloeosporioides (Garrido et al., 2009), to ensure that differences found in phenolic composition were not influenced by mixed infections with other pathogens.

The most common fungal species associated with olive wilt and decline in Southern Italy, Phaeomiella chlamydospora, Phaeoacremonium aleophilum and P. parasiticum (Carlucci et al., 2015) were also assayed by qPCR, using wood chips obtained from branches (Aroca et al., 2008; Martín et al., 2012). Wood chips were also tested by PCR for Botryosphaeria dothidea (Romanazzi et al., 2009), Diplodia seriata (Martín et al., 2014) and Phytophthora spp. (Drenth et al., 2006). Only leaves positive to Xfp from branches without discoloration in the xylem tissues and negative to tests against these fungal pathogens were used for further assays. The widespread occurrence of the fungi Stictis pinirizzi, Mycocentrospora cladosporioides, Spilocaea oleagina and the bacterium Pseudomonas savastanoi pv. savastanoi did not permit limitation of tests to totally symptomless trees, but branches showing symptoms of these pathogens were also excluded for leaf sampling.

Immediately after harvesting, leaves were frozen in liquid nitrogen and stored at -80°C until further analysis. Extraction and analysis of phenolic compounds were carried out following the methods of Taamalli et al. (2012). Total phenolic compounds were assayed using a spectrophotometric methods with Fast Blue BB (4-benzyloxyamino-2,5-dimethoxybenzenediazonium chloride hemi-[zinc chloride]) salt), and using a gallic acid standard dilution (Medina, 2011). Characterization of phenolics was carried out using an Agilent 1200 Liquid Chromatography system (Agilent Technologies) equipped with a standard autosampler. The HPLC column was an
Phenolic characterization of olive trees infected by Xylella fastidiosa

Agilent Extended C18 (1.8 μm, 2.1 × 50 mm). Separation was carried out at 40°C with a gradient elution programme at a flow rate of 0.5 mL min⁻¹. The mobile phases consisted of water plus 0.1% formic acid (A) and acetonitrile (B). The following multistep linear gradient was applied: 0 min, 1% B; 13 min, 25% B; 19 min, 40% B; 21 min, 90% B. The initial conditions were maintained for 5 min. The injection volume in the HPLC system was 5 μL. The HPLC system was coupled to an Agilent 6320 TOF mass spectrometer equipped with a dual ESI interface (both from Agilent Technologies), operating in negative ion mode using a capillary voltage of +3.5 kV. The other optimum values of the ESI–TOF–MS parameters were; drying gas temperature, 300°C; drying gas flow, 12 L min⁻¹; and nebulising gas pressure, 40 PSIG. Detection was carried out within a mass range of 50–1700 m/z. The accurate mass data of the molecular ions were processed through Mass Hunter software (Agilent Technologies). Five standard calibration graphs for the quantification of the phenolic compounds from olive leaves were prepared using commercial standards (quinic acid, oleuropein, hydroxytyrosol, luteolin and luteolin 7-O-glucoside) (Sigma-Aldrich).

The limit of quantification (LOQ) was determined as the signal-to-noise ratio of 10:1, and the limit of detection (LOD) was determined as signal-to-noise ratio of 3:1. Intra-day and inter-day precision was determined to assess repeatability of the methods, and were expressed by the relative standard deviations (RSD). An olive leaf extract was injected (n = 5) on one day (intraday precision) for three consecutive days (interday precision, n = 15).

Statistical analyses

Software R version 3.3.1 (R Foundation for Statistical Analysis) was used for all data analyses. Due to disease severities being on a 0–3 scale, before variance analysis, the Shapiro–Wilk normality test and the Levene’s test of homogeneity of variances were carried out to evaluate the applicability of analysis of variance (ANOVA). If data were not normally distributed, the same software was used to perform one-way or two-way ANOVA and pairwise multiple comparisons were made using the Duncan’s test. P value ≤0.05 was considered to be statistically significant.

Results

Disease severity assessments

Disease symptoms appeared 2 months after pruning in plants positive to qualitative PCR tests (Table 1). Disease severity data were not normally distributed (Shapiro-wilk test, P ≤ 0.05), so the non-parametric Kruskal-Wallis test was used to determine overall differences in means. At early stages there were large differences (approx. 7-9 fold increased severity) in Cellina di Nardò and Ogliarola di Lecce compared to Frantoio and Leccino. In the later (April) assessments, severity was about 3-4 fold greater, in June about 2.5–3 and in August about 1.5 fold greater in in the PCR positive plants. However, canopies with desiccated branches were found on each cultivar from April.

Relative qPCR assay

The quantification of Xfp in the different cultivars was shown with the TaqMan and SYBR Green qPCR assays, using the XF-F/R (Harper et al., 2010) set of primers employing the O. europaea chalcone synthase gene (Chs) as the internal calibrator.

To assess the differences among olive cultivars in relation to Xfp multiplication in xylem tissues, a qPCR assay was performed 4 months after pruning (April). For better evaluation, two different chemistries for fluorescent detection (TaqMan and SYBR) were used. The TaqMan qPCR with XF-F/R primers (Harper et al., 2010) generated an efficiency slope similar to that observed in SYBR Green qPCR tests (Figure 1). Both fluorescent methods gave a similar amplification efficiency (102.9 ± 2.6 for TaqMan PCR and 101.3 ± 2.4% for SYBR Green, n = 7), but a greater y-intercept was observed with TaqMan assay (31.6) compared with the SYBR Green assay (29.1) (Figure 1). As reported by Cao and Shockey (2012), the y-intercept corresponds to the theoretical limit of detection of the reaction, or the C value expected at the lowest copy number of target sequences gives significant amplification. Since the difference between
Table 1. Disease progression in four olive cultivars, expressed as mean disease severity scores based on a visually assessed scale from 0 to 3. Observations were assessed 2, 4, 6 and 8 months after pruning. The data comprised an average of 12 *Xylella fastidiosa* subsp. *pauca* (*Xfp*) infected plants per cultivar.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>February</th>
<th>April</th>
<th>June</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellina di Nardò</td>
<td>0.73 ± 0.25</td>
<td>1.56 ± 0.19</td>
<td>2.00 ± 0.26</td>
<td>2.10 ± 0.33</td>
</tr>
<tr>
<td>Ogliarola di Lecce</td>
<td>0.73 ± 0.13</td>
<td>1.73 ± 0.23</td>
<td>2.06 ± 0.22</td>
<td>2.17 ± 0.37</td>
</tr>
<tr>
<td>Frantoio</td>
<td>0.10 ± 0.13</td>
<td>0.50 ± 0.11</td>
<td>0.77 ± 0.20</td>
<td>1.38 ± 0.33</td>
</tr>
<tr>
<td>Leccino</td>
<td>0.08 ± 0.12</td>
<td>0.46 ± 0.21</td>
<td>0.60 ± 0.31</td>
<td>1.33 ± 0.22</td>
</tr>
</tbody>
</table>

Multiple comparisons

<table>
<thead>
<tr>
<th></th>
<th>z-score</th>
<th>P-value</th>
<th>z-score</th>
<th>P-value</th>
<th>z-score</th>
<th>P-value</th>
<th>z-score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellina - Ogliarola</td>
<td>0.0226</td>
<td>1.0000</td>
<td>-0.9828</td>
<td>0.9771</td>
<td>-0.4289</td>
<td>1.0000</td>
<td>-0.3030</td>
<td>1.0000</td>
</tr>
<tr>
<td>Cellina - Frantoio</td>
<td>4.1835</td>
<td>0.0001</td>
<td>3.6333</td>
<td>0.0008</td>
<td>3.7118</td>
<td>0.0006</td>
<td>3.6430</td>
<td>0.0008</td>
</tr>
<tr>
<td>Ogliarola - Frantoio</td>
<td>4.3716</td>
<td>0.0001</td>
<td>4.6161</td>
<td>0.0000</td>
<td>4.1407</td>
<td>0.0001</td>
<td>3.9460</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cellina - Leccino</td>
<td>4.3716</td>
<td>0.0000</td>
<td>3.9609</td>
<td>0.0002</td>
<td>4.3773</td>
<td>0.0000</td>
<td>4.0199</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ogliarola - Leccino</td>
<td>4.3491</td>
<td>0.0000</td>
<td>4.9437</td>
<td>0.0000</td>
<td>4.8061</td>
<td>0.0000</td>
<td>4.3228</td>
<td>0.0000</td>
</tr>
<tr>
<td>Frantoio - Leccino</td>
<td>0.1881</td>
<td>1.0000</td>
<td>0.3276</td>
<td>1.0000</td>
<td>0.6655</td>
<td>1.0000</td>
<td>0.3769</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Figure 1. Real-time PCR standard curve graphically represented as a semi-log regression line plot of Ct value (Y-axis) vs. log of input nucleic acid (X-axis). Five serial dilutions of DNA (extracted from 1 g of leaf petioles) between the range of 500 and 0.8 ng were used to design the standard curve.
y-intercepts was 2.5, this indicates that SYBR Green chemistry is 5.6-times more sensitive than TaqMan reagents. A similar (4-times) greater sensitivity of SYBR Green was observed also using primers for the calibrator Chs gene.

The copy number of the calibrator Chs was very stable among all the tested samples, and no differences were registered among cultivars (Table 2). The C_t values from the SYBR Green assay among whole samples was 25.6 ± 1.0, while using TaqMan the C_t was set at 23.9 ± 1.1.

For better interpretation of qPCR data, the level of infection of Frantoio cultivar was chosen as a baseline. The relative level of infection of the other cultivars was calculated as Fold Changes (FC) relative to Frantoio (Figure 2).

Using TaqMan chemistry, the relative level of infection of Leccino was very similar to that for Frantoio infection (FC_{Lecc} = 1.56). Conversely, Cellina and Ogliarola di Lecce showed greater levels of infection (FC_{Cel} = 3.48, FC_{Ogl} = 4.29).

As highlighted above from calculation of primer efficiency, SYBR Green chemistry was also more sensitive than TaqMan for detection of differences of infection among the cultivars. FC_{Lecc} obtained using SYBR Green chemistry was 3.25 ± 0.4, whereas the FC for Cellina di Nardò was 9.85 ± 1.0, and for Ogliarola di Lecce 21.11 ± 1.5. Moreover, statistical analyses of data from TaqMan assays resulted in two statistical groups, where Cellina di Nardò and Ogliarola di Lecce were characterized by twice greater relative levels of infection compared to Leccino and Frantoio (Figure 2). The output from SYBR Green assays resulted in four statistically different groups, one for each cultivar exhibiting a different relative level of infection, in increasing order of Frantoio, Leccino, Cellina di Nardò and Ogliarola di Lecce (Figure 2).

Analyses of phenolic compounds

Leaf samples from trees with OQDS symptoms were analyzed to evaluate the effect of X. fastidiosa infection on leaf phenolic composition. Leaves or wood chips were harvested from branches and were analyzed for the most common fungal species (according to previously reported lists of fungi) associated with olive wilt. This was to avoid detection of differences in phenolic compounds conditioned by the presence of other pathogens. Phaeonomiella chlamydospora (in 18.5% of analysed plants) Botryosphaeraceae (12.5%) and Colletotrichum spp. (8.3%) were detected most abundantly. Presence of Phaeoacremonium spp. was sporadic (2.1% of analysed plants), while Verticillium dahliae was never found in the analysed olive tissues. Only leaves that tested negative against previously described fungi were used for the phenolic assays.

The calibration plots indicated good correlations between peak areas and analyte concentrations, and the regression coefficients were greater than 0.988 in all cases. LOD was within the range 0.002 and 0.005 mg mL^{-1} while LOQ was within 0.005 and 0.009 mg mL^{-1}. The greatest intraday repeatability of the peak area among all peaks, expressed by RSD, was 0.82%, whereas the greatest interday repeatability among

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>TaqMan</th>
<th>SYBR Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellina di Nardò</td>
<td>25.7 ± 0.1 a</td>
<td>24.0 ± 0.1 a</td>
</tr>
<tr>
<td>Ogliarola di Lecce</td>
<td>25.4 ± 0.3 a</td>
<td>23.9 ± 0.2 a</td>
</tr>
<tr>
<td>Frantoio</td>
<td>25.8 ± 0.1 a</td>
<td>23.6 ± 0.2 a</td>
</tr>
<tr>
<td>Leccino</td>
<td>25.6 ± 0.1 a</td>
<td>24.0 ± 0.1 a</td>
</tr>
</tbody>
</table>

* Means in the same column followed by the same letter do not differ significantly (P ≤ 0.05), according to Duncan’s test.

Figure 2. Relative levels of Xylella fastidiosa in four olive cultivars, expressed as fold changes (2^{-ΔCt}) calculated using the cultivar Frantoio as an arbitrarily chosen baseline and chalcone synthase as calibrator. Letters a, b, c and d indicate statistical groups that differ significantly (P ≤ 0.05).
Table 3. Parameters of calibration curves, limit of detection (LOD), limit of quantification (LOQ) and relative standard deviation (RSD) for the HPLC method validation of phenolic assays.

<table>
<thead>
<tr>
<th>Standard phenolic compound</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>LOD (µg mL$^{-1}$)</th>
<th>LOQ (µg mL$^{-1}$)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinic acid</td>
<td>2.19E+05</td>
<td>-7.17E+04</td>
<td>0.999</td>
<td>1.81</td>
<td>6.03</td>
<td>0.82</td>
</tr>
<tr>
<td>Hydroxytyrosol</td>
<td>1.95E+05</td>
<td>-2.57E+05</td>
<td>0.999</td>
<td>2.60</td>
<td>8.66</td>
<td>0.78</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>1.81E+06</td>
<td>5.01E+05</td>
<td>0.997</td>
<td>0.77</td>
<td>2.56</td>
<td>0.81</td>
</tr>
<tr>
<td>Luteolin 7-O-glucoside</td>
<td>1.37E+06</td>
<td>1.53E+06</td>
<td>0.988</td>
<td>1.25</td>
<td>4.15</td>
<td>0.77</td>
</tr>
<tr>
<td>Luteolin</td>
<td>1.61E+07</td>
<td>3.91E+06</td>
<td>0.989</td>
<td>0.13</td>
<td>0.45</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 4. Characterization of olive leaf extract by HPLC-ESI-TOF (ion M-H). Exp = experimental; Clc = calculated.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>RT (min)</th>
<th>(M-H) Exp</th>
<th>m/z Exp</th>
<th>m/z Clc</th>
<th>Diff. (ppm)$^a$</th>
<th>Score$^b$</th>
<th>Reference$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*Quinic acid</td>
<td>0.365</td>
<td>C$<em>{12}$H$</em>{20}$O$_6$</td>
<td>191.0571</td>
<td>191.0561</td>
<td>-5.89</td>
<td>90.44</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>2</td>
<td>*Hydroxytyrosol glucoside</td>
<td>2.965</td>
<td>C$<em>{14}$H$</em>{24}$O$_6$</td>
<td>315.1085</td>
<td>315.1085</td>
<td>-1.26</td>
<td>96.62</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>3</td>
<td>Secologanoside is 1</td>
<td>3.960</td>
<td>C$<em>{16}$H$</em>{27}$O$_{11}$</td>
<td>389.1095</td>
<td>389.1089</td>
<td>-1.11</td>
<td>88.91</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>4</td>
<td>Secologanoside is 2</td>
<td>6.116</td>
<td>C$<em>{16}$H$</em>{27}$O$_{11}$</td>
<td>389.1101</td>
<td>389.1089</td>
<td>-2.62</td>
<td>96.13</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Elenolic acid glucoside</td>
<td>6.630</td>
<td>C$<em>{17}$H$</em>{27}$O$_{11}$</td>
<td>403.1262</td>
<td>403.1246</td>
<td>-3.68</td>
<td>80.9</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Oleuropein aglycon derivate</td>
<td>7.194</td>
<td>C$<em>{18}$H$</em>{27}$O$_{10}$</td>
<td>377.1459</td>
<td>377.1453</td>
<td>-1.23</td>
<td>92.94</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Unknown</td>
<td>7.825</td>
<td>C$<em>{19}$H$</em>{27}$O$_{10}$</td>
<td>415.1621</td>
<td>415.1610</td>
<td>-2.44</td>
<td>95.92</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Hydroxyoeluropein</td>
<td>9.036</td>
<td>C$<em>{21}$H$</em>{30}$O$_{14}$</td>
<td>555.1732</td>
<td>556.1803</td>
<td>-2.04</td>
<td>97.55</td>
<td>2, 4</td>
</tr>
<tr>
<td>9</td>
<td>*Luteolin 7-O-glucoside is 1</td>
<td>9.119</td>
<td>C$<em>{21}$H$</em>{30}$O$_{11}$</td>
<td>447.0952</td>
<td>447.0933</td>
<td>-3.93</td>
<td>77.64</td>
<td>3, 2</td>
</tr>
<tr>
<td>10</td>
<td>Luteolin rutinoside</td>
<td>9.517</td>
<td>C$<em>{23}$H$</em>{30}$O$_{15}$</td>
<td>593.1517</td>
<td>593.1512</td>
<td>-0.87</td>
<td>97.79</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>*Luteolin 7-O-glucoside is 2</td>
<td>9.998</td>
<td>C$<em>{23}$H$</em>{30}$O$_{11}$</td>
<td>447.0948</td>
<td>447.0933</td>
<td>-3.03</td>
<td>96.13</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>12</td>
<td>Apigenin 7 glucoside</td>
<td>10.010</td>
<td>C$<em>{21}$H$</em>{27}$O$_{10}$</td>
<td>431.0988</td>
<td>431.0984</td>
<td>-0.79</td>
<td>97.82</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>13</td>
<td>Oleuropein diglucoside is 1</td>
<td>10.545</td>
<td>C$<em>{23}$H$</em>{30}$O$_{8}$</td>
<td>701.2307</td>
<td>701.2298</td>
<td>-0.6</td>
<td>93.83</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>14</td>
<td>Oleuropein diglucoside is 2</td>
<td>10.728</td>
<td>C$<em>{31}$H$</em>{39}$O$_{8}$</td>
<td>701.2306</td>
<td>701.2298</td>
<td>-0.49</td>
<td>94.85</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>15</td>
<td>Oleuropein diglucoside is 3</td>
<td>10.893</td>
<td>C$<em>{31}$H$</em>{39}$O$_{8}$</td>
<td>701.2291</td>
<td>701.2298</td>
<td>3.2</td>
<td>98.67</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>16</td>
<td>2-methoxy oleuropein is 1</td>
<td>11.175</td>
<td>C$<em>{29}$H$</em>{39}$O$_{14}$</td>
<td>569.1898</td>
<td>569.1876</td>
<td>-3.76</td>
<td>85.77</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>2-methoxy oleuropein is 2</td>
<td>11.258</td>
<td>C$<em>{31}$H$</em>{39}$O$_{14}$</td>
<td>569.1899</td>
<td>569.1876</td>
<td>-3.64</td>
<td>97.16</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>Oleuropein</td>
<td>11.406</td>
<td>C$<em>{35}$H$</em>{52}$O$_{13}$</td>
<td>539.1772</td>
<td>539.1770</td>
<td>0.03</td>
<td>97.14</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>19</td>
<td>*Luteolin</td>
<td>11.939</td>
<td>C$<em>{18}$H$</em>{26}$O$_{6}$</td>
<td>285.0419</td>
<td>285.0405</td>
<td>-0.87</td>
<td>97.08</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>20</td>
<td>Ligstroside</td>
<td>12.611</td>
<td>C$<em>{32}$H$</em>{42}$O$_{12}$</td>
<td>523.1823</td>
<td>523.1821</td>
<td>-0.03</td>
<td>97.55</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>*Apigenin</td>
<td>13.382</td>
<td>C$<em>{18}$H$</em>{26}$O$_{5}$</td>
<td>269.0461</td>
<td>269.0455</td>
<td>-1.77</td>
<td>98.7</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>Diosmetin</td>
<td>13.929</td>
<td>C$<em>{18}$H$</em>{26}$O$_{6}$</td>
<td>299.0566</td>
<td>299.0561</td>
<td>-1.43</td>
<td>98.5</td>
<td>1</td>
</tr>
</tbody>
</table>

$^a$ Relative difference: the difference between the observed mass and the theoretical mass of the compound (ppm).

$^b$ Isotopic abundance distribution match: a measure of the probability that the distribution of isotope abundance ratios calculated for the formula matches the measured data.

$^c$ Reference list: 1 = Taamalli et al. (2013); 2 = Fu et al. (2010); 3 = Taamalli et al. (2012); 4 = Talhaoui et al. (2014); 5 = Quirantes-Piné et al. (2013).

$^d$ Confirmed by authentic chemical standard.
Table 5. Mean amounts of phenolic compounds (mg g\(^{-1}\) fresh weight) in olive leaves infected by *Xylella fastidiosa* susp. *pauca* (I) compared to negative samples (H). Data are relative to 12 infected or healthy plants per cultivar. Two-way ANOVA was carried out to evaluate statistical differences and interactions among factors (cultivar (Cv) and health status (HS)). Letters indicate statistical groups that differ significantly (Duncan’s test, \(P \leq 0.05\)).

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Cellina di Nardò</th>
<th>Ogliarola di Lecce</th>
<th>Frantoio</th>
<th>Leccino</th>
<th>Statistic analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>I</td>
<td>H</td>
<td>I</td>
<td>Cv</td>
</tr>
<tr>
<td>01. Quinic acid</td>
<td>0.77 ±0.07(^c)</td>
<td>3.99 ±0.51(^b)</td>
<td>1.20 ±0.12(^c)</td>
<td>5.68 ±0.88(^a)</td>
<td>1.10 ±0.22(^c)</td>
</tr>
<tr>
<td>02. Hydroxytyrosol glucoside</td>
<td>4.09 ±0.40(^c)</td>
<td>0.20 ±0.07(^d)</td>
<td>4.12 ±0.52(^c)</td>
<td>0.24 ±0.05(^d)</td>
<td>5.33 ±0.40(^b)</td>
</tr>
<tr>
<td>03. Secologanoside</td>
<td>0.10 ±0.03</td>
<td>0.11 ±0.04</td>
<td>0.09 ±0.04</td>
<td>0.12 ±0.03</td>
<td>0.09 ±0.05</td>
</tr>
<tr>
<td>05. Oleuropein aglycone</td>
<td>0.08 ±0.02(^c)</td>
<td>0.08 ±0.03(^c)</td>
<td>0.10 ±0.03(^c)</td>
<td>0.08 ±0.02(^c)</td>
<td>0.30 ±0.06(^c)</td>
</tr>
<tr>
<td>08. Luteolin 7-O-glucoside</td>
<td>0.79 ±0.29</td>
<td>0.76 ±0.24</td>
<td>0.71 ±0.23</td>
<td>0.66 ±0.22</td>
<td>0.81 ±0.21</td>
</tr>
<tr>
<td>10. Oleuropein diglucoside</td>
<td>0.68 ±0.31</td>
<td>0.88 ±0.59</td>
<td>0.73 ±0.55</td>
<td>0.69 ±0.47</td>
<td>0.71 ±0.51</td>
</tr>
<tr>
<td>12. 2-methoxy oleuropein</td>
<td>0.52 ±0.28</td>
<td>0.49 ±0.38</td>
<td>0.35 ±0.21</td>
<td>0.27 ±0.22</td>
<td>0.43 ±0.25</td>
</tr>
<tr>
<td>14. Luteolin</td>
<td>0.05 ±0.03</td>
<td>0.06 ±0.03</td>
<td>0.10 ±0.06</td>
<td>0.08 ±0.02</td>
<td>0.09 ±0.06</td>
</tr>
</tbody>
</table>

NS, Not significant; *, \(P < 0.05\); **, \(P < 0.01\); ***, \(P < 0.001\).
all peaks was 1.1%. The different parameters of the calibration curves are summarized in Table 3.

Characterization of olive leaf extracts using HPLC-ESI-TOF is reported in Table 4. At 4 months after pruning, differences in constitutive levels of phenols were observed for oleuropein aglycone and hydroxytyrosol glucoside (Table 5). The cultivars Frantoio and Leccino showed levels of oleuropein aglycone that were, respectively, 4-fold or 2-fold greater compared to the putative susceptible cultivars. Frantoio and Leccino showed levels of hydroxytyrosol glucoside that were, respectively, 12.8–12.9 or 14.8–14.9% greater compared to the putative susceptible cultivars. No differences in quinic acid, secologenoside, luteolin-7-O-glucoside, oleuropein diglucoside, 2-methoxy oleuropein or luteolin were observed among healthy plants of the four cultivars.

Xfp-positive samples showed significant differences in hydroxytyrosol glucoside and quinic acid levels. For both compounds, the interaction between health status and cultivar was observed. For hydroxytyrosol glucoside, strong reductions were observed in the putative susceptible infected plants of Cellina di Nardò (95.1% reduction) and Ogliarola di Lecce (94.2%), or in the putative resistant Frantoio (97.6% reduction) and Leccino (98.2%). Only quinic acid had greater amounts in Xfp-infected plants, with large increases (5-fold increase) in Cellina di Nardò and Ogliarola di Lecce, and lesser (4-fold increases) in Frantoio and Leccino (Table 5). A representative chromatogram of phenolic compounds with regard to health status is shown in Figure 3 (A and B).

**Discussion**

In naturally Xfp-infected olive plants, disease severity was up to 3-fold greater in the cultivars Cellina di Nardò and Ogliarola di Lecce compared to Frantoio and Leccino, where mean disease severity was constantly less than for the other two cultivars (Saponari and Loconsole, personal communication; Giampetruzzi et al., 2016). Using level of infection of Frantoio as a baseline, the relative level of infection expressed as FC was significantly less in Leccino (1.6-fold in TaqMan assays) from those observed in putative susceptible cultivars such as Cellina di Nardò (3.5-fold in TaqMan) or Ogliarola di Lecce (4.3-fold in TaqMan), according to preliminary results in bacterial concentration observed in Leccino (1.3 × 10^4 CFU mL^-1 tissue extract vs 2.1 × 10^5 CFU mL^-1 tissue extract in Ogliarola di Lecce) (Saponari and Loconsole, personal communication; Martelli, 2016). This was supported by evaluation of disease severity, which was less in Leccino and Frantoio during the sampling period. Disease severity of naturally infected plants could be influenced by fungal pathogens, because many fungal species can be found in olive plants with decline symptoms in Southern Italy, and old olive plants are frequently infected by *P. chlamydospora* (Nigro et al., 2013), *Phaeoacremonium* spp. or *Botryosphaeriaceae* (Carlucci et al., 2015). Our results confirmed the presence of such fungi in olive trees naturally infected by Xfp, and no cultivar was unaffected by at least one fungus related to decline. The most frequently detected fungi were *P. chlamydospora* and *Botryosphaeriaceae*, which were detected, respec-
Phenolic characterization of olive trees infected by Xylella fastidiosa

Xylella Fastidiosa primers designed by Harper et al. (2010) responded positively to SYBR Green. Dissociation curve analysis was used to determine that the intercalating dye produced single, specific products. No qualitative differences with PCR or TaqMan methods were observed. Both the qPCR assays gave high correlation coefficients and similar primer amplification efficiency, although SYBR Green showed a better limit of detection (5.6-times less) than TaqMan, using X. fastidiosa-specific primer pairs (Figure 1). The differences between TaqMan and SYBR Green qPCR assay results derived from the different chemistry employed: in SYBR Green, the amount of signal is dependent on the mass of double stranded DNA produced in the reaction, while TaqMan chemistry requires the use of a fluorogenic probe and a single fluorophore is released for each amplified molecule synthetized. Multiple dyes binding a single amplified molecule increase sensitivity for detecting amplification products. The SYBR green qPCR method was as reliable as TaqMan for determining X. fastidiosa in petioles of olive leaves, independently from cultivars, and may represent an improved diagnostic method since no probe is required reducing costs for assay setup and running. The designed internal calibrator gene Chs did not fluctuate during tests (constant cycle threshold values); therefore, this could be efficiently employed as a calibrator to control each sample for the presence of PCR inhibitors, to determine a cut-off value of sensitivity for negative samples and to normalize positive samples for the efficient DNA recovery. That procedure was necessary to discern between infected and uninfected (healthy) olive plants and to accurately evaluate the levels of Xfp infection.

Phenolic compounds have been associated with host resistance to bacterial and other diseases (Hammerschmidt, 2004; Gutha et al., 2010; Markakis et al., 2010; Zine El Aabidine et al., 2010; Rusjan et al., 2012). For the two phenolic compounds mainly affected by the presence of Xfp, hydroxytyrosol has been shown to inhibit or delay the rate of growth of a range of bacteria and fungi that cause disease of animals (Bisignano et al., 1999; Sousa et al., 2006) or plants (Yangui et al., 2010a, 2010b; Medina et al., 2011). The present study did not reveal significant effects of Xfp on the phenolics commonly present at greatest concentrations in olive leaves, such as secoiridoids. Phenolics associated to disease resistance in olive, such as oleuropein (Zine El Aabidine et al., 2010), were not affected by the presence of Xfp. Infection, however, modified concentrations of some minor phenolic compounds, including hydroxytyrosol glucoside and quinic acid. Furthermore, significant interactions were observed among health status and cultivars. The olive cultivars Ogliarola di Lecce and Cellina di Nardò showed lower constitutive levels of hydroxytyrosol glucoside compared to the cultivars Frantoio and Leccino, confirming different baselines of this compound (in healthy plants), as has been observed in other olive cultivars (Petridis et al., 2012a). Bacterial infection was associated with a statistically significant decrease of hydroxytyrosol glucoside in mature naturally-infected olive trees (Table 5), with lower amounts in Leccino (1.8- and 2.2-fold less, respectively, than in Cellina di Nardò and Ogliarola di Lecce) and in Frantoio (1.5- and 1.8-fold less respectively, than in Cellina di Nardò or Ogliarola di Lecce). The decrease in hydroxytyrosol glucoside concentration has also been observed in olive trees under high salinity stress (Petridis et al., 2012a), or after cold stress (Ortega-Garía and Peragón, 2009). In the case of grapevines with Pierce’s diseases, symptomatic leaves developed lower water potentials than healthy plants around noon each day, which was related to a reduction in the leaf osmotic potential (Goodwin and Meredith, 1988). Moreover, hydroxytyrosol levels decreased with water stress in four Greek olive tree cultivars (Petridis et al., 2012b). Petridis et al. (2012b) suggested that since hydroxytyrosol is one of the main phenolic compounds in olive, and this compound has high antioxidant activity, increased concentrations under adverse conditions would be expected. Involvement of hydroxytyrosol in oleuropein synthesis could explain this behaviour (Petridis et al., 2012b), but conversely to water stress tests, the present study did not reveal increases in oleuropein levels in X. fastidiosa-infected plants.

Constitutive levels of quinic acid were similar in the four olive cultivars analyzed. However, statistically significant interactions between health status and cultivar was observed for this compound, because greater amounts of quinic acid were detected in putative susceptible cultivars (5-fold greater) compared to Leccino and Frantoio (4-fold increases) (Table 5).

Wallis and Chen (2012) indicated that in grapevine, even though plants may initially respond to X. fastidio-
sa infections with increased production of phenolic compounds, the bacteria ultimately cause plants to decline because they no longer have resources to support secondary metabolite production, including defense-associated phenolic compounds. These authors defined symptomatic plants as entering "survival mode", where most photosynthate is allocated to basic growth and maintenance (also see Bonello et al., 2006). Our results suggest similar behaviour in olive trees; the only phenolic compound for which the concentration was greater in Xfp-infected olive trees was quinic acid, which is associated with suppression of disease development caused by fungi in tomato (Liu, 2001; Bartz et al., 2013). Wallis et al. (2013b) demonstrated that quinic acid was the only compound positively associated with Pierce's Disease symptoms in grapevine, although other molecules were positively associated with bacterial concentration. Their results suggested a further linkage between behaviour of Xfp-infected olive trees and infected grapevines. Due to lack of effective protocols for inducing OQDS in healthy olive plants, the present study focused on 1-year-old branches in naturally-infected plants, aiming to evaluate relationships between phenolic levels with disease. As proposed for grapevine (Wallis et al., 2013b), future studies should consider monitoring earlier shifts in phenolics in olive that occur during Xfp infections, and how those relate to progression.

In future, we will develop additional data to support the possibility that changes in the levels of hydroxytyrosol glucoside and quinic acid could be used as markers of Xfp infection in olive trees, and to reinforce the hypothesis that constitutive amounts of hydroxytyrosol glucoside could be associated with olive cultivars that are tolerant to OQDS.

Conflict of interest. The authors declare that they have no conflicts of interest.

Acknowledgments

This research was partially financed by the Apulia Region “Cluster Tecnologici Regionali” TAPASS Project. We thank farmers/growers for their support in the field observations.

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*Accepted for publication: 22 August 2017*
SHORT NOTES

Diplodia scrobiculata: a latent pathogen of Pinus radiata reported in northern Spain

Tania MANZANOS, Ana ARAGONÉS and Eugenia ITURRITXA

Plant Protection Department, NEIKER, Granja Modelo de Arkaute. Apdo 46. 01080 Vitoria-Gasteiz, Spain

Summary. Pinus radiata is a tree species native to the Central Coast of California and Mexico, which has been widely introduced in Europe for wood production. In Spain, especially in the northern region, it was introduced in the nineteenth century. Plantations located in the Basque Country (northern Spain) showing symptoms of Diplodia shoot blight were studied to confirm the causative pathogen species. Symptomatic and asymptomatic trees were sampled, and more than 150 fungal isolates obtained were morphologically characterized, with identities confirmed by sequencing the internal transcribed spacer (ITS) and the translation elongation factor 1-α (EF1-α) regions. Species-specific primers for Diplodia sapinea and D. scrobiculata were used to differentiate these fungi. Diplodia scrobiculata was detected on samples from asymptomatic trees, and BLASTN comparison was performed using the NCBI database. Lesions on P. radiata seedlings under controlled conditions were proved to be more substantial from D. scrobiculata than from D. sapinea. This is the first report of virulent D. scrobiculata in asymptomatic P. radiata trees in Spain.

Key words: Pinus radiata, Diplodia shoot blight, asymptomatic, aggressiveness.

Introduction

Diplodia scrobiculata has been reported in Europe and Spain (Stanosz et al., 1999; Moret and Muñoz, 2007). This fungus is known to coexist and interact with D. sapinea, one of the most common fungi found on pine trees (Burgess et al., 2004), but D. scrobiculata has a much more limited distribution and host range (Bihon et al., 2010). In its native range, Pinus radiata D. Don (Monterey pine) has been suggested to be exclusively associated with D. scrobiculata (Burgess et al., 2004). Previous pathogenicity studies have shown D. scrobiculata to be less virulent than D. sapinea (Palmer et al., 1987; Blodgett and Stanosz, 1999; Blodgett and Bonello, 2003), and biocontrol experiments have proved its ability to reduce Diplodia shoot blight (Muñoz et al., 2008). Nevertheless, D. scrobiculata has been reported to be as virulent as D. sapinea in South Africa (Bihon et al., 2010).

This paper presents results which identified the fungal species associated with Diplodia blight in northern Spain.

Material and methods

A survey for incidence of wood fungal pathogens was conducted in Pinus radiata plantations showing Diplodia shoot blight symptoms, located in the Basque Country (northern Spain, 42.989625°N, -2.618927°E). Samples were obtained from diseased and symptomless trees ranging from 9 to 52 years old. Wood cores were collected with a Pressler’s increment borer (diameter = 5 mm) at 130 cm height (Grissino-Mayer, 2003). The cores were placed into sterilized tubes, labelled, transported to the laboratory, and stored at 4°C.

The wood cores were surface-sterilized for 2 min with sodium hypochlorite (1% active chlorine) and rinsed with sterile deionized water. Thin disks cut from whole cross sections of the cores were placed on potato dextrose agar (PDA, Oxoid) and incubated...
in darkness for 7 d, and the cultures were placed into clear plastic boxes in an incubator at 20ºC. Developing fungal colonies were transferred to PDA and incubated in darkness for 7 d.

Botryosphaeriaceae isolates were incubated with sterilized pine needles according to the modified method of Smith et al. (1996) and were examined weekly for formation of pycnidia and conidia.

The growth rates and colour of the isolates growing on PDA at 23ºC in darkness were measured using colonies generated from 5 mm² diam. mycelial plugs obtained from the margins of 5-d-old PDA colonies. Fungal species were identified by colony and conidium morphology (de Wet et al., 2003; Phillips et al., 2013).

Genomic DNA was extracted from mycelia cultured on PDA at 23ºC, using a commercial kit (Analytik Jena AG, Life Science). A total of two partial gene regions were used in this study: internal transcribed spacer (ITS) and the translation elongation factor 1-α (EF1-α). The ITS region was amplified with primer pairs ITS1 and ITS4 (White et al., 1990) as described by Alves et al. (2004). The primers EF1-728F and EF1-986R (Carbone and Kohn, 1999) were used to amplify part of the translation elongation factor 1-α (EF1-α), as described by Phillips et al. (2005), Alves et al. (2006) and Alves et al. (2008). PCR products were purified (Nucleospin®, Macherey-Napel) and sequenced (Eurofins. Genomics, Germany).

Related sequences of *D. scrobiculata* used in the dendrograms were downloaded from the NCBI database. BLASTN comparison of the sequences was performed using the NCBI database and E value, and identity percentages were determined. Sequences were aligned with multiple sequence comparison by log-expectation (MUSCLE), using Mega v 7.0.26 software (Tamura et al., 2016).

Phylogenetic analyses using Maximum Likelihood (ML) and Neighbour-Joining (NJ) (Saitou and Nei, 1987) were performed using MEGA v. 7.0.26 (Tamura et al., 2016) with a Kimura 2-parameter model and statistical bootstrapping procedure involving 500 replicates.

To confirm the identity of fungal strains, the specific primers BotR, DpF, and DsF were used. These were developed for differentiation of the fungal pathogens *Diplodia sapinea* and *D. scrobiculata*, as described by Smith and Stanosz (2006).

Pathogenicity of fungal strains was tested by stem inoculation of each isolate on five *P. radiata* seedlings (18 months) maintained in a greenhouse at 22 ± 4ºC and 55–60 % relative humidity (Figure 1a). For each inoculation, a mycelial plug (3 to 4 mm² diam.) taken from the margin of an actively growing colony on PDA was placed in a shallow wound made by cutting the apical meristem of the seedling, and removing the first 5 cm.

**Results**

More than 150 isolates were obtained from *P. radiata* plantations suffering from Diplodia shoot blight throughout the Basque Country. *Diplodia sapinea* was the most common fungus isolated from symptomatic trees and is considered a widespread pathogen (99% of the cultures). *Diplodia scrobiculata* was isolated from an asymptomatic tree located in Bizkaia (43.352889ºN, -2.929818ºE).

Five weeks after incubation of fungi with sterilized pine needles, conidia appeared. They were brown, internally roughened without septa, clavate

![Figure 1. Disease caused by Diplodia scrobiculata on Pinus radiata seedlings. a: Uninoculated control. b: Symptoms produced 16 d after inoculation. c: at 30 d. d: at 55 d. e: at 75 d. f: Mycelium of the pathogen growing on PDA. g: Conidia of Diplodia scrobiculata.](image-url)
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with truncate bases (Figure 1g). Conidium dimensions were: length, 31 to 44 μm (mean = 38.1, standard error ± 3.06) and width 9 to 13 μm (mean = 11.1 ± 1.03), with a length/width ratio of 3.46 ± 0.41 (n=32).

When cultured on PDA at 20°C, the colonies were white with sinuate edges and appressed mycelium that became dark grey after 4 d (Figure 1f).

The strain was submitted to the Spanish Type Culture Collection (CECT-Universitat de Valencia, reference number CECT 20966).

Phylogenetic relationships among Diplodia scrobiculata isolates were assessed using the Neighbour-Joining method from the DNA sequences of the ITS region and EF1-α gene, and were constructed with MEGA v.7.0.26 software. Bootstrap values (500 replications) are provided to indicate support levels for tree nodes (Figures 2 and 3).

These dendrograms are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the dendrogram. The analysis for ITS phylogenetic tree involved 15 nucleotide sequences (Figure 2) and eight for the EF1-α gene (Figure 3). GenBank accession numbers of each reference sample are provided.

BLAST analysis of the ITS sequences revealed an E value 0, and 99% of sequence homology, with the external D. scrobiculata sequences from the GenBank database included. The representative ITS sequence of D. scrobiculata obtained in this study was deposited in GenBank (Accession No. KX363798).

BLAST analysis of the EF1-α gene sequences have E values of KF766399,1.67 x 10^{-6}; EU392260 and DQ458884,1.41 x 10^{-4}; AY624253,1.12 x 10^{-6}; AY624254,1.11 x 10^{-6}; HM100270,1.07 x 10^{-3}; HM100268, 1.04 x 10^{-3}, respectively, and 98% of sequence homology with the external D. scrobiculata sequences from the GenBank database. The representative EF1-α gene of D. scrobiculata obtained in this study was deposited in GenBank (Accession No. KY962007).

In the pathogenicity test, infections first became visible 2 weeks after inoculation the needles in the top of the P. radiata seedlings became light brown. Although the inoculated plants produced resin, in almost all cases it was not enough to avoid the spread of the disease. The infections extended down through the main stems, the needles became dark brown, began to fall, and the stems turned tan.

Twelve weeks after inoculation, the seedlings displayed dark brown discoloration lesions (Figure 1e), located in both the bark and the wood tissues of the main stems ranging from 2.3 to 15 cm (mean = 6.3 ± 5.9 cm) for D. scrobiculata and from 0.6 to 11.5 cm (4.05 ± 0.99) for D. sapinea. The pathogens were successfully re-isolated onto PDA from symptomatic tissues and identified by the colony morphology and production of characteristic conidia, thus fulfilling Koch’s postulates. The control seedlings treated with sterile PDA plugs (non-inoculated) remained asymptomatic.

**Discussion**

This is the first report of D. scrobiculata affecting P. radiata in Spain. Although the fungus was isolated...
from an asymptomatic tree, in greenhouse conditions it showed a high aggressiveness. Further investigations should be undertaken to determine the distribution and impacts of this pathogen, and its interactions with *D. sapinea*. The roles of biotic and abiotic factors in the development of disease caused by *D. scrobiculata* on *P. radiata* should also be investigated.

**Acknowledgements**

This study was funded by the Ministerio de Economía y Competitividad (Subprograma de Proyectos de I+D orientado a los Recursos y Tecnologías Agrarias en coordinación con las Comunidades Autónomas), Project: RTA 2013-00048-C03-03 INIA, the Basque Government (Departamento de Desarrollo Económico y Competitividad) Project: FORPATO15 and the Life Healthy Forest Project: LIFE14 ENV/ES/000179.

**Literature cited**


ABSTRACTS

Abstracts of invited talks, oral and poster presentations given at the 15th Congress of the Mediterranean Phytopathological Union, June 20–23, 2017, in Córdoba, Spain

The 15th Congress of the Mediterranean Phytopathological Union entitled “Plant health sustaining Mediterranean Ecosystems”, was held in Córdoba, Spain on June 20–23, 2017. The mission of the meeting was to promote dissemination of the latest scientific advances and encourage dialogue, interaction and collaboration between researchers from different disciplines interested in all aspects of Phytopathology. More than 200 participants from 26 countries attended the congress, making this an outstanding scientific event. The presentations covered a broad range of aspects related to plant diseases including Genome Analysis, Invasive Emerging Pathogens, Integrated Disease Management, Food Safety, New Tools In Diagnostics and Management, Molecular Pathogen-Host Interactions, Biocontrol, Epidemiology and Modelling, and Microbiomes and their Role in Plant Health. Abstracts of the invited talks, and the oral and poster presentations are given in this issue.

Key note lectures

Olive quick decline and Xylella fastidiosa in Southern Italy: the state of the art. D. BOSCIA, M. SAPONARI. CNR – Institute for Sustainable Plant Protection, University of Bari, Via Amendola 122/D, 70126, Bari, Italy. E-mail: donato.boscia@ipsp.cnr.it

The identification in 2013 of an outbreak of Xylella fastidiosa (Xf) in olive groves in the Salento peninsula (southern Italy) resulted in a plant health emergency of unprecedented proportions for the EU. Infected olive trees show extensive canopy desiccation and severe quick decline symptoms. In the outbreak area, the bacterium was found to be efficiently spread by the meadow spittlebug Philaenus spumarius, abundant on the olive canopies during the dry season. The initial demarcated foci rapidly expanded over the following 4 years, establishing a new demarcation line 80 km from the first reported outbreak; while few species were found infected in 2013 the currently known susceptible hosts reached approx. 30 different plant species. Phytosanitary measures to combat the spread and mitigate the impact of the bacterial infections, included restrictions for new plantations and movement of propagating materials, and removal of infected trees. The severe damage and the imposed phytosanitary restrictions caused severe economic and social impacts in the local community, raising concerns against the containment measures and failure to implement timely, effective and coordinated preventive measures. Due to the novelty of the Xylella-associated disease in olives and the new outbreak in the EU, the EU Commission mobilized dedicated resources to build research activity to address research gaps for this emerging pathogen. Between 2015 and 2016, two projects in the H2020 framework have been funded. These are: “Pest Organisms Threatening Europe” (POnTE) and “Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy” (XF-ACTORS), the latter exclusively targeting Xf. From the increased research activity developed in the past 3 years, new knowledge is providing data on the genetic and biological properties of the Xf population, the host range, vector identification and biology, and identification of olive cultivars with promising resistance traits.
Keeping up with the plant destroyers in the post-genomics era. S. KAMOUN. The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom. http://www.KamounLab.net

Infectious plant diseases cause severe losses in world agriculture, and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a rapidly increasing world population. Pathogens such as the rice blast fungus, wheat stripe and stem rusts, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far-reaching consequences. When faced with these threats, knowledge of the pathogens is essential. The genome sequence of a plant pathogen is a deep look into its soul. From important and often unexpected insights into the biology of the pathogen to the tools for surveillance and diagnosis, the genome is an invaluable resource that accelerates research and output delivery. The costs of genome sequencing are rapidly decreasing, and genome sequences can rapidly provide knowledge to develop DNA markers, outline population structures, and indicate pathogen origin. These This paper discussed ways in which genome biology impacts plant pathology, particularly, how pathogen genomics drive basic and applied plant pathology, and how new findings on pathogen biology can be exploited for development of new approaches to breeding disease-resistant crops. Detailed knowledge of pathogen genomes coupled with novel methods of plant genome editing is ushering the era of next-generation disease resistance breeding in plants.

Fusarium pathogenomics: understanding fungal pathogenicity through genomics. L.-J. MA. Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst MA, USA. E-mail: lijun@biochem.umass.edu

The root-infecting fungal pathogen Fusarium oxysporum causes vascular wilt in over 100 different plant species. These pathogens produce thick-walled resting structures that remain viable for long periods, making disease control challenging. This study takes a systems biology approach to dissect the molecular mechanisms underlying fungal pathogenesis and host defense using the F. oxysporum- Arabidopsis pathosystem. Comparative genomics and comparative meta-transcriptomics were employed to study compatible (inoculation of a F. oxysporum strain results in diseased plants) and incompatible (F. oxysporum inoculation has no negative effect on plant health) interactions by inoculating the same plant host (Col-0) with different F. oxysporum isolates. The study focused on the “primary determinative phase”, including fungal penetration and colonization from the cortex to xylem. Comparative study enables identification of genes and pathways that contribute to the co-evolutionary arms race between wilt pathogens and their hosts. Distinct sets of genes from two F. oxysporum strains contribute to the different disease phenotypes. Plant genes involved in pathogen-associated molecular patterns triggered immunity (PTI) were induced in compatible and incompatible interactions, while there were more distinct expression profiles for genes involved in effector-triggered immunity (ETI) in two different interactions.

This research was supported by a National Science Foundation CAREER award (1652641), a Burroughs Wellcome Fund Investigator award (1014893), a National Institute of Food and Agriculture National Research Initiative Hatch Grants Program grant (MAS00441), competitive Grants (2008-35604-18800 and MASR-2009-04374) and a seed grant from Massachusetts Green-energy High Performance Computing Center.

Understanding host specificity via comparative genomics. D.S. GUTTMAN. Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada. E-mail: david.guttman@utoronto.ca

The Pseudomonas syringae species complex includes diverse lineages that can infect numerous agricultural and wild plant species. The recent widespread application of next-generation sequencing technology has greatly expanded understanding of the genetic structure of this species complex, although there is still limited understanding of the specific genetic factors underlying niche specificity. We present a comparative and evolutionary genomic analysis of >400 strains of P. syringae, including 62 type and pathotype strains. We specifically focussed on the compositional dynamics of the genome, the impact of selection and recombination on genes associated with host adaption, and the use of association tests to identify previously unrecognized genes with host-specific associations. We found that virulence-associ-
Will climate change affect IPM in the Mediterranean environment? I. PERTOT, E. ECCEL, A. ALIKADIC, C. DOLCI, A. CAFFARRA, R. DE FILIPPI, A. CAFFARRA, R. DE FILIPPI, R. OFELLI, 1 Center Agriculture Food Environment, University of Trento via E. Mach 1, 38010 TN, Italy. 2 Predictive Models for Biomedicine and Environment, Fondazione Bruno Kessler, via Sommarive 18, 38123 Povo, Trento, Italy. 3 Research and innovation Centre, Fondazione Edmund Mach, via E. Mach 1, S. Michele all’Adige, 38010 Tn, Italy. E-mail: ilaria.pertot@fmach.it

In the nearfuture, climate change is expected to have significant influences on the agricultural sector, and particularly on plant protection, due to temperature increase and variation in precipitation. Temperature, rain and relative humidity are the main environmental factors influencing disease epidemiology. Several studies have assessed, global and regional scales, the effects of temperature increase in the last century, and predicted future trends of climate change. Although there is uncertainty in climate-modeling, especially at the regional scale, there is general consensus that global average surface temperature will increase and precipitation will vary. However, understanding of short and long term effects of climate on the complex interactions among plants, pathogens and their biocontrol agents, and the lack of accurate models that capture this complexity; ii) the natural seasonal variability of weather; iii) inability to accurately predict temperature and relative humidity changes at local and microenvironment levels; and iv) unpredictable or unrecognized factors that may affect disease epidemiology (e.g. variation in pathogen virulence). The uncertainty of predictions and the impacts of climate change on the efficient implementation of IPM in the Mediterranean region will be discussed.

Forest pathogen invasion: pathways, surveillance and early detection. A. SANTINI, L. GHELARDINI, D. MIGLIORINI, A. L. PEPO, N. LUCHI. 1 Institute for Sustainable Plant Protection, CNR, Via Madonna del Piano, 10 50019 Sesto Fiorentino, Italy. 2 Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente DiSPAA, Università di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. 3 Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. E-mail: alberto.santini@cnr.it

Human-driven species expansion has greatly increased during the last century, as a consequence of the unprecedented growth of international travel and trade, resulting in disturbance to ecosystems and severe socio-economic impacts. In plants, emerging infectious diseases (EIDs) are tightly linked to biological invasions. More than half of the world plant EIDs in the last few decades have resulted from the arrival of previously unrecognized pathogens. Many studies confirm that the main pathway of entrance of pathogens was the trade of living plants, and the trade of ornamental woody plants plays a role of primary concern. These observations should focus attention on the risk inherent in the trade of ornamental plants for planting in soil, which also constitutes the main pathway of introduction of pests. This pathway is particularly insidious because invasive harmful organisms are not easily detectable in soil, and they are, in addition, almost unknown and neglected in their native ranges. Several unexpected introduction pathways are becoming of increasing importance. Eradication is likely to be impossible, so increasing surveillance and prevention by early detection of new introductions are among the few reliable prevention measures, although detection is difficult in the face of global mobility and climate change.

Emerging pathogens as a consequence of globalization and climate change. M.L. GULLINO, G. GILARDI, A. GARIBALDI. 1 Centre of Competence for Agro-Environmental Innovation (AGROINNOVA) and 2 Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy. E-mail: marialodovica.gullino@unito.it
Many new diseases caused by soil-borne and foliar pathogens have been recorded for the first time worldwide, on a number of crops, resulting from globalization and climate change. The horticultural sector is one of the most affected by the spread of new diseases. Production of leafy vegetables (including lettuce, rocket, spinach, basil and lamb’s lettuce, grown as ready-to-eat sector products, is a good case study. Italy is the second largest producer of fresh-cut leafy vegetables in Europe. During recent years many new pathogens have been recorded, causing severe losses. Among foliar diseases, those caused by *Fusarium equiseti* on wild and cultivated rocket and lettuce, *Allaphoma tropica* on lettuce, *Colletotrichum kahawae* on cultivated rocket, *Myrothecium roridum* on lamb’s lettuce and *M. verrucaria* on spinach and wild rocket were observed. *Fusarium oxysporum* f. sp. *lactucae*, present in Europe since 2002, is spreading in new countries. A new race has been recently isolated from lettuce for the first time in the Netherlands, while race 1 of this pathogen has been found very recently in France. Some of these new pathogens are seed transmitted. The globalization of the seed market is a major cause of the rapid spread of such pathogens. Some of the newly introduced pathogens, typical of warmer areas, are spreading due to the increased in temperatures. The possible influences of globalization and climate change on the appearance and spread of new pathogens will be discussed.

This research was supported by the European Union’s Horizon 2020 research and innovation programme “Effective Management of Pests and Harmful Alien Species - Integrated Solutions” (EMPHASIS).

**Integrated management strategies for prevention and control of mycotoxins.** M.D. KAMINIARIS, M.C. ILIADI, C.S. LAGOGIANNI, O. RAFTOPOULOU, I. DANELI, M. ANDRIOLATOU, A.A. GKATZOUNI, E.F-N. VARVOUNI, A.S. ARSENI, A. BENAKIS, M.G. DIMAKOPOULOU, D.I. TSITISIGIANNIS. Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. E-mail: dimtsi@aua.gr

Mycotoxin contamination of agricultural commodities poses one of the greatest threats for safety and quality of food and feed. There is a continuous risk of mycotoxins “from the farm to the fork”, and climate conditions and/or production practices have impacts by favouring growth of mycotoxigenic fungi and mycotoxin production. The economically effective solutions are those that, with assistance from agricultural precision technology, will contribute to the exclusion of fungi from the plant hosts. Restriction of mycotoxin production in plants, or downstream in production lines, with Integrated Pest Management (IPM) systems, will provide effective, durable and environmentally sustainable mycotoxin control. IPM strategies of mycotoxigenic fungi and mycotoxins in pistachios (aflatoxins), grapes (ochratoxins), corn (aflatoxins, fumonisins) and barley are outlined, that are based on epidemiology, breeding of less susceptible plant genotypes and evaluation of resistant/tolerant varieties, evaluation of biocntrol products and fungicides. For biocontrol, a large number of endemic isolates of yeasts, bacteria and non-toxigenic *Aspergillus flavus* were tested in laboratory and field studies. Isolates were found to inhibit production of mycotoxins. Additional experiments to evaluate several fungicides of different chemical groups led to effective commercial formulations that can reduce the aflatoxin contamination by up to 100%. Epidemiological data and identification of critical pre- and post-harvest control points points that influence the mycotoxin production is crucial for developing predictive systems to reduce the mycotoxin biosynthesis.

**Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi.** F. BI1,2, S. BARAD2, A. DUBEY1, D. KUMAR2, V. CASADO3, J. DIAZ MÍNGUEZ2, E. ESPESO4, D. PRUSKY1. 1Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel. 2Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, and Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Guangzhou 510640, China. 3Department of Microbiology and Genetics, CIALE, Universidad de Salamanca, Salamanca, Spain. 4Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas (C.I.B.), Madrid, Spain. E-mail: dovprusk@volcani.agri.gov.il

Fruit pathogens can contribute to acidification or alcalization of host environments. This capability has
been used to categorize fungal pathogens into acidifying and/or alkalizing classes. We have shown that diverse classes of fungal pathogens, including Colletotrichum gloeosporioides, Penicillium expansum, Aspergillus nidulans, and Fusarium oxysporum, secrete small pH-affecting molecules. These molecules modify environmental pH that dictates acidic or alkaline colonizing strategies, and induce expression of PACC-dependent genes. In many organisms, acidification is induced under carbon excess, i.e. 175mM of sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e., less than 15mM sucrose. The carbon source is metabolized by glucose oxidase (gxo2) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (gdh2) results in the secretion of ammonia. Sucrose concentration also affects secondary metabolite accumulation, suggesting the importance of fruit sugar content for fungal metabolism. Our results have indicate that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

Using population genomics to identify genes affecting pathogen adaptation. B.A. McDONALD1.

1Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland. E-mail: bruce.mcdonald@usys.ethz.ch

Zymoseptoria tritici causes Septoria tritici blotch, the most damaging wheat disease in Europe. This fungus is globally distributed and is well adapted to a wide range of climatic conditions. Zymoseptoria tritici populations in Europe and North America evolved rapidly to become resistant to fungicides and virulent on resistant wheat cultivars. Little is known about the genetic basis of pathogen adaptive traits such as melanization, thermal sensitivity, host specialization and fungicide resistance. We used QTL mapping, based on 700 progeny from two crosses among four Swiss parental strains, and analyses of genome-wide associations (GWAS) based on 150 strains drawn from four global field populations, to elucidate the genetic architecture of these and other adaptive traits. Finished genome sequences and extensive RNA-seq profiles from across the infection cycle were obtained for all four parents used to make the mapping populations. RADseq generated more than 17,000 segregating SNP markers for the QTL mapping analyses, while comparisons of entire genome sequences provided over 700,000 informative SNPs for the GWAS. While genotyping has become easy, phenotyping remains difficult and limits progress in most marker-trait association studies. We developed high-throughput phenotyping methods to accelerate progress. Automated analyses of digital images generated approx. 2.7 million phenotypic measurements associated with melanization, virulence, host specialization, fungicide sensitivity and thermal adaptation. Significant QTLs and marker-trait associations were found for every trait, and several candidate genes were identified. Several of the candidate genes have been functionally validated, including three encoding small secreted proteins acting as pathogen effectors.

This research was supported by the Swiss National Science Foundation and ETH Zurich.

Pseudomonas syringae pathogenesis of plants: effectors and immunity. J.R. ALFANO. Center for Plant Science Innovation, University of Nebraska, Lincoln, Nebraska, 68588 U.S.A. E-mail: jalfano2@unl.edu

The bacterial pathogen Pseudomonas syringae uses a type III secretion system to inject type III effector proteins into plant cells to favour pathogenicity. When plants are infected by pathogens, two types of plant immunity can be triggered. Conserved molecules, known as pathogen-associated molecular patterns (PAMPs), can be recognized by surface-localized receptors known as pattern recognition receptors (PRRs), which induce pattern-triggered immunity (PTI). Pathogen effectors can be recognized by specific NOD-like receptors (NLRs) leading to effector-triggered immunity (ETI). The majority of type III effectors in P. syringae pv. tomato DC3000 can suppress PTI and/or ETI. An update is presented on type III effectors currently being investigated. One of these is HopE1, which was recently shown to use the calcium sensor calmodulin as a co-factor, and to target MAP65-1, a microtubule-associated protein 65, which functions in the Arabidopsis microtubule network. A recently commenced project is focussing on
Arabidopsis orosomucoid (ORM) proteins and their involvement in plant immunity. ORMs are known regulators of sphingolipid biosynthesis. Arabidopsis plants over-expressing ORM1 or ORM2 lack signaling from the FLS2 PRR, and are of increased susceptibility to P. syringae. Moreover, plants over-expressing ORM1/2 are greatly reduced in their FLS2 levels; ORM1/2 mutants have enhanced levels of FLS2. The molecular explanation behind these phenotypes is presented.

New bio-inspired treatments derived from microbiome and metabolome studies. M. LORITO, S.L. WOO. 1Department of Agricultural Sciences, and 2 Department of Pharmacy, University of Naples Federico II, Via Università, 100, 80055 Portici (Naples) Italy. E-mail: lorio@unina.it

A new generation of bio-inspired products, useful for disease control and bio-fertilization, is reaching market development phases. Some are derived from formulations and technologies, mainly based on microbes and bioactive molecules, applied for a long time in biological control. However, the new products have been optimized for efficacy, reliability and cost, also using data generated by recent -omics and plant-microbe interaction studies. Other products are based on novel concepts, which may be designed and constructed by assembling small beneficial microbiomes, where microbe combinations are defined by using new knowledge on factors and molecular mechanisms positively affecting crop vigour, yield and resistance to biotic and abiotic stresses. Two new formulations have been recently marketed in about 70 countries for specific application on wheat and corn. More products are in the pipeline, particularly for application on Mediterranean crops. Their development will be supported by the recently established initiative Partnership for Research and Innovation in the Mediterranean Area (PRIMA), a large (approx. 500M Euro) research programme involving about 20 countries. PRIMA is devoted to the use of “innovative solutions” and to “promote their adoption for improving the efficiency and sustainability of food production and water provision” in Mediterranean agriculture. The programme has a major focus on novel and sustainable approaches to reduce the impacts of pests and pathogens.

Can we breed for durable disease resistance in pea and faba bean? The cases of broomrapes and powdery mildews. D. RUBIALES, M. FERNANDEZ-APARICIO, A.M. VILLEGAS-FERNANDEZ, S. FONSEVILLA. Institute for Sustainable Agriculture, CSIC, Avda. Menendez Pidal s/n, 14004, Córdoba, Spain. E-mail: diego.rubiales@ias.csic.es

Legume crops, such as pea and faba bean, can be damaged by a number of diseases, but insufficient levels of host resistance are available in most instances. As a result, only cultivars with moderate levels of resistance are available to farmers. Two important groups of diseases are caused by distinct groups of biotrophic pathogens: parasitic weeds (the broomrapes) and airborne fungi (the powdery mildews). Several clearly distinct species of these pathogens are known (the broomrapes Orobanche crenata, O. foetida and O. aegyptiaca; or the powdery mildews Erysiphe pisi and E. trifolii). These pathogens can infect the same legume crops. Historic and recent achievements are reviewed for pea and faba bean, and compared with those experienced in other non-legume crops. Implications in resistance breeding will be critically discussed, with a special focus on potential durability of host resistance.

This research was supported by the Project AGL2014-52871-R.

Plant disease epidemics and food security. S. SAVARY. AGIR, INRA, Université de Toulouse, INPT, INP-E1 PURPAN, Castanet-Tolosan, Centre Inra Occitanie-Toulouse, France. E-mail: serge.savary@inra.fr

There is increasing interest in linking plant disease epidemics and crop health with global food security. Recent studies have revisited the possible linkages between the occurrences of plant disease epidemics and major historical events. Aside from their dramatic effects on food systems, plant disease epidemics may also cause regular, progressive attrition in agrosystem performance. A useful way to address the impacts of plant diseases on food security is through the different components of food security, including: (1) primary food production; (2) imports and stockpiles; (3) physical access to food; (4) economic access to food; (5) stability of food availability; and (6) quality and nutritive value of food. All six components
can be affected by plant diseases, to varying degrees, depending of individual cases. Another approach considers different spatio-temporal types of plant disease epidemics, including: (1) chronic (occurring regularly over very large areas, usually leading to moderate losses); (2) acute (occurring occasionally over limited areas, leading to important losses); or (3) emerging (occurring over expanding areas, leading to variable losses). Epidemiological modelling can contribute to assessment and comparison of plant disease impacts on food security. This assessment can then contribute to informing decisions in policy-making and research prioritization and planning. Examples of epidemiological analyses are provided for some major world food crops.

Role of epidemiological models in decision making for crop protection. V. ROSSI1. 1Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84 29122 Piacenza, Italy. E-mail: vittorio.rossi@unicatt.it

Traditionally, growers strongly rely on calendar applications of chemical pesticides. Directive 128/2009/EC makes integrated pest management (IPM) mandatory across Europe, to reduce the negative impacts of pesticides on human health and the environment. A key principle of IPM is to protect crops only when it is necessary, i.e., when there is risk for pathogens to develop, attack plants and cause damage. Weather is one of the main drivers for disease development. Relationships between weather conditions and pests and diseases have long been studied. In recent years, however, new approaches have increased our ability to investigate and understand these complex relationships. Similarly, advanced modeling techniques have made it possible to incorporate this knowledge in a new generation of mechanistic models, able to produce accurate and robust disease predictions. Advances in information and communication technologies have allowed incorporation of models into decision support systems (DSSs), and to effectively deliver these to growers. DSSs are now characterized by: (i) holistic vision of crop management problems and their interactions; (ii) incorporation of mathematical prediction models for plant growth and development, disease development, and fungicide modes of action of fungicides; (iii) provision of information on the focus of decisions as easy-to-understand decision supports; (iv) easy and fast access through the Internet; and (v) two-way communication between users and providers. These characteristics make it possible to consider context-specific information, such as crops and varieties, and soil characteristics, in addition to weather data.

The plant microbiota: mycorrhizal fungi and all the others. P. BONFANTE. Department of Life Science and Systems biology, University of Torino, Viale Mattioli 25, 1025, Torino, Italy. E-mail: paola.bonfante@unito.it

Plants have specific microbiontia, which may exert powerful effects on their health. Several studies of plant microbiota have focused on identification of microbial biodiversity on roots or epigeous organs, and have detected influences of plant genotype on the microbiota composition. Bacteria and fungi with beneficial functions, such as root symbionts and plant growth-promoting rhizobacteria, coexist with endophytes, saprotrophic microbes, and also with some pathogens. However, studies seeking to understand how plants build up their microbiota, or whether there are relationships between the microbiota and plant genotypes, are rare. Arbuscular mycorrhizal (AM) fungi are common members of root microbiota in wild and agricultural ecosystems, where they improve mineral plant nutrition, and in turn receive reduced carbon. They offer good tools for unravelling how plants respond to beneficial microbes. Using a combination of cellular, genetic and molecular approaches, we have demonstrated how phosphate is uptaken by the AM Gigaspora margarita, and is released to host plants through activity of fungal and plant phosphate transporters active in different rhizospheric and root compartments. A transcriptomic data set developed for tomato fruit led to the characterization of an additional phosphate transporter, which well responds to phosphate availability and mycorrhization. We conclude that improving the nutritional status and by affecting the source-sink relationships of whole plants, mycorrhizal fungi, as plant microbiota members, have strong impacts on plant nutrition and health.

This research was supported by the Project Mycoplant (CSP and Unito), Mycoceres, Green-Rice and 60% UNITO funds.
The plant microbiome: beyond collecting stamps. J.M. RAAIJMAKERS1,2, M. MEDEMA3, V. TRACCAÑA3, M. DE HOLLANDER3, J. PEREZ-JARAIMILLO3, V. CARRION BRAVO3. 1Netherlands Institute of Ecology (NIOO-KNAW), Netherlands. 2Institute of Biology, Leiden University, Netherlands. 3 Wageningen University, Department of Bioinformatics, Netherlands. E-mail: J.Raaijmakers@nioo.knaw.nl

Plant roots are colonized by many microorganisms, populations of which can reach cell densities much greater than the number of plant cells. Various studies have shown that members of the plant microbiome contribute to plant tolerance to abiotic (e.g., drought) and biotic (e.g., pathogens) stress factors, but also to plant nutrition, growth and development. For the majority of plant-associated microorganisms, however, there is limited knowledge on their support functions and the mechanisms involved. Novel -omics technologies have provided in-depth knowledge of the diversity and functioning of plant microbiomes and significant advances are being made to uncover mechanisms, genes and metabolites involved in the multi-trophic interactions in these microbiomes. To better understand this complexity, reductionist and systems approaches are needed to identify the biotic and abiotic factors involved in microbiome assembly and activity. New results are presented on the role of rhizosphere and endosphere bacteria in protection of plants against soil-borne pathogens. For rhizosphere bacteria, we have shown that representatives of the Proteobacteria protect plants from pathogen infection by the production of chlorinated peptides and alteration of root architecture and plant growth via modulation of sulfur assimilation. In-depth metagenomic sequencing of the endosphere allowed de novo assembly of high quality bacterial genomes, and revealed various yet unknown biosynthetic genes and pathways with potential for plant protection and antibiotic discovery. An overview is presented on the wealth of genes and functions of the plant microbiome.

Control of Xylella fastidiosa, once established in a territory, is difficult to attain, so efforts should focus on development of preventive measures. Remote sensing has been shown to be a useful decision support tool for crop management, through early detection and implementation of surveillance programmes that assist limitation of pathogen spread to new areas. A collaboration between JRC-European Commission and the POnTE consortium was established to develop a robust and accurate method for the automatic classification of X. fastidiosa infection and disease severity at large scales. Remote sensing information can be combined with species distribution models (SDMs), that determine relationships between sampled locations for a species and associated environmental variables, and these are used to estimate the ecological requirements of the species. SDMs provide realistic scenarios to explain the influence of bioclimatic variables on the epidemiology of plant diseases, particularly those caused by “new” plant pathogens. We used correlative niche models to quantify and map the global patterns of the potential geographic distribution of Xylella fastidiosa. Overall, projected potential distribution from estimated models conformed well with the current known distribution of X. fastidiosa. The application of SDMs to the most prevalent X. fastidiosa subspecies will be discussed.

This study was supported by the European Union’s Horizon 2020 research and innovation programme, under grants agreement No. 635646 Pest Organisms Threatening Europe (POnTE) and No. 727987 Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy (XF-ACTORS).

Grapevine trunk diseases: a need for clarity in concepts and definitions. G. SURICO, L. MUGNAI. Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: giuseppe.surico@unifi.it

Grapevine wood diseases (more than 20 of these can be listed), usually called Grapevine Trunk Diseases (GTD), became a major and increasing problem for vinegrowers in the last decades. Besides the economic impacts of GTDs, there is in recognition, description, attribution and nomenclature for some of them. At the beginning of the 20th century, several authors
paid considerable attention to various grape diseases, but there was confusion in their attempts to explain symptoms and development over time. Examples include “mal nero”, folletage and California disease. Symptoms of what are now known to be virus diseases were frequently attributed to the actions of fungal pathogens. In the case of esca, all symptoms recorded on the leaves (i.e., the well-known “leaf stripes” and others) were thought to be the effects of white rot of vine wood. Something similar is happening today with the introduction of some “new” GTDs. Different reports and lack of knowledge has led to the introduction and use of disease names that should now be revised and updated in the light of new knowledge for the esca complex, and for grapevine trunk diseases in general. It is important to clarify what was traditionally linked to wrong interpretations, and to use disease names that can be useful to share knowledge. These disease names should be based on official parameters applied in the naming of plant diseases.

**Communications**

**Invasive pathogens and new emerging plant diseases**


Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is the main fungal disease of citrus worldwide, causing external fruit blemishes and yield losses. The Mediterranean Basin is free of the disease, so phytosanitary measures are in place to avoid the entry of *P. citricarpa* in the EU. However, the suitability of Mediterranean climates for CBS establishment is debated. As a case study, an analysis of climate types and environmental variables in South Africa was conducted to identify potential associations with CBS distribution. In 1950, CBS was confined to climates with summer rainfall (Cw, Cf). The disease later spread to drier regions, and the hot arid steppe (Bsh) is the main climate region where CBS now develops. The disease was not detected in the Mediterranean-type climates (Csa, Csb). Arid steppe (Bs) climates are common in important citrus areas in the Mediterranean Basin. Hierarchical Bayesian analyses were also conducted by considering latent Gaussian analyses, which allowed the use of the integrated nested Laplace approximation (INLA) methodology. The spatial effects were implemented with the stochastic partial differential equation (SPDE) approach. Spatial models outperformed non-spatial models in the 1950 dataset. Problems of model convergence were detected in 2014 due to the strong spatial structure of CBS. Spatial models with principal components for 1950 had better classification accuracy of CBS distribution in 2014 than non-spatial ones. Therefore, previous models based solely on climate may underestimate the potential geographical distribution of this disease.

DC and ALQ were supported by the research grant MTM2016-77501-P from the Spanish Ministry of Economy and Competitiveness, and JMM by the grant VALi+d ACIF/2016/455 from the Generalitat Valenciana.

**Advances on the study of emerging Southern tomato virus infecting tomato crops in the Mediterranean basin.** L. Elvira-González, C. Carpino, A.V. Puchades, A. Alfaro-Fernández, M.I. Font-San Ambrosio, L. Rubio, L. Galipienso. 1Centro de Investigaciones Agrarias (IVIA), Ctra. CV-315, 46113 Moncada, Valencia, Spain. 2Department of Agricultural and Forestry Science, University of Palermo, Piazza Marina 61, 90133 Palermo, Italy. 3Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain. 4Euro-Mediterranean Institute of Science and Technology (IEMEST), Vía Michele Miraglia 20, 90139 Palermo, Italy. 5Departamento de Biotecnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.

Southern tomato virus (STV; genus Amalgavirus, family Amalgaviridae), has a double stranded RNA genome. STV has been detected in different tomato (*Solanum lycopersicum*) varieties showing symptoms of stunting, and fruit discoloration and reduced size. This virus was first detected in North America, and recently
in Asia and the Mediterranean basin (Italy, France and Spain). The role of STV in symptom development remains unclear, since the virus is frequently detected in mixed infections with other viruses, and it has been found in some asymptomatic tomato plants. STV is seed transmitted at high rates, but “horizontal” transmission by vectors is unknown. We developed sensitive methods for STV detection and quantification, to study the role played by STV in symptom development, to test horizontal transmission by insect vectors, and to implement sanitation programmes. Molecular hybridization and nucleic acid isothermal amplification (RT-LAMP) enabled sensitive detection of STV from different tomato plant tissues. The virus was detected in field samples collected from different production regions of Spain and Italy. A real time PCR assay after reverse transcription (RT-qPCR) was also developed for STV detection and quantification. STV titre remained constant over time, as for other persistent viruses. The virus was detected in individual tomato seeds, and in seed coats and embryos, making seed disinfection difficult. Nucleotide sequencing of different STV isolates showed very low genetic variation, which may be related to a strong symbiotic-mutualistic interaction between STV and the tomato host.

This research was supported by the INIA project E-RTA2014-00010-C02 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain) co-funded by FEDER 2014-2020 funds.

Development of new potato varieties with late blight and potato cyst nematode resistance, reduced bruising and improved processing quality.

M. PAIS1, K. WITEK1, L. TOMLINSON1, A. WITEK1, F. JUPE1, M. SMOKER1, S. PERKINS1, S. MARILLONNET3, P.E. URWIN3, N. CHAMPOURET1, C. RICHAEL4, J.D.G. JONES1. 1The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom. 2Department of Cell and Metabolic Biology, Leibniz Institute of Plant Biochemistry, Halle, Germany. 3Centre for Plant Sciences, University of Leeds, Leeds, United Kingdom. 4Simplot Plant Sciences, J.R. Simplot Company, Boise, Idaho, USA. E-mail: marina.pais@tsl.ac.uk.

Solutions were sought for several important problems faced by potato industries, including: late blight (LB), potato cyst nematodes (PCN), potato browning, acrylamide formation and blackening upon cooking. These are linked to significant crop losses, increased production costs, extensive use of agrochemicals and food wastage. We followed a genetic modification approach, based on: the use of a 3-resistance (R)-gene stack that combines genes from Solanum venturii (Rpi-vnt1) and Solanum americanum (Rpi-amr3i and Rpi-amr1e) to control LB; the use of genes encoding a rice cystatin and a synthetic repellent peptide that confer resistance to PCN by two different mechanisms; and the use of silencing constructs to reduce browning, the cold-induced accumulation of reducing sugars and the levels of asparagine (therefore reducing the potential for acrylamide formation and blackening). The Golden Gate cloning technique is used to generate constructs for potato transformation, and the binary vector was used to decrease chances of backbone integration. Several constructs are in the transformation pipeline. The three LB-resistance genes have been cloned individually or as a 3-R-gene stack, and the two genes conferring resistance to PCN as a single module or in combination with the 3-R-gene stack, with or without the silencing modules for improved tuber quality. The first transgenic lines obtained have been evaluated for resistance to different isolates of the LB pathogen in detached leaf assays, and some are being tested with PCN in glasshouse conditions. A field trial is planned to assess resistance against LB strains in field conditions.

This research is supported by the British Biotechnology and Biological Sciences Research Council (BBSRC) and by The Gatsby Charitable Foundation.

Screening of European potato varieties for resistance to pathotype 18(T1) of Synchytrium endobioticum in Greece. I. VLOUTOGLOU3, K.B. SIMOGLOU3, H. ELEFTHERIADIS2, D. TSIROGIANNIS1, C. KRITIKOS3, I. VLOUTOGLOU3, I. KAGIAS3, D. GKIANTZI3. 1Benaki Phytopathological Institute, Department of Plant Pathology, Laboratory of Mycology, 8 St. Delta Street, 145 61 Kifissia, Athens, Greece. 2Region of Eastern Macedonia & Thrace, Regional Unit of Drama, Rural Economy & Veterinary Directorate, Department of Quality and Phyto sanitary Control, Dioikittirion, 661 00 Drama, Greece. 3Hellenic Ministry of Rural Development and Food, Directorate General of Plant Produce, Department of Plant Protection.

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The quarantine pathogen Synchytrium endobioticum (which causes potato wart) was detected for the first time in Greece in two commercial potato (Solanum tuberosum L.) fields, in Kato Nevrokopi (Regional Unit of Drama, Northern Greece), during the 2011 official surveys. The pathotype in both fields was identified as 18(T1), an aggressive and rare pathotype. In compliance with the EU and National legislation, phytosanitary measures were implemented in the area, including demarcation of a buffer zone (c. 200 ha) around the infested fields. In addition, field trials and bioassays (pot tests) were carried out according to the EPPO Standard PM 07/28(1) for identifying resistant potato varieties to be used in the buffer zone. A total of 50 commercial potato varieties, eight of which were reported by other EU Member States and/or European potato breeding companies to be resistant to pathotype 18(T1), were evaluated in field trials over four consecutive years (2013-2016). Results showed that most of the commercial potato varieties tested were very susceptible to this pathotype. Only four varieties, out of the eight varieties reported elsewhere as resistant, constantly exhibited resistance. Based on the results of the bioassays conducted under controlled environment conditions using very high S. endobioticum inoculum pressure, only two of the varieties constantly showing field resistance to pathotype 18(T1) could be potentially used in the buffer zone, as, in line with Council Directive 69/464/EEC, they provided adequate protection against secondary infections by S. endobioticum.

This research was supported by the Hellenic Ministry of Rural Development and Food.

Is Calonectria pauciramosa established in Portugal? Occurrence in ornamental nurseries and in public gardens. A.P. RAMOS1, T. VALADA2, F. MAIA2, B. FERREIRA2, M.F. CAETANO2, A. LIMA1. 1LEAF Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. 2Laboratório de Patologia Vegetal “Veríssimo de Almeida”, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: pramos@isa.ulisboa.pt

Myrtus communis plants, from an historical garden and from an ornamental nursery, showing severe symptoms of chlorosis and wilting of growing tips, root and crown rot, were received for diagnosis in 2016. Cylindrocladium-like isolates were obtained from roots, crowns, branches and leaves of diseased plants. Eight representative isolates were selected to confirm their identity using morphological, cultural and molecular methods. Sporulating cultures on synthetic nutrient-poor agar, incubated in darkness at 25°C, had macroconidiophores with stipe extensions terminating in broadly ellipsoidal to obpyriform vesicles, with the widest dimension below the mid-points and a penicillate arrangement of fertile branches each terminating in 2-6 phialides. Phialides produced clusters of cylindrical conidia (38.1-64.4 × 3.5-5.4 μm), rounded at both ends, 1-sepate (thirty structures measured). On malt extract agar, the isolates grew faster at 25°C (growth rate 6.3 mm d⁻¹) than at 15°C (3.0 mm d⁻¹). None of the isolates grew at 35°C. These features agree with descriptions of Calonectria pauciramosa. To further confirm the identity of the fungus, the rDNA-ITS and the β-tubulin gene regions were amplified. Comparison of the sequences with other sequences available in the GenBank database showed they were identical to the Ca. pauciramosa 1031-ITS6 isolate from M. communis in Italy (AM749819), and to the Ca. pauciramosa CYL1/04 isolate from Polygala myrtifolia in Spain (AY923867). After the first record of Ca. pauciramosa in Portugal in 2003, our results indicate that the disease may now be spread in ornamental nurseries as well as in historical gardens.

Spread of Tomato leaf curl New Delhi virus in Italy: a new challenge for the cultivation of zucchini squash. S. DAVINO2, M. LUIGI2, S. BERTIN2, A. MANGILI3, S. PANNO3, A. CARUSO3, E. TROIANO3, L. OTGIANU4, M. NANNINI4, G. PARRELLA3, L. TOMASSOLI3. 1Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED, 5 - 90128 Palermo, Italy. 2Consiglio per il Mezzo Agrario e Forestale, Laboratorio di Patologia Vegetale “Veríssimo de Almeida”, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. 3Laboratório de Patologia Vegetal “Veríssimo de Almeida”, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. 4Laboratorio de Patologia Vegetal “Veríssimo de Almeida”, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: pramos@isa.ulisboa.pt

In compliance with the EU and National legislation, phytosanitary measures were implemented in the area, including demarcation of a buffer zone (c. 200 ha) around the infested fields. In addition, field trials and bioassays (pot tests) were carried out according to the EPPO Standard PM 07/28(1) for identifying resistant potato varieties to be used in the buffer zone. A total of 50 commercial potato varieties, eight of which were reported by other EU Member States and/or European potato breeding companies to be resistant to pathotype 18(T1), were evaluated in field trials over four consecutive years (2013-2016). Results showed that most of the commercial potato varieties tested were very susceptible to this pathotype. Only four varieties, out of the eight varieties reported elsewhere as resistant, constantly exhibited resistance. Based on the results of the bioassays conducted under controlled environment conditions using very high S. endobioticum inoculum pressure, only two of the varieties constantly showing field resistance to pathotype 18(T1) could be potentially used in the buffer zone, as, in line with Council Directive 69/464/EEC, they provided adequate protection against secondary infections by S. endobioticum.

This research was supported by the Hellenic Ministry of Rural Development and Food.
Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (family Geminiviridae) that was first identified in Asia in tomato plants, and then spread to several countries worldwide. The virus is responsible for economic damage to cucurbitaceous and solanaceous crops. After the virus was first recorded in Spain (2012) and Tunisia (early 2015), ToLCNDV was detected in Italy in October 2015. The rapid spread of the virus through the Mediterranean region was likely mediated by the vector, *Bemisia tabaci*. In Italy, the first ToLCNDV outbreak occurred in Sicily on zucchini squash, and immediately prompted intensified monitoring in the southern and central regions where *B. tabaci* occurs. In summer 2016, ToLCNDV-infected zucchini plants were found in Sardinia, Campania and Lazio regions. Phylogenetic analyses of the coat protein (CP) sequences showed that Italian ToLCNDV isolates split were in two well-supported groups. A cluster grouped all isolates from Sicily together with the reference isolates from Tunisia and Spain and a few isolates from Campania and Lazio. This cluster included a subgroup represented by the Sardinian isolates. The second cluster only included isolates from Campania and Lazio. These results suggest that in Sicily at least two independent introductions of ToLCNDV occurred; the first in 2015 from Spain and the second in 2016 from Tunisia. The pathogen spread from Sicily to Sardinia and to Campania and Lazio, but divergent isolates have been introduced in these regions through a different route, likely from Spain.

This research was supported by the Italian Ministry of Agriculture (MiPAAF) in the frame of Project ASPROPI (Azione a supporto della protezione delle piante), and by Regione Campania, 2016 Plan of Phytosanitary Action, URCOFI project.

Deciphering copper resistance in *Xanthomonas citri* pv. *citri*. D. RICHARD$^{1,3,7}$, V. RAVIGNÉ$^{1}$, A. RIEUX$^{1}$, B. FACON$^{4,5}$, C. BOYER$^{1}$, K. BOYER$^{1}$, P. GRYGIEL$^{1}$, S. JAVEGNY$^{1}$, M. TERVILLE$^{1}$, B. I. CANTEROS$^{6}$, I. ROBÈNE$^{1}$, C. VERNIÈRE$^{7}$, A. CHABIRAND$^{2}$, O. PRUVOST$^{1}$, P. LEFEUVRE$^{1}$. 1 CIRAD, UMR PVB-MT, F-97410 St Pierre, Réunion, France. 2 ANSES, Plant Health Laboratory, F-97410 St Pierre, Réunion, France. 3 Université de la Réunion, UMR PVBMT, F-97490 St Denis, Réunion, France. 4 INRA, UMR PVBMT, F-97410 St Pierre, Réunion, France. 5 INRA, UMR CBGP, F-34090 Montpellier, France. 6 INTA, Estación Experimental Agropecuaria Bella Vista, Argentina. 7 CIRAD, UMR BGPI, F-34398 Montpellier, France. E-mail: olivier.pruvost@cirad.fr

Copper-based compounds are widely used in integrated pest management (IPM) programmes aiming to control important plant bacterial pathogens, which have adapted in response to this selective pressure. Copper resistance of *Xanthomonas citri* pv. *citri* (*Xcc*), a major citrus pathogen worldwide causing Asiatic citrus canker, was first observed in Argentina two decades ago, and was subsequently reported as a *copLAB*-based, plasmid-encoded system. The emergence of resistant strains has since been reported in Réunion (South West Indian Ocean) and Martinique (Eastern Caribbean Sea). Disease severity was markedly increased in groves established with susceptible cultivars and infected with copper-resistant *Xcc*. Using tandem repeat-based genotyping and *copLAB* PCR, we demonstrated that the genetic structure of the copper-resistant strains from these three regions included two distant clusters, and varied for the detection of *copLAB* amplicons. We sequenced six copper-resistant *Xcc* strains from Argentina, Martinique and Réunion, together with reference copper-resistant *Xanthomonas* and *Stenotrophomonas* strains, using long-read sequencing technology. Genes involved in copper resistance were found to be strain-dependent, with the novel identification in *Xcc* of *copABCD* and a *cus* heavy metal efflux resistance-nodulation-division system. The genes providing the adaptive trait were part of a mobile genetic element similar to Tn3-like transposons, and included in a conjugative plasmid. The mining of all bacterial genomes available from public databases suggested that the mobile elements containing copper resistance genes and their plasmid environments were primarily detected in the Xanthomonadaceae family.

This research was supported by the European Regional Development Fund (ERDF project number GURDTI 2016-
Is Xanthomonas citri subsp. citri (Xcc) knocking at the doors of the Mediterranean region? P. CARUSO1, R. PAVONE1, C. LICCIARDELLO1, M.P. RUSSO1, V. CATARA2, G. LICCIARDELLO2, O. PRUVOST3, I. ROBENE1, J. CUBERO3, C. REDONDO3, Y. AYSAN3, R. CETINKAYA-YILDIZ4, S. HORUZ4, A. URSO5, G. TIMPANARO2. 1Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale, Italy. 2Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania. 3CIIRAD, UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBM), 7 chemin de l’irat - 97410 Saint Pierre, La Réunion, France. 4INIA, Departamento de Protección Vegetal, Ctra De La Coruna Km 7.5, 28040 Madrid, Spain. 5PPD-CU, 01330 Balcali, Sariyer - Adana, Turkey. 6BCRI, Kisla Mah. Yuregir - 01321 Adana-Turkey. 7Erciyes universite Köşk Mahallesi, Talas Bkt., 38030 Melikgazi/Kayseri, Turkey. E-mail: paola.caruso@crea.gov.it

The Mediterranean region is free of citrus bacterial canker (CBC), a disease caused by Xanthomonas citri subsp. citri (Xcc) and X. fuscans subsp. auratifolii (Xfa). In 2014, EFSA highlighted ornamental rataceous species (ORS) as a possible pathway for CBC entry. The ORPRAMed research project is underway to evaluate the risk of introduction of Xcc and Xfa through ORS in Europe. ORPRAMed partners are focusing on ORS not covered by 2000/29EC Directive through phytopatological, molecular, genetic, detection and economic approaches. We have analysed the trade flows in vegetal material for non-food uses. We have considered the import flows from countries where Xcc is classified as present. From the UN-ComTrade dataset (2015), with Code 06, Mediterranean countries reported imports of over 8.7 million kg from areas where Xcc is present, for an overall value of €46.0 million. The re-exportation from these countries of plant material must also be included, which is estimated at 156,384 kg for a value of $0.276 million. A field survey was also conducted in Turkey, to exclude the presence of CBC, due to its vicinity to infected areas. In the survey carried out in Adana, Mersin and Hatay provinces, 61 commercial nurseries and approx. 8500 ha of citrus orchards were screened, and disease symptoms were not observed. Growers and the nurserymen were also informed about the possible introduction risk of this severe citrus disease.

This research is part of the ORPRAMed Project, funded through the ERA-Net - ARIMNet2 2015 Call (EU FP7 grant no. 618127) by the following funding agencies: MI-PAAF; INIA; ANR; GDAR.

Phytophthora capsici emerging simultaneously in different greenhouse crops in Southeast Spain. M. DE CARA-GARCÍA1, A.M. AGUILERA-LIROLA2, A. PÉREZ-HERNÁNDEZ1, I. ESPITIA-VÁZQUEZ1, J.M. GÓMEZ-VÁZQUEZ2. 1IFAPA Centro La Mojonera, Camino de San Nicolás, 1, 04745, La Mojonera, Spain. 2S.C.A. Campoaldea, Avda. de la legión española, 2, 04779, Adra, Spain. E-mail: franciscom.cara@juntadeandalucia.es

From 2014 to 2017, a general survey of greenhouse crops in western Almeria province (Spain) was performed to detect soil-borne pathogens associated with wilting and/or root and crown rot. Fifty-three farms with diseased plants were surveyed. Four symptomatic plants and rhizospheric soils per greenhouse were sampled and analyzed. Symptomatic plants were only observed in sweet peppers for the first season, but extended to other crops (melon, watermelon, cucumber, tomato and zucchini) in the following three years. The prevalent pathogen isolated was Phytophthora sp., showing distinguishing features of P. capsici. Phytophthora sp. was isolated from 44.1% of pepper greenhouses, 90% of melon greenhouses and 100% of greenhouses with the other crops. One fourth of the surveyed greenhouses had been partially or completely flooded before the occurrence of symptoms and subsequent sampling, and greater association was noted between symptoms and the presence of Phytophthora sp. in these greenhouses. Wilted plants and Phytophthora sp. were present in 75% of flooded pepper greenhouses, 80% for melon, and 100% for flooded greenhouses containing watermelon or zucchini. Mating type was checked for 57 Phytophthora sp. isolates (obtained from all the host species). All isolates belonged to the A1 group. Molecular identification as P. capsici was
confirmed for 24 isolates by sequencing ITS-rDNA region. These isolates were inoculated on pepper, and four on the other plant species. All of the isolates were pathogenic. These results demonstrate simultaneous emergence of *P. capsici* causing soil-borne diseases in different economically important greenhouse crops in Almería.

This research was supported by European Regional Development Fund (ERDF) and European Social Fund (ESF) through the research project PP.AVA.AVA201601.7 and the fellowship granted to M. de Cara by IFAPA.

**Assessment of the host status of ornamental rutaceous species to Xanthomonas citri pathovars causing citrus bacterial canker.** G. LICCIARDELLO¹, O. PROUVOST², I. ROUBENE³, J. CUBERO³, C. REDONDO³, A. CARUSO³, C. LICCIARDELLO³, P. CARUSO³, V. CATARA¹. ¹Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. ²CIRAD, UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PV/BMT), 7 chemin de l’irat - 97410 Saint Pierre, La Réunion, France. ³INIA, Departamento de Protección Vegetal, Ctra De La Coruña Km 7.5, 28040 Madrid, Spain. ⁴Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acreale, Italy. E-mail: vcatara@unict.it

*Xanthomonas citri* pv. citri (*Xcc*) and *X. citri* pv. *aurantifolii* (*Xca*) cause citrus bacterial canker (CBC), a severe disease responsible for defoliation and fruit blemish and drop, requirings costly control measures. *Xcc* and *Xca* are quarantine pathogens for the UE, and are not recorded in the Mediterranean region. The probability of their entry, via import of ornamental rutaceous plants, through the commercial trade and passenger pathways, is rated as likely by EFSA (2014). To provide useful information for pest risk assessment, 25 ornamental rutaceous plants in the genera *Atalantia*, *Balsamocitrus*, *Claussenia*, *Eremocitrus*, *Glycosmis*, *Melicope*, *Microcitrus*, *Murraya* and *Vespiris*, not covered by Directive 2000/29EC, as well as *Citrus* and *Fortunella*, were tested for resistance to strains of *Xcc* (pathotypes A, A* and A") and *Xca* (pathotypes B and C), in controlled environment detached leaf assays. Nine plant species were presumptively classified as non-hosts, among them *Murraya paniculata*. Only *M. ovatifoliolata* and *Eremocitrus glauca* were susceptible to all pathotypes. The remaining species were susceptible at least to one of the pathotype A strains. Bacterial population densities ranged from $10^7$ to $10^8$ cfu mL$^{-1}$ in plants showing HR or no response, and $10^7$ to $10^8$ cfu mL$^{-1}$ in plants showing typical CBC lesions. Crystal violet staining showed aggregation of citrus canker strains on *M. paniculata* leaves similar to that on citrus species but different to that found for a non-citrus *Xanthomonas*. A *de novo* sequencing of the *M. paniculata* genome, already completed, will serve for RNAseq studies on both *Murraya* species.

This research is part of the ORPRAMed (Ornamental Rutaceous Plants Xcc Risk Assessment in Mediterranean) Project funded through the ERA-NET - ARIMNet2 2015 Call (EU FP7 grant no. 618127) by the following funding agencies: MIPAAF, INIA, ANR and GDAR.

**Evaluation of the presence of Gnomoniopsis smithogilvyi (syn. castanea) in chestnuts, rootstocks and grafts of six varieties of chestnut trees.** M. CONTI¹, J. CROVADORE¹, B. COCHARD¹, R. CHABLAIS¹, M. JERMINI², F. LEOFORT². ¹Plants and Pathogens Group, Institute Land Nature Environment, hepiia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland. ²Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch.

*Gnomoniopsis smithogilvyi* is an endophytic fungus, recently identified in Europe and Switzerland as the main cause of chestnut brown rot and as a cause of chestnut canker. The pathogen causes high plant mortality in chestnut nurseries and orchards. The presence of this fungus and of the chestnut canker agent *Cryptonectria parasitica* was assessed in the propagation material of six chestnut varieties, used by the Ticino Cantonal Nursery to restore fruit orchards. Sixty root samples, 41 shoot samples from germinated chestnuts and 17 chestnut rootstock samples were analysed, along with 112 samples from 56 rootstock/graft pairs, to determine whether the pathogen was transmitted by rootstocks or grafts. DNA extraction was followed by specific amplification primers for *G. smithogilvyi* and *C. parasitica*. *Gnomoniopsis smithogilvyi* was detected as an endophyte, but *C. parasitica* was never detected. Six of the 60 roots analysed from seed chestnuts were contaminated with *G. smithogil-
vyi (in varieties Lüina, Torcione Nero, Marrone Michelangelo, Marrone Lattecaldo and Bouche de Bétizac), as well as two of 41 shoots from seed chestnuts (Lüina and Bouche de Bétizac), and two of 17 rootstocks (Lüina and Torcione Nero varieties). For 112 samples from 56 rootstock / graft pairs, G. smithogilvyi was found in 12% of the rootstocks and 60% of the grafts. These results showed low incidence of G. smithogilvyi in rootstock propagation material, and high contamination of grafting material in all varieties, and confirm that G. smithogilvyi is an endophyte.

This research was supported by the strategic research fund of the University of Applied Sciences and Arts Western Switzerland.

Characterization of Elsinoë ampelina, the causal agent of grapevine anthracnose in Brazil. R.F. SANTOS, M. CIAMPI-GUILLARDI, L. AMORIM, N. S. MASSOLA JÚNIOR, M. B SPÓSITO. Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, 13418-900 Piracicaba, SP, Brazil. E-mail: ricardofeliciano@usp.br

Anthracnose, caused by Elsinoë ampelina, is an important disease in vineyards in South and Southeast Brazil, the main grape-producing regions in the country. This study characterized E. ampelina isolates associated with grapevine anthracnose in Brazil through molecular analysis, morphological characterization and pathogenicity tests. Thirty-nine E. ampelina isolates were obtained from leaves, stems and berries with anthracnose symptoms collected in the Rio Grande do Sul and São Paulo States. Fungus characterization was carried out using molecular analysis based on ITS, TEF 1-α and HIS3 regions, in combination with cultural and conidial morphology. For pathogenicity tests, ten isolates were inoculated onto Vitis labrusca cv. Niagara Rosada. ITS sequences showed only two polymorphic sites within the 602 bp sequenced and TEF 1-α sequences were monomorphic. However, HIS3 was the most informative region showing 55 polymorphic sites. Haplotype network analysis based on multilocus alignment (ITS, TEF 1-α and HIS3) grouped the isolates into seven haplotypes. Colonies of E. ampelina isolates showed slow growth (23 to 28 mm diam. at 30 d), variable colouration and wrinkled texture on PDA medium. Conidia were cylindrical to oblong with rounded ends, hyaline, aseptate, 3.6 to 7.0 μm long and 2.0 to 3.4 μm wide. Inoculations on ‘Niagara Rosada’ confirmed the pathogenicity of all isolates inoculated. These caused reductions of shoot dry weight by up to 80%, and severity of leaf disease reached a maximum of 72%.

This research was supported by São Paulo Research Foundation (FAPESP Projects 2013/24003-9 and 2014/24472-1).

Genetic and phenotypic diversity of Verticillium dahliae populations from sunflower in Europe. A. MARTÍN-SANZ1, A. GARCÍA-CARNEROS2, S. RUEDA1, P. MIRANDA-FUENTES2, L. MOLINERO-RUIZ2. 1Pioneer Hi-Bred International, Inc., Campus Dupont – Pioneer, Ctra. Sevilla-Cazalla (C-433) km 4,6, 41309 La Rinconada, Spain. 2Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. E-mail: lmolinero@ias.csic.es

The incidence of Verticillium dahliae (Vd) affecting sunflower in France, Italy, Spain and countries around Black Sea has greatly increased in the last five years, becoming a major constraint for sunflower production in some regions. Twenty Isolates of Vd collected in these countries, and one from Argentina, were characterized under a multidisciplinary study. The isolates were inoculated, by root immersion in suspensions of conidia, to seven sunflower genotypes with different phenotypic responses according to previous experiments. Some of the isolates were also inoculated on different hosts (artichoke, eggplant, cotton, tomato and lettuce) to determine the host pathogenicity spectrum of Vd from sunflower. The vegetative compatibility groups (VCGs) were determined through complementation between nit mutants of the fungal isolates and VCG reference strains. Phenotypic and genetic data indicated that the isolates from Black Sea countries were distinguishable from those from West Europe and Argentina, which could be due to the presence of at least two different races. Artichoke was very susceptible to all the isolates and significant crop × Vd isolate interactions were found for disease variables. Ongoing experiments using SSR reference markers for Vd will provide extensive information about the molecular structure of populations from sunflower and the re-
relationships with populations from other crops. This study is the first attempt to increase understanding of the genetics, virulence and phenotypic characteristics of the Vd isolates affecting sunflower in Europe.

This research was partially supported by grants from the Spanish Ministry of Economy, Industry and Competitiveness (AGL2010-17909 and AGL2016-80483-R) and the European Regional Development Fund (ERDF).

Identification of two species belonging of Polerovirus in hot pepper (Capsicum spp.) in Italy: a new phytosanitary risk. A. TIBERINI1, I. ADAMS2, A. FOX2, A. FOWKES2, S. DAVINO3, L. TOMASSOLI4. 1Università degli Studi “Mediterranea” di Reggio Calabria, Fuo di Vito, 89121 Reggio Calabria (RC) Italy. 2Food and Environmental Research Agency (FERA), Sand Hutton, York, UK. 3Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED. 5, 90128 Palermo, Italy. 4Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di ricerca Difesa e Certificazione (CREA-DC), Via C.G. Bertero 22 - 00156 Roma, Italy. E-mail: antonio.tiberini@unirc.it

Diseases caused by aphid-borne poleroviruses (genus Polerovirus, family Luteoviridae) are emerging threats to the production of important crops. During the current decade, several first outbreak and survey studies have been reported for poleroviruses in Capsicum annum. In Italy, two simultaneous detections of Pepper vein yellow leaf virus (PeVYV) occurred in 2015 in central Italy (Lazio) in hot pepper in open fields, and southern Italy (Sicily) in greenhouse-grown sweet pepper. During recent investigations, hot pepper has been found affected by multiple viruses, causing a range of symptoms including leaf yellowing, brittleness, crinkling, mosaic and necrosis. Most of the viruses were endemic (Tomato spotted wilt virus, Alfalfa mosaic virus, Broad bean wilt virus 2, Potato virus Y, Pepper mild mottle virus) but Chilli veinal mottle virus (ChiVMV) and PeVYV were new for Italy. The concern that other alien viruses could be introduced through intensive but free exchange and trade of foreign germplasm has led to the use the NGS technique to analyse the whole viroma of some severely symptomatic chilli plants of other Capsicum spp. A new isolate has been identified sharing high nucleotide sequence similarity with the putative species Pepper yellow leaf curl virus (PYCV) in the genus Polerovirus, for which taxonomy is under debate to be considered as new species or a PeVYV strain. On the basis of preliminary partial genome analysis, this isolate showed a mosaic sequence related to PeVYV and Tobacco vein distorting virus as previously reported in the first PYCV outbreak in Israel. This study aims to clarify the taxonomic position of this putative Polerovirus.

Range of expansion and genetic diversity of Bemisia tabaci populations in Italy, under the recent threat of Tomato leaf curl New Delhi virus spread. S. BERTIN1, G. PARRELLA2, M. GIORGINI2, M. NANNINI2, S. DAVINO4, M. LUGI1 and L. TOMASSOLI1. 1Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di ricerca Difesa e Certificazione (CREA-DC), Via C.G. Bertero 22 - 00156 Roma, Italy. 2Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Sede di Portici; via Università 133, Portici, Napoli, Italy. 3Agris Sardegna, Servizio Ricerca Studi ambientali, Difesa delle colture e Qualità delle produzioni, Viale Trieste 111 - 09123 Cagliari, Italy. 4Università degli Studi di Palermo, Dipartimento Scienze Agrarie e Forestali, Viale delle Scienze ED. 5 - 90128 Palermo, Italy. E-mail: sabrina.berlin@crea.gov.it

After the introduction of Tomato leaf curl New Delhi virus (ToLCNDV; Begomovirus: Geminiviridae), the insect vector Bemisia tabaci (Hemiptera: Aleyrodidae) strengthens its harmfulness to the horticultural crops in the Mediterranean basin. Within the B. tabaci complex, Mediterranean (Med) and Middle East–Asia Minor 1 (MEAM1) species (formerly referred to as biotypes Q and B) are widespread in the endangered areas, and are regarded as the main vectors responsible for ToLCNDV transmission. In Italy, B. tabaci has raised the status of the virus in warm areas, including the southern regions, Sicily and Sardinia, and the north-western coast (Liguria). During the last decade, the level of Med populations has progressively increased, and Med displaced MEAM1 in those areas where intensive farming occurs. Following ToLCNDV outbreaks in Italy, surveys were carried out to investigate the infestations of B. tabaci in the affected areas. ToLCNDV-associated Med populations of B. tabaci were found to be established in the Lazio region (central Italy), where begomovirus epidemics had never occurred and vector presence was thought to be only occasional. Nevertheless, single and mixed
populations of Med and MEAM1 species were found in other ToLCNDV-free locations, suggesting that agro-ecological factors still limit Med outbreaks in this central region. Further south, in the Campania region, MEAM1 has been displaced for a long time and only Med was found. The spreading pattern and the genetic diversity of B. tabaci populations are also under investigation in other regions (Sardinia and Sicily), in view of their effects on ToLCNDV epidemiology and disease management.

This research was supported by the Italian Ministry of Agriculture (MiPAAF) in the frame of Project ASPROPI (Azione a supporto della protezione dell’aliment), and by the Regione Campania, 2016 Plan of Phytosanitary Action, in view of their effects on ToLCNDV epidemiology and disease management.

New emerging viruses in pepper crops in Turkey. N. BUZKAN1, B.B. ARPACI2. 1Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey. 2Kilis Yedi Aralık University, Department of Horticulture, Kilis, Turkey. E-mail: nbuzkan@gmail.com

In Turkey, pepper (Capsicum annuum) is economically cultivated in 81,500 ha producing approx. 2-2.5 million tons per year. High incidence of yellow dwarfed pepper plants was observed in major pepper growing areas from the summer of 2013 onwards. Infected plants were mostly found in open-field crops, with symptoms of leaf interveinal yellowing and narrowing, suggestive of polerovirus infections. The fruits of diseased plants were smaller than normal and discoloured, resulting in reduced commercial value. Total RNA was extracted from the infected samples using Trizol. Two-step reverse transcription polymerase chain reaction (RT-PCR) was then performed with primers Pol-G-F/Pol-G-R designed for universal detection of poleroviruses. PCR amplicons were directly sequenced with both primers and subjected to a BLASTn search to identify which virus species each presented. Some sequences were similar to that of Pepper vein yellows virus (PVYV) (9%) and others of Beet western yellows virus (BWYV) (10%) in the genus Polerovirus. However, some sequence chromatograms showed double peaks, suggesting mixed infections with Paprika mild mottle virus (PaMMV) (Tobamovirus) and Broad bean wilt virus -2 (BBWV-2) (Fabavirus). cDNAs from all samples were then subjected to RT-PCR using a primer pair specific to PaMMV and a universal primer pair to detect fabaviruses. BLASTn analysis of the sequenced PCR amplicons proved the presence of PaMMV (4%) and BBWV-2 (6%) for the first time in pepper plants in Turkey.

This research was partly supported by TUBITAK (113 O 423).

Races of Fusarium oxysporum f. sp. niveum in the Aydın Province, Turkey. B. GEÇIOĞLU ERİNCİK1, M.T. DOKEN2. 1Adnan Menderes University, Koçarlı Vocational School, 09100, Aydın, Turkey. 2Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09100, Aydın, Turkey. E-mail:bgerincik@adu.edu.tr

Fusarium wilt, caused by Fusarium oxysporum f. sp. niveum (Fon), is a common soil-borne disease in the watermelon production areas of the Aydın Province in Turkey. A total of 73 pathogenic Fon isolates were sampled from that Province in 2010 and 2011. Races of Fon isolates were determined using the differential watermelon cvs ‘Sugar Baby’, ‘Charleston Gray’, ‘Calhoun Gray’ and ‘PI-296341-FR’. Two-week-old seedlings of the cultivars were root-dipped in spore suspensions (1 × 10^6 microconidia mL^-1) of each isolate. Plants were incubated in a growth chamber and evaluated for the presence of disease symptoms (yellowing, vascular discolouration, and wilting) at 14 d after inoculation. Three races of Fon were detected from the Aydın Province. Among of 73 isolates, 21 were designated as race 0, 27 as race 1 and 25 as race 2. No race 3 isolates were identified.

This research was supported by the Scientific Research Fund of Adnan Menderes University through the project no: ZRF-12011.

Polyphasic characterization of Ralstonia solanacearum strains isolated in Spain from different geographical origins. P. CARUSO4, E.G. BIOSCA3, E. BERTOLINI1, E. MARCO-NOALES4, M.T. GORRIS4, C. LICCIARDELLO3, M.M. LÓPEZ4. 1CREA-Centro di ricerca per l’agricolturcita e le culture mediterrane (CREA-ACM), Corso Savoia, 190 – 95024 Acireale (Catania) Italy. 2Departamento de Microbiología y Ecología, Universitat de València, Av. Dr. Moliner 50, 46100-Burjassot, Valencia, Spain. 3Departamento de Fitosanidad,

Phytopathologia Mediterranea
Potato brown rot and bacterial wilt are caused by the bacterium *Ralstonia solanacearum*, that at global level is one of the world’s most important phytopathogenic bacteria. An extensive survey revealed presence of this quarantine pathogen in some Spanish regions. We report the characterization and intraspecific diversity of a selection of 48 *R. solanacearum* strains isolated in Spain, from different sources and geographical origins. Phenotypic and genotypic analyses were performed by a polyphasic approach, to evaluate the influence of site and host on strain diversity. All the strains were compared using biochemical and metabolic profiles, and serological relationships were evaluated by Indirect-ELISA using polyclonal and monoclonal antibodies. Molecular analyses included partial sequence analysis of *hrpB* and *egl* genes, repetitive sequences (rep-PCR), amplified fragment length polymorphism (AFLP) profiles and macrorestriction with *XbaI* and *SpeI* followed by pulsed field gel electrophoresis (PFGE). Biochemical and metabolic characterization showed that all analysed strains belonged to phylotype II sequvar 1, and shared homogeneous profiles. Strain homogeneity was confirmed by serological tests, rep-PCR typing and phylogenetic analysis. However, differences among strains were found by AFLP and PFGE techniques, some profiles being related to the geographical origins of the strains. Our results support the hypothesis that several clones of the pathogen have been introduced into Spain.

**First report of cobweb disease on shiitake and oyster mushrooms in Spain caused by *Cladobotryum dendroides* and *C. mycophilum***

Several species of *Cladobotryum* cause cobweb disease in mushroom-growing countries worldwide. Recently, *C. mycophilum* was detected in *Agaricus bisporus* (white button mushroom) and *Pleurotus eryngii* (king oyster mushroom) crops from Castilla-La Mancha (Spain). In 2016, symptoms of cobweb were also observed on *Lentinula edodes* (shiitake) and *Pleurotus ostreatus* (oyster mushroom) crops. The disease appeared at the end of the shiitake crop cycle, first on the substrate before spreading to the nearest fruit bodies by means of a fine grey-white mycelium. In oyster mushroom crops, cobweb appeared on fruit bodies at the end of the crop cycle. Eight isolates of *Cladobotryum* recovered from the substrate and diseased fruit bodies of shiitake, and two isolates from diseased oyster fruit bodies, were used to identify the cobweb causal agent. Genomic DNA from the fungal cultures was isolated, and the ITS DNA barcode region was amplified and sequenced. The obtained sequences were combined with sequences from *Cladobotryum* spp. isolated from different edible mushroom crops for phylogenetic analysis. *Cladobotryum dendroides* was identified as the cause of cobweb in shiitake, and *C. mycophilum* in oyster mushroom. Pathogenicity tests on fruit bodies of shiitake were performed using conidial suspensions of two *C. dendroides*, and two *C. mycophilum* isolates on oyster mushroom. *Cladobotryum dendroides* and *C. mycophilum* were re-isolated from the inoculated fruit bodies, while the control mushrooms remained symptomless. This is the first report of *C. dendroides* and *C. mycophilum* causing cobweb in shiitake and oyster mushroom in Spain.

This research was supported by Project E-RTA2014-00004-C02-01 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain), the European Regional Development Fund (ERDF) and by the Royal Botanic Gardens, Kew (London, UK).

**Phytophthora mekongensis and *P. prodigiosa*, two new species associated with citrus in Vietnam.**

*Phytophthora mekongensis* and *P. prodigiosa* are the first reports of new species associated with citrus in Vietnam. The first was detected in 2016 in the Mekong Delta province, which is one of the largest and most fertile citrus growing areas in Vietnam. The second species was detected in 2017 in the northern province of Lang Son. These species have the potential to cause significant economic damage to citrus crops in Vietnam.
Two new *Phytophthora* species were found to be associated with brown rot of pomelo (*Citrus grandis*) and root rot of trees of ‘King’ mandarin (*Citrus nobilis*) and pomelo in the Mekong River Delta area of Vietnam. The two species were characterized from morphological traits, and using the ITS1-5.8S-ITS2 region of the rDNA and the cytochrome oxidase subunit 1 (COI) as barcode genes. One of the two species clustered in the *Phytophthora* Clade 2 and was designated as *P. mekongensis*. It was closely related to, but distinct from, *P. meadii* and produced papillate, often bi- and tri-papillate, caducous sporangia. The second species resided in Clade 9 and was designated as *P. insolita*. It was closely related to, but distinct from, *P. insolita* produced non-papillate, internally and externally proliferating, persistent sporangia, chlamydospores and hyphal swellings with bizarre shapes. In pathogenicity tests, both species induced fruit brown rot on various citrus species. In contrast, only *P. mekongensis* induced typical symptoms of *Phytophthora* gummosis on artificially inoculated citrus trees. *Phytophthora mekongensis* can be regarded as an aggressive pathogen of citrus, while *P. prodigiosa*, although quite common as a soil inhabitant in citrus groves of the Mekong River Delta area, is likely to be an opportunistic pathogen. This is the first report of a *Phytophthora* species from Clade 2 other than *P. citricola* and *P. citrophthora*, as causal agents of citrus diseases worldwide and the first report of a species in Clade 9 in Vietnam.

This research was funded by an initiation grant of STINT (The Swedish Foundation for International Cooperation in Research and Higher Education) and the Project SMAGRUMI (Sensori Ambientali per il Miglioramento della Qualità delle Produzioni Agrumicole)–PO. FESR 2007-2013–Sicily.

**First Report of ‘Candidatus Liberibacter solanacearum’ in carrot in Italy.** V. CATARA, G. LIC-CIARDELLO, M. LINGUAGLOSSA, F. SALONIA, C. RAPISARDA, R. LA ROSA, , G.E. COCUZZA MASSIMINO. Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. E-mail: vcatara@unict.it

In Europe, ‘*Candidatus Liberibacter solanacearum*’ has been found, with its various psyllid vectors, associated to members of the family Apiaceae, including carrot, celery and parsnip. Symptoms on carrot plants include leaf yellowing or purpling, stunting and hairy growth of secondary roots. No such problems were reported by carrot growers to date in a large area of cultivation in Sicily (Southern Italy). Nevertheless, a survey was undertaken in spring 2017, due to the report of ‘*Ca. Liberibacter solanacearum*’ associated to carrot in countries bordering the Mediterranean Sea, such as France, Greece, Spain, Israel and Morocco. Leaves showing yellowing and purple discoloration were observed in three out of five carrot fields visited, although with a very low incidence. Total DNA was extracted from petiole tissues of symptomatic and asymptomatic plants using the DNeasy Plant Mini Kit-Qiagen. DNA extracts positive for ‘*Ca. L. solanacearum*’ by real-time PCR with the Lso-HLBp-HLBr primer-probe set, and with cycle threshold values between 21.75 and 36.59, were obtained from three field carrot samples. Positive amplifications for ‘*Ca. L. solanacearum*’ were also obtained by conventional PCR using primer pairs LsoF/OI2c targeting a portion of the 16S rDNA. Amplicons obtained from the PCR assays were directly sequenced (BMR, Italy). BLAST analysis of the 16S rDNA sequences (approx. 1000 bp) showed 99% nucleotide identity with ‘*Ca. L. solanacearum*’ strains amplified from carrot in Finland (GenBank: GU373048.1). To our knowledge, this is the first report of ‘*Ca. L. solanacearum*’ in Italy. Numerous psyllids (Hemiptera), presently under identification, have been also collected in the investigated fields.

**Exploring the potential invasiveness of *Hymenoscyphus fraxineus* in Mediterranean mountains.** C. AGLIETTI¹, F. CANTINI², P. CAPRETTI², N. LUCHI², S. PAPINI³, A. SANTINI³, L. GHELARDINI¹². ¹Departament of Agrifood Production and Environmental Sciences (DiSPAa), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy. ²Institute for Sustain-
New emerging viruses in the genus Polerovirus in vegetable growing areas in Turkey. N. BUZKAN1, B.B. ARPACI2, F. YARALI, M. KOÇ3, A. APALAK3.

1Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey. 2Kilis Yedi Aralık University, Department of Horticulture, Kilis, Turkey. 3Directorate of Provincial Food Agriculture and Livestock, Kilis, Turkey. E-mail: nbuzkan@gmail.com

There are 17 formally accepted virus species in the genus Polerovirus (Luteoviridae) (ICTV released in 2014). Two poleroviruses, Pepper vein yellows virus (PVYV) and Beet western yellows virus (BWYV) were recently detected in field-grown pepper plants in Turkey. During an extensive survey in June 2015, symptoms including chlorosis of young leaves and bright yellow colour of older leaves, suggestive of polerovirus infections, were observed in plants of spinach (Spinacia oleracea), muskmelon (Cucumis melo), cucumber (Cucumis sativus), lettuce (Lactuca sativa), field bean (Phaseolus vulgaris), summer squash (Cucurbita pepo var. pepo) and broad bean (Vicia faba) in some provinces of the eastern Mediterranean and Southeast Anatolia regions. Leaf tissues from symptomatic plants were first tested in DAS-ELISA for the presence of poleroviruses, then in RT-PCR to amplify a 1.1-kb portion of the polerovirus genome with the general polerovirus primer pairs. PCR amplicons were subsequently sequenced and subject to a BLASTn search to identify the polerovirus species. According to multiple alignment of the obtained nucleotide sequences from RdRp region, some broad bean isolates showed nucleotide identity to Cucurbit aphid-borne yellows virus (CABYV) and Chickpea chlorotic stunt virus (CpCSV) isolates. Broad bean and squash isolates also had nucleotide identity with BWYV isolates from Capsicum annum plants from Turkey (HE978259.1). To our knowledge, this is the first report in Turkey of CpCSV and CABYV in broad bean, and BWYV in broad bean and squash plants. CABYV infection in broad bean is also the first global record of this association.

This research was partly supported by TUBITAK (113 O 423).

Daylily rust (Puccinia hemerocallidis), a new disease of Hemerocallis spp. in Europe that entered through the West. P. TALHINHAS, R. CARVALHO, E. SILVA, J.P. MELO E ABREU, A. MONTEIRO, A.P. RAMOS. LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt

Daylilies (Hemerocallis spp.) are garden plants appreciated for their dense and bright green foliage and their long flowering periods. While originating from
East Asia, they are adapted to diverse climates, from the tropics to high latitudes, and very many cultivars are available in catalogues. Daylily rust (Puccinia hemerocallidis) also originated in East Asia, and was reported in Oceania, Africa and the Americas in the early years of the 21st century. The European Plant Protection Organisation has listed this fungus in the “EPPO A1 List of pests recommended for regulation as quarantine pests”, and recognised the existence of routes for its potential introduction into Europe. Starting in November 2015, rust symptoms were observed on daylily plants in several gardens in Portugal, in the Lisbon area and in the Algarve region, as well as in Madeira island, attaining high prevalence, incidence and severity. In cool climates in Europe the disease cycle is naturally broken in the absence of the aecial host (Patrinia spp.), because daylilies lose their leaves and urediniospores are not able to survive. However, in Mediterranean conditions, most Hemerocallis spp. genotypes retain their leaves, providing conditions for the maintenance and multiplication of inoculum. The relevance of this disease for European ornamental horticulture industry and its potential spread according to agroecological conditions will be discussed.

This research was supported by the Project PTDC/BIA-MIC/1716/2014 (Fundação para a Ciência e a Tecnologia, Portugal).

Integrated management of almond witches’ broom in endemic areas: does grafting represent a promising control measure? P. TAWIDIAN, Y. ABOU-JAWDAH. Department of Agriculture, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut 1107 2020, P.O.Box 11-0236 / (AGSC), Riad El-Sohl, Lebanon. E-mail: abujawya@aub.edu.lb

Almond witches’ broom (AlmWB), caused by “Candidateus Phytoplasma phoenicium”, is an invasive disease infecting almond peach and nectarine. In Lebanon, the disease spread rapidly leading to death or complete yield loss of over 200,000 trees. Severe yield losses were also reported in Iran. Planting resistant varieties would be the best approach for environmentally sound management of the disease. Since resistant or tolerant almond cultivars have not yet been identified, grafting on resistant non-host stone fruit rootstocks was assessed. Preliminary field results showed that no symptoms develop on the growth emerging from apricot or plum scions grafted on severely infected almond trees, for more than 2 years post-grafting with one exception: one of the apricot cultivars, initially developed symptoms but recovered 2 months later and remained symptomless thereafter. Preliminary results from greenhouse trials showed that when AlmWB-infected scions were grafted on plum and apricot seedlings, they developed symptomless shoots, except in one case which recovered after 3 months. One year post-grafting the phytoplasma was not detected by PCR in almond grafted on two out of three plum cultivars, and in one apricot cultivar out of the two tested. Results of real time PCR (qPCR) showed significant reductions of phytoplasma titre in the recovered tissue as compared to titres before recovery. These preliminary grafting results look promising, and long term field trials are planned in phytoplasma infested regions. Further studies are recommended to unveil the physiological/molecular mechanisms underlying the recovery phenomenon, which may pave the way for effective curative control measures.

This work was partially supported by a joint project (AID 9627) between the Ministry of Agriculture, the Italian cooperation and the Association of Volunteers in International Service (AVSI) and project LNCSSR 03-06-14 of The Lebanese National Council for Scientific Research.

A deep characterization of Grapevine Pinot Gris Virus by molecular and ultrastructural approaches. G. TARQUINI1, G.L. BIANCHI2, F. DE AMICIS2, M. MARTINI1, A. LOSCHI1, G. FIRRAO1, N. LOI1, R. MUSETTI1, P. ERMACORA1. 1Department of Agricultural, Food, Environmental and Animal Sciences. University of Udine. via delle Scienze, 206, I-33100 Udine, Italy. 2ERSA, Plant Protection Service. via Sabbatini, 5, I-33050 Pozzuolo del Friuli, Udine, Italy. E-mail: giulia.tarquini@spes.uniud.it

In 2003, an emergent disease characterized by symptoms of stunting and chlorotic mottling and deformation of leaves, has been reported in several grapevine varieties in different regions of Northern Italy. A new Trichovirus, named Grapevine Pinot gris virus (GPGV), was discovered in 2012 using an NGS approach. Despite increasing reports worldwide, the aetiology of GPG-disease is still unclear, since the virus was detected both in symptomatic and asymptomatic samples. The GPGV morphological and genetic charac-
teristics were investigated, to allow differentiation of virus isolates associated with symptomatic plants from those associated with asymptomatic plants. Ultrastructural observations and immunogold labelling detected filamentous flexuous viruses in phloem parenchyma cells, which also contained enlarged mitochondria, and vesicles hypothetically associated with endoplasmic reticulum alterations. No cytological differences were observed between symptomatic and asymptomatic tissues. Genome sequences of nine GPGV isolates from Friuli Venezia Giulia were obtained by Sanger sequencing and subjected to phylogenetic analysis, together with those available in GenBank. Results showed that GPGV isolates from asymptomatic plants clustered in a distinct clade, whereas isolates associated with symptomatic grapevines showed greater diversity. Further analysis of the sequence dataset highlighted features such as recombination breakpoints in the RdRp gene and a positively selected codon site in the CP gene. These analyses may assist understanding the population structure of the virus and the temporal dynamics of its interaction with hosts, although further studies are required to clarify the significance of these evolutionary events in the expression of disease symptoms.

This research was supported by Regione Friuli Venezia Giulia (CUP: F22I15000110002).


A new race of *Fusarium oxysporum* f. sp. *lactucae* that causes *Fusarium* wilt of lettuce. This is the first report of *F. oxysporum* f. sp. *lactucae* in Netherlands, and identifies a new race of *F. oxysporum* f. sp. *lactucae* using the IRAP technique. The primers FPUF and FPUR were designed based on a polymorphic band of the IRAP specific for the two Dutch isolates determined as a new race of the pathogen.

This project was supported from the EU Horizon 2020 research and innovation programme under grant agreement no. 633999 (EMPHASIS).

**Plectosphaerella species as new pathogens of basil and parsley cultivation.** M.L. RAIMONDO, A. CARLUCCI. Department of Science of Agriculture, Food and the Environment, University of Foggia, Via Napoli 25, 71122 Foggia, Italy. E-mail: antonia.carlucci@unifg.it

Since 2012, several basil and parsley samples collected from local markets in Foggia province (South Italy) were subjected to laboratory analyses to ascertain the main fungal pathogens occurring. The sampling consisted of young parsley and basil plants showing leaf yellowing, necrotic lesions on stems, collars and roots, and in some cases stunting of the entire plants. Mycological analyses revealed mainly a common presence of fungal isolates belonging to *Plectosphaerella* genus. Morphological and molecular studies identified four different species of *Plectosphaerella* including *P. cucumerina*, *P. pauciseptata*, *P. plurivora* and *P. ramiseptata*. To understand the pathogenic roles of these *Plectosphaerella* species, and another five reference species (*P. alismatis*, *P. citrulli*, *P. delsorboi*, *P. melonis* and *P. oratosquillae*), pathogenicity tests were
performed in vitro and in vivo, using basil cv. ‘Napoletona’ and parsley cv. ‘Gigante di Napoli’. Pathogenicity tests were carried out on detached leaves (in vitro conditions), and on 30-d-old basil and parsley plants grown in plots in a greenhouse (in vivo conditions). All four species isolated during this study caused symptoms both on basil and parsley leaves and on young plants, although producing different symptoms (necrotic spots, parenchymatic patches, hydropic areas, collar and root discolorations) and at disease severities. Plectosphaerella ramiseptata was the most aggressive species. To our knowledge, this is the first report of P. cucumerina, P. pauciseptata and P. ramiseptata on parsley, and the first report of P. pauciseptata, P. plurivora and P. ramiseptata on basil.

Occurrence of Xanthomonas campesstris pv. campesstris isolates on wheat in Algeria. This pathosystem has never been seen. B. KHENFOUS-DJEBARI1, M. KERKOU2, C. BRAGARD3, Z. BOUZNAD1. 1Laboratoire de Physiopathologie et Biologie Moleculaire, departement de Botanique, ENSA El Harrach, Alger, Algerie. 2DiagGene, 8, Rue le Nôtre 49066 Angers, France. 3Appliedmicrobiology-Phytopathology, Earth&Life Institute, Université catholique de Louvain. E-mail: b.djebari@ensa.dz

In a study of diseases caused by Xanthomonas translucens in Algerian wheat plots, two isolates were obtained, X7 in 2010 and X14 in 2011. Although they were morphologically identical on yeast dextrose chalk agar and nutrient agar, they were shown to differ from other isolates when sequencing was performed on the housekeeping genes gyrase subunitB (gyrB), RNA polymerase sigma factor (rpoD), Chaperone protein DnaK (DnaK) and ATP synthase subunit beta (atpD). The sequences obtained for the different genes closely link the isolates to Xanthomonas campesstris pv. campesstris and pv. Raphaani, with similarity indices of 98 to 99%. A pathogenicity test performed by inoculation onto the sensitive wheat cv. Acsad 885 (Ramada) at the three-leaf seedling stage, and by dip-inoculation, in order to fulfill all Koch’s postulates, was completed by inoculation of three cabbage cultivars (Chou de Milan de pontoise, Chou Milan gros de vertus and Lucien Clause chou rouge tête noire) and one cauliflower cultivar boule de neige.

Similar to that described for X. c. pv. campesstris on Brassicaceae, characteristic symptoms of black rot and V-shaped necrotic leaf lesions and blackening of vascular tissues were obtained on the four cultivars, while inoculated wheat seedlings showed water soaked lesions and necrotic to black lesions.

Genome analysis: applications to plant health

Experimental evolution in the fungal pathogen Fusarium oxysporum to study mechanisms of genome plasticity and host adaptation. C. LÓPEZ-DÍAZ1, D. HAZAL AYHAN2, J.J. GINÉS-RIVAS1, I. OKEKE-INFANTE1, LI-JUN MA3, A. DI PIETRO1. 1Department of Genetics, University of Córdoba, Spain. 2University of Massachusets, Amherst, USA. E-mail: g02lodic@uco.es

Filamentous plant pathogens undergo rapid evolution, leading to shifts or expansions in host ranges. The Fusarium oxysporum species complex collectively causes vascular wilt in more than a hundred different crops, provoking severe losses in global agriculture. The evolutionary mechanisms underlying host adaptation and host range dynamics in this pathogen remain poorly understood. We followed an experimental evolution approach, involving serial passages of the tomato pathogenic isolate Fol 42-87, either through plants or on artificial media plates. Independently evolved populations obtained after ten consecutive passages displayed notable phenotypic differences with respect to the initial clonal isolate. These included alterations in growth, sporulation and virulence. Four of the five plate-passaged populations had reduced virulence on tomato plants. Resequencing of the evolved populations revealed the presence of segmental duplications and deletions, particularly in the transposon-rich lineage-specific regions of the genome. In addition, single nucleotide changes and small indels were detected in the evolved lines, some of which affected genes with known functions in fungal development and virulence. Collectively, our findings suggest that chromosome plasticity acts as a major evolutionary driver in F. oxysporum, and provide new insights into the genetic mechanisms underlying host adaptation in this important fungal pathogen.

This research was supported by the Project BES-2014-070450 (Ministerio de Economía y Competitividad, MINECO, Spain) and a Burroughs Welcome Investigator Award.
Genome size variability across fungi and the occurrence of very large genomes among rust fungi. P. TALHINHAS1,2, A.P. RAMOS1, S. TAVARES1,2,3, M.C. SILVA1,2, J. LOUREIRO1, M. COELHO1. 1LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. 2Centro de Investigação das Ferrugens da Cadeira, Instituto Superior de Agronomia, Universidade de Lisboa, Quinta do Marquês, 2780-505 Oeiras, Portugal. 3Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg Copenhagen, Denmark. 4CFE, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal.

The completion of genome sequencing for some rust fungi indicates a link between biotrophic specialisation and genome size expansion. The measurement of genome sizes for 60 rust fungi, using flow cytometry, has revealed some of the largest genomes among fungi, with nine rust species with haploid genomes between 300 and 893 Mbp. The genome of Uromyces appendiculatus was 652 Mbp, Phakopsora pachyrhizi 716 Mbp, U. transversalis746 Mbp, Hemileia vastatrix 772 Mbp, and Gymnosporangium confusum 893 Mbp. The genome of U. bidentis, was 2489 Mbp. The overall average of the 60 rust fungi was ca. 380 Mbp. Genome size information is available for over 1800 fungal species, either arising from flow cytometry, genome sequencing, or other methods. This reveals an overall average of 44 Mbp. Departing from our genome size measurements of Pucciniales fungi, in this work we analyse genome size variability across representatives of the entire fungal phylogeny and attempt to relate such variations with relevant biological and genomic traits (e.g. life style, sexuality, nutrient use, composition in transposable elements). The analysis of genome size variation can unveil clues suggesting polyploidisation events or transposable elements activity of evolutionary/adaptive relevance. Such traits can be associated with reproduction strategies (sexual, asexual, parasexual and/or rare sexual) and substrate utilization (saprobic, mutualistic, obligate/facultative pathogenic, biotrophic/necrotrophic, and combinations of these).

This research was supported by the Project PTDC/BIA-MIC/1716/2014 (Fundação para a Ciência e a Tecnologia, Portugal).

UV damage repair in the Fusarium species complex. S. MILO-COCHAVI, S. KOVO. Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment. Hebrew University, Israel. E-mail: shay.covo@mail.huji.ac.il

Fungal plant pathogens are exposed to various sources of DNA damage that can cause cell death and genome instability. Recent genome resequencing projects demonstrated high degrees of genome instability in fungal plant pathogens, suggesting poor DNA repair capacity. However, we found that the major fungal plant pathogens are equipped with various redundant DNA repair genes. We have focused on the Fusarium species complex that causes severe diseases in crops throughout the Mediterranean region. This complex has a diverse host range, and causes wilt, fruit deformation and head blight diseases. Preliminary results showed that while the soil-borne pathogen F. oxysporum survived sunlight exposure to a great extent, the foliar pathogen F. mangiferae was sensitive. We recapitulated the sun exposure sensitivity results by UVC irradiating both species. Sequence comparison of the most important UV damage repair genes between F. oxysporum and F. mangiferae failed to reveal a major difference. In contrast, F. mangiferae is more resistant to the methyl methanesulphonate (MMS), indicating that its base excision repair capacity is greater. RNA sequencing of both species revealed strong transcriptional response to DNA damage. Unlike Saccharomyces cerevisiae, Fusarium species show over expression of the nedd8 pathway in the context of nucleotide excision repair, but lack activation of the ribonucleotide reductase pathway. There is no evidence for low capacity of DNA repair in the Fusarium species complex, suggesting that the high degree of genome instability stems from cis DNA elements.

Characterization of mating type genes of Alternaria alternata isolated from onion. W. BIHON1, L. KENYON2. 1World Vegetable Center, West and Central Africa, Samanko Research Station, BP 320, Bamako, Mali.
Alternaria is a cosmopolitan fungal genus comprising many saprophytic, endophytic and pathogenic species. Pathogenic Alternaria species cause major pre- and post-harvest losses on diverse agricultural crops including vegetables. Understanding the mode of reproduction in a plant pathogenic fungus is essential because it affects the population genetic structure, evolution and epidemiology, and so will influence effective disease management. Plant pathogenic fungi in general have different means of reproduction, including sexual, asexual and parasexual mechanisms. Sexual reproduction in ascomycetes is controlled by a mating type (MAT) locus. In this study, the complete genomes of two randomly selected strains of Alternaria alternata from onion were sequenced, and two genes (MAT1-1-1 and MAT1-2-1) at the MAT locus were identified and characterized. The high mobility group (HMG) and alpha-1 (α-1) box are highly conserved and the genes are, respectively, 1083 and 1217 base pairs in length. The flanking region of both idiomorphs contained DNA lyase. These results suggest that this fungus is heterothallic, since the two opposite mating type genes were found from two different strains. Sexual structures have not been observed in A. alternata and the presence of both mating types indicates the existence of cryptic sexual process. This would result in an increase in the genetic diversity of the pathogen and complicate the management practices used. A more detailed study of the frequency and distribution of the two genes in major onion growing locations is necessary to determine how frequent recombination occurs in these populations.

This research was supported by the World Vegetable Center core donors Republic of China (Taiwan), United Kingdom Department for International Development (DFID), United States Agency for International Development (US-AID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan.


Root lesion nematodes (RLN) are ranked third worldwide as plant parasitic nematodes with economic impacts. Pratylenchus penetrans, one of the most damaging species of this group, affects more than 400 hosts, and is considered a limiting factor in production of important crops (e.g. corn, potato), ornamentals (e.g. lily, roses) and fruit trees (e.g. apple, cherry orchards). Surveys conducted in Portugal have revealed different RLN species associated with important crops, with P. penetrans as one of the most abundant species found in potato fields. Host resistance to RLN is very limited, as only a few genetic loci have been linked to resistance/tolerance to some species. Effective and long-lasting control strategies based on current pesticide compounds are hampered by increasing regulations, due to their non-specificity and potential toxic effects to ecosystems and human health. A promising research area is the identification of critical metabolic and parasitism-related genes of these plant pathogens, in which silencing through RNA interference (RNAi) can promote lethal or inhibitory effects on nematode development or parasitism strategy. The main goal of the project PratyTech is to identify protein-coding genes in P. penetrans which may be established as new nematode targets for the development of specific and efficient crop resistance strategies. Another relevant aspect of this project is the study of the host gene expression profile and cellular changes upon P. penetrans infection in potato, which should provide important insights into the molecular mechanisms involved in RLN parasitism.

This work was supported by National Funds through FCT—Foundation for Science and Technology under the Projects PTDC/AGR-PRO/2589/2014 and UID/AGR/00115/2013.

Tolerance of olive (Oleae europaea) cv. Frantoio to Verticillium dahliae relies on differential basal and pathogen-induced transcriptomic responses.
Verticillium wilt (VW) is one of the most serious biotic constraints for olive trees. Knowledge of the genetics of tolerance/resistance to this disease is very limited. To analyze the susceptibility/tolerance of olive cultivars Frantoio (tolerant) and Picual (susceptible) to Verticillium dahliae, a comparative transcriptomic analysis (RNA-seq) was carried out in root host tissues. Results showed that a large number (27,312 unigenes) of differentially-expressed genes (DEGs) were found between ‘Frantoio’ and ‘Picual’ non-manipulated control roots. Dissimilar root system architecture was also observed between the two cultivars. Upon infection with V. dahliae, ‘Picual’ and ‘Frantoio’ plants also responded in completely different ways. Genes induced in ‘Picual’ roots were basically different to the DEGs observed in ‘Frantoio’ non-manipulated/uninoculated roots. Transcriptome changes occurring in each cultivar at early stages of V. dahliae infection were also very dissimilar. When targeting for tolerance/resistance-related genes, the most noticeable expression differences between the cultivars were: i) a pathogenesis-related protein of the Bet v I family, likely encoding a major latex protein; ii) a dirigent-like protein involved in lignification; iii) several BAK1 (Brassinosteroid insensitive 1-Associated receptor Kinase) and NHL1 (Harpin-Induced protein-like) unigenes; iv) six unigenes involved in ROS stress response (stronger in ‘Picual’ but no expression in ‘Frantoio’); and v) an overall induction of BAM unigenes (involved in starch degradation) in ‘Picual’ in contrast to ‘Frantoio’. These results show that tolerance of ‘Frantoio’ plants to VW is a complex polygenic plant trait.

This research was supported by grants AGR-5948 from Junta de Andalucía (Consejería de Economía, Innovación y Ciencia) and AGL2009-07275 and AGL2016-75729 from Ministerio de Economía y Competitividad/Agencia Estatal de Investigación, Spain (co-financed by the European Regional Development Fund, ERDF). Technical and personnel support was provided by CICT of Universidad de Jaén (UJA, MINECO, Junta de Andalucía, ERDF).

**Expressional and positional candidate genes for resistance to Didymella pinodes in pea.** S. FONDEVILLA, M.D FERNANDEZ-ROMERO, D. RUBIALES, Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, 14004 Córdoba, Spain. E-mail: sfondevilla@ias.csic.es

Didymella pinodes, causing Ascochyta blight, is the most destructive foliar pathogen of dry peas. Resistance identified so far is incomplete, and more frequent in wild pea relatives than in cultivated pea. One of these wild relative resistant accessions is the P. sativum ssp. syriacum accession P665. Quantitative Trait Loci (QTLs) associated with resistance to Ascochyta blight have been identified in the recombinant inbred lines (RIL) population P665 × Messire and in other crosses, but the resistance genes underlying these QTLs are unknown. expressional and positional candidate genes for resistance to D. pinodes were identified by selecting 15 candidate genes to be mapped in the P665 × Messire RIL population. They were differentially expressed in resistant reactions in previous transcriptomic studies, or putatively located into QTLs associated with resistance to this disease according to other pea maps. Thirteen QTLs were successfully amplified in the parental lines. Two were monomorphic, direct polymorphism was found for another, and CAP markers were developed for the remaining ten genes. Therefore, eleven genes could be analysed and mapped in the available P665 × Messire map. Four genes were located within the confidence interval of previously described resistance QTLs or highly associated with resistance parameters. These are therefore suggested as putative candidate genes for resistance to Ascochyta blight.

This research was supported by the Project AGL2014-52871-R.

**Genomic analysis of nontoxigenic strains of Pseudomonas syringae pv. phaseolicola.** P. LLOPÉS, L. BARDAJÍ, M. ECHEVERRÍA, P. RODRÍGUEZ-PALENZUELA, J. SÁNCHEZ-COLMENERO, C. RAMOS, J. MURILLO. Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Universidad Regional de Murcia.

Vol. 56, No. 2, August, 2017 303
**High-frequency rearrangements of virulence plasmids from Pseudomonas syringae are mediated by MITEs and IS801.**

L. BARDAJÍ¹, M. ANORGÁ¹, M. ECHEVERRÍA¹, D. RAMÍREZ-ZAPATA¹, C. RAMOS², J. MURILLO¹. ¹Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Universidad Pública de Navarra, 31006 Pamplona, Spain. ²Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora”, Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias, Campus Teatinos s/n, 29010 Málaga, Spain. E-mail: jesus.murillo@unavarra.es

**Pseudomonas syringae pv. phaseolicola (Pph)** is an economically important pathogen of bean (*Phaseolus vulgaris*), causing halo blight, and is a relevant research model. Efficient control of halo blight is difficult because the pathogen is transmitted by seed and has very high epidemic potential, and is primarily based on the use of pathogen-free seed and resistant cultivars. However, the efficacy of these methods can be compromised by the variability of local pathogen populations. At least 16 Pph races have been identified, facilitating breakdown of resistance. Likewise, most field isolates of the pathogen from Spain are nontoxigenic and cannot be detected using commercial ELISA antibodies or by PCR targeting the phaseolotoxin cluster. We therefore undertook a comparative genomics approach to better characterize the Spanish pathogen Pph populations, and to develop appropriate detection methods, for a better control of halo blight. Representative Pph isolates were sequenced using Illumina MiSeq with paired-end technology, and their genomes are being compared to the closed genome of the race 6 model strain *P. syringae* pv. *phaseolicola* 1448A. As expected for this highly clonal pathovar, the genomes are highly conserved, with very high sequence identity, which hampers epidemiological studies. The Type III effector repertoire is highly conserved, with variations in only five effectors between strain CYL314 (nontoxigenic) and the reference strain 1448A, including *avrPphF* (*hopF1*). We confirmed that strain CYL314 completely lacks the phaseolotoxin cluster, containing an alternative genomic island. The conservation of other virulence genes will be presented and discussed.

This research was supported by projects AGL2014-53242-C2-1-R and AGL2014-53242-C2-2-R (Plan Nacional MINECO, Spain), co-financed by FEDER.

The *Pseudomonas syringae* complex includes several species of Gram negative bacteria causing economically relevant diseases in many cultivated plants. Most isolates of *P. syringae* contain native plasmids collectively carrying many pathogenicity and virulence genes, which are readily exchanged intraspecifically. Gene flow is promoted by a diverse array of repeated sequences, among which insertion sequences and miniature inverted-repeat transposable elements (MITEs) are particularly abundant and active. The virulence plasmid pPsv48C from *P. syringae* pv. *savastanoi* NCPPB 3335 is extremely stable, and we showed that it contains two independent functional replicons (*repA* and *repJ*) and 29.5% of its sequence occupied by putative mobile elements. This plasmid spontaneously suffers the deletion of an 8.3 kb fragment, with a frequency greater than $10^{-3}$, by recombination between two direct copies of MITEPsy2. Likewise, we showed that insertion sequence IS801 promotes deletions of pPsv48C by one-ended transposition, with an average frequency greater than $10^{-4}$, half of these resulting in the loss of a virulence gene. These deletion derivatives were maintained in the population by replication mediated by *repJ*, which is adjacent to IS801. We demonstrated that IS801 also promotes deletions in plasmid pPsv48A and in the large plasmid from *P. syringae* pv. *phaseolicola* 1448A, either by recombination or failed transposition. The accumulation of these types of deletions in vivo is prevented by the occurrence in these plasmids of functional post-segregational killing systems, contributing to the maintenance of pathogenicity genes in *P. syringae* populations.

This research was supported by projects AGL2014-53242-C2-1-R and AGL2014-53242-C2-2-R (Plan Nacional MINECO, Spain), co-financed by FEDER.
Detection and phylogenetic analysis of **Grapevine virus A** from important vineyards in Iran. R. MORADI1, D. KOOLIVAND1, O. EINI1, M. HAJIZADEH2. 1Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran. 2Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran. E-mail: Koolivand@znu.ac.ir

**Grapevine virus A** (GVA) belongs to the **Vitivirus** genus (family Betaflexiviridae). This virus is one of the most destructive agents in vineyards worldwide. From 2016 to 2017, leaf samples from grapevines with leafroll, reddening of leaf margins and petioles, as well as samples from symptomless grapevines, were collected from vineyards in different regions in west Iran (Abhar, Zanjan and Tarom). Total RNA was extracted from the different tissues of samples according, and was subjected to reverse transcription polymerase chain reaction (RT-PCR) with random hexamer primers. The synthesized cDNAs was used as template to amplify a DNA fragment (865 bp) in PCR by specific primers (GVA-HSS7/GVA-C7273) corresponding to coat protein (CP) gen of GVA. The full-length CP gene was amplified from suspected samples, and the amplified products were sequenced by Macrogen (Korea). The obtained sequences were aligned with other sequences from Genbank, and a phylogenetic tree was prepared using the MEGA6 program. We conclude that the most common leafroll were observed in grapevines as well as growth reductions in vineyards, and these symptoms were reported in grapevines infected with GVA. The sequence identities between the GVA isolates from Iran and the other isolates were 85 to 90% at the nucleotide level. CP-based phylogenetic trees also showed that the new Iranian isolates grouped in a subclade together with GVA isolates from diverse geographical regions, including China, Israel, and Greece.

This research was supported by the University of Zanjan.

Distribution of ToxA, the necrosis virulence gene of the wheat tan spot agent, in North African and Middle East wheat-growing areas. N. OUAAR3, A. YAHYAOUI2, A. BENBELKACEM3, H. BENSILMANE1. 1Ecole Nationale Supérieure d’Agronomie, Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1 Avenue Pasteur, Hassen Badi, Algiers, Algérie. 2International Maize and Wheat Improvement Center, Apdo. Postal 6-641, 06600, Mexico DF, Mexico. 3Institut National de la Recherche Agronomique d’Algérie, Unité de Recherche de Constantine, Station ITGC, Elkhroub, Algérie. E-mail: n.ouaar@st.ensa.dz

Tan spot of wheat (caused by **Pyrenophora tritici-repentis**) is an economically important disease worldwide, and has long been important in North African and Middle East wheat-growing wheat regions. These areas are close to the wheat origin centre. **Pyrenophora tritici-repentis** uses at least three host-specific toxins, PtrToxA, PtrToxB and PtrToxC. Virulence of an isolate is correlated with the presence of these toxins, and host resistance is associated with absence of the sensitivity loci. Synthesis PtrToxA is under control of a ToxA gene, which is the most common virulence gene. To improve knowledge of pathogen populations in northern Africa, and because breeding for resistance to tan spot can be improved by knowledge of the distribution of toxin-encoding genes, 238 isolates sampled from Algerian, Tunisian and Syrian wheat-growing areas were analyzed. Using PCR, a molecular test was applied to the isolates populations to screen for the ToxA gene. ToxA occurred in isolates from all three countries, and the distribution of the ToxA gene was mapped in the study areas. These results allow breeders to better target genotypes in field, and use them according to deployment of the ToxA virulence gene.

This research was supported by Ecole Nationale Supérieure d’Agronomie (ENSA), Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1, Avenue Pasteur, Hassen Badi, Algiers, Algeria.

Allelic diversity analysis for Verticillium wilt resistance candidate genes in olive (**Olea europaea**). A. SERRANO, L. LEÓN, A. BELAJ, B. ROMÁN. Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Centro Alameda del Obispo, Avda, Menéndez Pidal s/n. 14004, Córdoba, Spain. E-mail: alicia.serrano.gomez@juntadeandalucia.es.

Verticillium wilt (caused by **Verticillium dahliae**) is a destructive soil-borne disease affecting olive crops in traditional production areas. The use of genomic tools could help to overcome some of the difficulties associated with pathogen infestation homogeneity, differences in colonization, ambiguous symptom ex-
pression or lack of high throughput screening techniques in selection for host resistance. Different Verticillium resistance genes from olive have been identified in recent transcriptomic studies. We explored the allelic diversity of four of these genes among 77 olive genotypes with different levels of resistance. The olive collection belongs to the World Olive Germplasm Bank, the Wild Olive tree collection and the Olive Breeding Program of IFAPA (Córdoba). The selected genes are among those identified in a suppression subtractive library already reported: a disease resistance-responsive family protein (DRR), a transcription factor (GRASS), a caffeoyl-o-methyltransferase (CO-MT) and an acetone-cyanohydrin lyase (ACL). Primers for amplification of exonic regions of these four genes were designed, and amplified fragments were subjected to allele specific sequencing that allowed SNP detection. The overall nucleotide diversity of the identified alleles was determined, and the ratio of synonymous and non-synonymous substitution per respective site were calculated. Predicted proteins and phylogenetic analysis among alleles of each gene were also examined. The information from this study can be used for association analysis in wider germplasm collections. If validated, this knowledge may be useful for enhancing host resistance and/or assisting selection in olive breeding programmes.

This research was supported by INIA project RTA2013-00019, partially funded by European Regional Development Fund (ERDF).

Elucidation of the rust resistance genetic control in Portuguese common bean through a genome-wide association study. S.T. LEITÃO1, D. RUBIALES2, M.C. VAZ PATTO1. 1Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Avenida da República, 2780-157 Oeiras, Portugal. 2Instituto de Agricultura Sostenible, CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain. E-mail: sleitao@itqb.unl.pt

Common bean (Phaseolus vulgaris) is the most important grain legumes for human consumption worldwide, with recognized nutritional and environmental benefits. However, its vulnerability to several diseases leads to significant yield losses and limited cultivation in Europe. It is therefore essential to identify disease resistant sources and to uncover the genetic control of resistance to improve production. Portugal holds unique common bean germplasm resulting from more than five centuries of natural adaptation and farmer’s mass selection, not yet fully explored in breeding. We screened 158 accessions from this germplasm against Uromyces appendiculatus, the fungus responsible for bean rust. Each host accession was inoculated three times and, on average, 14 plants per accession were tested. Infection type (IT, scored using a 0-4 visual scale) and disease severity (% leaves covered by pustules) were analysed 12 d after inoculation. The most frequent IT was 4, indicating compatible plant-pathogen interactions. Disease severity of plants with IT = 3-4 ranged from <1 to 80%. Three accessions showed low (0-2) IT scores, indicative of incompatible interactions, and were considered resistant or partially-resistant. Eighty-six accessions showed chlorotic halos surrounding rust pustules (IT = 3). The same germplasm collection was also screened with 12k single nucleotide polymorphisms, uniformly distributed throughout the genome. Currently, we are searching for genomic regions controlling rust resistance through a genome-wide association study, using a mixed linear model accounting for genetic structure and familial relatedness. This approach will allow the development of molecular selection tools to assist future precision breeding of rust resistance in common bean.

The Research Unit of Biotechnology and Genetic Resources, INIAV, Oeiras, Portugal provided the common bean seeds. This research was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through the grant SFRH/BD/92160/2013, IF/01337/2014 FCT Investigator contract, the project Exploiting bean genetics for food quality and attractiveness innovation (PTDC/AGR-TEC/3555/2012), the Research unit GREEN-it "Bioreources for Sustainability" (UID/Multi/04551/2013), and by COST Actions FA1208 and FA1306.

Phylogenetic and recombination analysis of the partial silencing suppressor NSs gene of Tomato spotted wilt virus from Iran. M. ABADKHAH, D. KOOLIVAND and O. EINI. Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan-Iran. E-mail: koolivand@znu.ac.ir

Tomato spotted wilt virus (TSWV), is the type member of Tospovirus (family Bunyaviridae). This virus contains three single-stranded RNA segments (S,
M and L RNAs). Non-structural NSs protein has been identified as the suppressor silencing and is located on the S segment. Samples suspected to be infected by TSWV were collected from tomato fields, and total RNAs were extracted using the RNX plus kit (SinaClone), based on the manufacturer’s instructions. Reverse transcription-polymerase chain reaction was carried out using a pair of specific primers (TSWV-NSsF/TSWV-NSsR) corresponding to a part of NSs gene of TSWV. A DNA fragment (724bp) was amplified in the PCR. The best result was obtained when the annealing temperature was set to 50°C. The amplified DNA fragment was sequenced and the new sequence was aligned with the reported sequences in the GenBank. The identities between the Iranian and other reported isolates were 96%-99%. Alignment was performed with other international sequences using Clustal W software, then a phylogenetic tree was generated using the MEGA7 program and the Neighbour-Joining method. Phylogenetic analysis showed that the new isolate was in a subclade with three isolates from France (LYE40, LYE47 and STMB3). Recombination events were identified using the Maxchi method in the RDP4 Beta 80 program, and showed that there was evidence for recombination of the Iranian isolate with other isolates (TSW(RT)/T15-2/wt).

The genome of *Fusarium oxysporum* is highly dynamic and contains lineage specific (LS) regions rich in transposable elements that are involved in pathogenic behavior. Unexpectedly, Southern blot hybridisation and sequence analysis in the tomato pathogenic isolate *F. oxysporum* f. sp. *lycopersici* 4287 revealed a high degree of structural and physical conservation in the telomeric and subtelomeric regions, extending approximately 30 kilobases from the chromosome ends. The evolutionary origin of this highly conserved region is currently unknown. Our main goal has been to analyse the function of the conserved telomeric and subtelomeric regions in the maintenance and plasticity of the chromosome structure, as well as in the regulation of the adjacent genes.

This research was supported by the Spanish Ministerio de Ciencia e Innovacion (grants BIO2016-78923-R).
Integrated disease management

Development of the root-knot nematodes in zucchini and associated yield losses. S. VERDEJO-LUCAS, M. TALAVERA, A. PÉREZ-DE-LUQUE, IFAPA. 1Camino de San Nicolás 1, 04745, La Mejorona, Almería, Spain. 2Camino de Purchil s/n, 18004 Granada, Spain. 3Alameda del Obispo, 14080 Cordoba, Spain. E-mail: soledad.verdejo@juntadeandalucia.es

The interaction of Cucurbita pepo genotypes and Meloidogyne populations resulted in a poorer host condition from M. incognita than from M. javanica. The critical event in the M. incognita-zucchini interaction was the development from fourth stage juveniles to adult females. Meloidogyne incognita-induced feeding sites contained more and larger highly vacuolated giant cells than those of M. javanica, but 74% of the M. incognita feeding sites deteriorated before life cycle completion. In contrast, 96% of the invading M. javanica reached the egg-laying female stage. The extent of yield losses in zucchini depended on the interaction of (large) initial population densities, planting time and length of the growth period. Average yield losses in zucchini range from 20-36%, although densities below 125 nematodes/250 cm² of soil have negligible impact. Populations greater than 1100 nematodes/250 cm² of soil reduced yield by 52%. Population increases were greater in autumn cropping cycles than in spring, but yield reductions were less in autumn than spring cycles. Limited yield losses were observed in growth periods of ca. 2 months, in contrast to those of ca. 3-4 months. Root galling and yield were negatively related, indicating that the root-knot nematode damage was critical for plants, as it decreased yield. The leaf chlorophyll content decreased with increased population densities and post-infection time, with significant reductions at densities greater than 450 nematodes/250 cm² soil. Modification of planting date and cropping nematode resistant or non-host crops before planting zucchini effectively reduced nematode damage in zucchini.

This research was supported by IFAPA (Instituto de Investigación y Formación Agraria y Pesquera, Spain) project PPTRA. TRA 2016009. INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain) RTA 2014-00078-00-00, and the European Regional Development Fund (ERDF).

Meta-analysis of the effect of the application period in the management of Botrytis bunch rot in vineyards. G. FEDELE, E. GONZÁLEZ-DOMÍNGUEZ, L. DELIÈRE, P. SAURIS, E. DÍAZ-LOSADA, J.L. RAMOS SÁEZ DE OJER, D. GRAMAJE, V. ROSSI. 1Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84 29122 Piacenza, Italy. 2SAVE, Bordeaux Sciences Agro, INRA, 33140 Villenave d’Ornon, France. 3Estación de Viticultura e Enoloxía de Galicia - INGCAL. Ponte San Clodio s/n, 32427, Leiro, Ourense, Spain. 4Consejería de Agricultura, Ganadería y Medio Ambiente. Finca La Grajera, Ctra. LO-20 salida 13, 26071, Logroño, Spain. 5Instituto de Ciencias de la Vid y del Vino (Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Gobierno de La Rioja). Finca La Grajera, Ctra. LO-20 salida 13, 26071, Logroño, Spain. E-mail: vittorio. rossi@unicatt.it

Botrytis cinerea is one of the most important pathogens of grapevines. The management of B. cinerea is challenging, mainly because the pathogen produces large numbers of conidia on multiple inoculum sources, grapevines are susceptible at multiple growth stages, and different infection pathways exist. This complexity has caused growers to rely heavily on routine application of fungicides at four growth stages: flowering (A), pre-bunch closure (B), veraison (C) and before harvest (D). Recently, a weather-driven model has been developed to predict the development of Botrytis bunch rot. The model was validated in 21 epidemics using a discriminant function analysis (DFA) with 81% accuracy; the DFA also showed that the infections occurring during flowering and fruit set may play a key role in determining the severity of B. cinerea rot on mature bunches. These results are in apparent contrast with recommendations from the research carried out mainly in the 1990s, which suggested that sprays in growth stages B and in C to D as the most important for disease control. To better understand the contributions of the fungicides applied either in A, B, C or D on final Botrytis bunch rot, we performed a meta-analysis of 115 studies, conducted from 1970 to 2016 in eight countries, covering a wide range of epidemics. Raw data showed that average efficacy of treatments in A was 31% (range from 0.98 to -0.30), B, 22% (range from 0.84 to -0.67), C, 40% (range from 0.96 to -0.81) and D, 39% (range from 0.92 to -0.76), average
efficacy of a four-treatment schedule (at stages A, B, C and D) was 79% (range from 0.79 to 0.23). Studies were weighted in inverse proportion to their sampling variances for model fitting purposes. Results of the meta-analysis, combined with the epidemiological model, provide new information on how to schedule fungicides treatments for controlling B. cinerea in grapes.

Copper: a basic active ingredient in the control of olive diseases. L.F. ROCA, J.R. VIRUEGA, A. ÁVILA, J. MORAL, F. MARCHAL, J. ROMERO, P. MIRANDA, C. AGUSTÍ-BRISACH, A. TRAPERO. Dpto. de Agronomía (Patología Agroforestal), Universidad de Córdoba. Campus de Rabanales, Edificio C-4, 14071, Córdoba. E-mail: trapero@uco.es

Copper is extensively used in the control of olive diseases. More than 80% of the authorized fungicides in this crop in Spain contain this element as the active ingredient. Among the main diseases affecting the aerial part of olive trees are leaf spot (caused by Venturia oleaginea), cercosporiose (Pseudocercospora cladosporioides) and anthracnose (Colletotrichum spp.) These diseases cause defoliation and weakness of the trees and anthracnose also produces fruit rot, being especially severe in humid, temperate autumns. Other diseases, such as leprosy (Phytophthora vagabunda) or tuberculosis (Pseudomonas savastanoi pv. Savastanoi) are becoming increasingly relevant, mainly due to the intensification of olive culture. All of these diseases have been traditionally controlled using copper products. Copper treatments are preventive. Persistence and mode of action, which prevent development of pathogen resistance, are responsible for the success of these products. Future limitation of the amount of copper applied per hectare and year in olive plantations, imposed by the European Union, will force optimization of fungicide applications, with reduction of copper doses, use of alternative active ingredients and disease prediction models. Strategies of mixing copper and systemic fungicides (e.g. triazoles and strobilurins), have allowed reductions of up to 67% the amount of copper in the control of olive leaf spot. Dodine and bentiavalicarb are also examples of alternative and efficient active ingredients against olive leaf spot. Research must be intensified to achieve effective control of anthracnose, leprosy and tuberculosis.

This research was supported by several public projects (MINECO, Junta de Andalucía) and private phytosanitary companies (including Arysta LifeScience, Basf, Bayer Crop Science, Isagro, Nufarm, Syngenta, and UPL).

Tomato defence responses to nematodes and viruses induced by ozone treatments. M.I. PRIGIGALLO, P. VERONICO, F. CILLO, M.T. MELILLO, N. SASANELLI, G. BUBICI. Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, 70126, Italy. E-mail: giovanninicola.bubici@cnr.it

Ozone is widely used as a disinfectant, and ozonated water has been known to confer some protection of plants against several biotic stresses. By applying four foliar spray treatments of ozonated water (10 ppm ozone) on tomato seedlings, i.e. two pre- and two post-inoculation with Tomato spotted wilt virus (TSWV), we observed reduction of disease incidence and severity by 20%, as well as a virus titre reduction by 80% at 19 days post-inoculation. The same treatments also reduced the number of galls induced by root knot nematode (RKN; Meloidogyne incognita) by 29%. Soil drenching with ozonated water for four consecutive days before inoculation reduced RKN gall formation by 60%, but not TSWV infection. Overall, in mock-inoculated plants, foliar sprays induced PR1b1 expression in leaves, though other salicylate- (PAL and PR-5x) or jasmonate-dependent genes (LoxD, AOS and PinI) were substantially unaffected. Soil drenching promptly enhanced transcription of PAL and PR1b1 in roots and leaves, down-regulated PR-5x and did not affect expression of LoxD and AOS. PinI was significantly down-regulated only in leaves. The impact of ozonated water applications on the expression of these genes did not correlate with that of benzothiadiazole, a known inducer of systemic acquired resistance. This demonstrates that ozonated water may protect tomato from two very different biotic stresses, especially when applied at the sites of their infection, and modulates salicylate and jasmonate pathways differently from benzothiadiazole.

This research was supported by the Fondazione Cassa di Risparmio di Puglia, Italy, within the Project ‘Risposte di difesa contro nematodi e virus indotte da trattamenti di ozono in pomodoro’.
Early assessment of late wilt of maize (Harpophora maydis) and the control effect of Lycium europaeum extracts. C. RODRÍGUEZ-MALLOL1, R. TEJ1,2, L. MOLINERO-RUIZ1. 1Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. 2Physiology and Biochemistry of Plant Response to Abiotic Stresses Unit, Faculty of Sciences of Tunis, University of Tunis El Manar, 1060 Tunis El Manar, Tunisia. E-mail: lmolinero@ias.csic.es

Maize late wilt (MLW), caused by the soilborne fungus Harpophora maydis, is characterized by the sudden appearance of symptoms from plant flowering onwards. Since genetic resistance is the most effective control method, protocols for the early evaluation of maize lines are required. As well, extracts of Lycium europaeum inhibiting the in vitro growth of H. maydis are promising as a potential control method. Three experiments were conducted under greenhouse conditions. In the first two experiments, inoculated plants were grown in different artificially colonized substrates for 6 weeks. The most disease conducive substrate was used in the third experiment, where effects of methanolic leaf extracts of L. europaeum were assessed. In the first experiment, plant development after inoculation was delayed an average of 3 d in phenological stages V4 to V5. In the second experiment, reductions of weights of above-ground parts and roots were recorded in inoculated plants compared with the controls. Necrotic lesions were apparent in the roots of the plants as early as 4 weeks after inoculation. In the third experiment, weight reductions only occurred in the plants inoculated with H. maydis. When the plants were inoculated and treated with different extracts of L. europaeum, one extract with a high chlorogenic acid content resulted in weights of roots that did not differ to those of the controls. This study has established a protocol for early evaluation of MLW, and has demonstrated bioactivity of L. europaeum against this disease.

This research was supported by the Grant P12-AGR1281 (Andalusian Government, Spain) and the European Regional Development Fund (ERDF). R. Tej was supported by the Tunisia Ministry of Higher Education and Scientific Research.

Maternal and paternal effects on the heritability of Verticillium wilt resistance in olive progenies.

P. VALVERDE, C. TRAPERO, D. BARRANCO, C.M. DÍEZ, FCO.J. LÓPEZ ESCUDERO. Departamento de Agronomía. Escuela Técnica Superior de Ingenieros Agrónomos y Montes Universidad de Córdoba. Edificio C-4, Planta Baja. Campus Universitario de Rabanales. 14071, Córdoba. Spain. E-mail: pedrovalverde@uco.es

Verticillium wilt, caused by the fungus Verticillium dahliae, is currently the most important disease of olive trees, causing important losses in olive growing countries worldwide. The use of genetic resistance is likely to be the most efficient, economically convenient and environmentally friendly control method to combat the disease, and will be a key component in integrated disease management. Twelve reciprocal crosses corresponding to all pairwise combinations were performed with the olive cultivars ‘Picual’, ‘Frantoio’, ‘Sikitita’ and ‘Arbosana’ acting as male or female parents. Additionally, the crosses ‘Koroneiki’ × ‘Arbosana’, ‘Arbosana’ × ‘Koroneiki’, ‘Arbequina’ × ‘Arbosana’ and ‘Arbosana’ × ‘Arbequina’ were investigated. The main goal was to evaluate the differential effect of the cultivars acting as mothers or fathers, in the level of resistance of the progenies to Verticillium wilt. Fruits were harvested during October and then seeds were sown and germinated in controlled conditions. Five-week-old olive seedlings were inoculated by dipping their bare root systems in conidial suspensions of the defoliating V. dahliae isolate V117. Weekly, during 10 weeks, plants were evaluated for symptom development, using a 0 to 4 scale, and every 2 weeks plant growth was also measured. The evaluated parameters presented variable values, depending on the reciprocal crosses. For instance, no differences in disease severity were recorded in the reciprocal crosses of ‘Arbosana’ and ‘Arbequina’, but differences up to 50% occurred in the reciprocal crosses of ‘Frantoio’ and ‘Sikitita’. No differences plant death were recorded in the progeny of the reciprocal crosses of ‘Arbosana’ × ‘Sikitita’, but important differences up to 40% occurred in the reciprocal crosses of ‘Sikitita’ and ‘Picual’.

Evaluation of selected soils for suppression of Fusarium diseases. M. SANTOS1, F. DIÁNEZ1, F. CARRETERO1, FJ. GEA1. 1Agronomy Department, University of Almeria, Carretera Sacramento s/n. Almería 04120, Spain. 2Departamento de Agronomía, Escuela Politécnica Superior, Universidad de Almería, Almería, Spain. E-mail: msantos@ual.es
The study of naturally occurring disease-suppressive soils has produced significant progress towards acquiring understanding of the biotic and abiotic forces that inhibit plant disease development in such soils. This research evaluated possible suppressiveness of four different soils where Fusarium diseases have low severity (disease-suppressive, S). Soils used in all experiments were collected from a commercial organic carnation production glasshouse from Cádiz (Spain) (S1, S2 and S3) and Sevilla (Spain) (S4), and were inoculated with *Fusarium oxysporum* f. sp. *melonis* (Fom; race 0) and *Fusarium oxysporum* f. sp. *niveum* (Fon; race 0), at concentration 10³ or 10⁶ cfu g⁻¹. Experimental controls of Conductive soils (C), disinfested soils (D) and vermiculite (V) were included with and without inocula. The suppressiveness of the soils to Fusarium yellow of melon and Fusarium wilt of watermelon were evaluated for 50 d. Severity of Fom was reduced by 0%, and and Fon by -50%, in comparison to inoculated vermiculite (*P* < 0.05). The most suppressive soil was S4 for both pathogens. The growth of both pathogens in S-soils was suppressed compared with C soils and V, which suggested that S-soils displayed greater fungistasis than C-soils or V. The suppressiveness to the pathogen was reduced in D-soils. The suppressiveness to the pathogen was therefore likely to be due to microorganisms colonizing the roots grown in S-soils, and the suppression may be due to antagonistic microorganisms.

**Reaction of some watermelon varieties to the races of Fusarium oxysporum f. sp. niveum.** B. GEÇİOĞLU-ERİNCİK, 1 M.T. DÖKEN, 2 Adnan Menderes University, Koçarlı Vocational School, Aydın. 2 Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection, Aydın. E-mail: bgerin cik@adu.edu.tr

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (Fon), is one of the major limiting factors for watermelon production. In recent surveys, this disease has been found to be widespread in the Province of Aydın. One of the best control measures against the disease is the use of resistant cultivars. The watermelon varieties (Crimson Sweet, Crimson Tide, Galaxy, Wonder and Anthem F1), commonly grown in the Aydın Province, were tested against the three races (Race 1, 2, and 3) of Fon. Root dip inoculation was used, and the experiment was conducted in a growth chamber. The reaction of each watermelon variety differed, depending on the race of the pathogen. Cultivar Wonder exhibited the least disease severity, ranging from 9% to 35% depending on the races. Crimson Sweet was the most susceptible cultivar, developing up to 78% disease severity.

This research was supported by the Scientific Research Fund of Adnan Menderes University through the project no: ZRF-12011.

**Identification of optimal cereal/legume combinations for Mediterranean rainfed farming systems.** A. VILLEGAS-FERNANDEZ, A. AMARNA, D. RUBIALES. Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: diego.rubiales@ias.csic.es

Modern agriculture based on monocultures suffers from a lack of biodiversity. Increasing the diversity of crop systems offers opportunities to improve yield stability, reduce pest and disease damage, and to enhance stress resilience in agricultural systems. This diversification should be adapted to each situation, considering a wide range of factors from the specific crop to local weather conditions. In an attempt to optimize cereal/legume combinations for Mediterranean rainfed farming systems, two field experiments were established at Córdoba in growing season 2016/2017 that will be extended to other areas in 2017/18. Crop combinations of wheat/Faba bean and barley/pea were tested, respectively, in the two experiments. Mixed intercropping was chosen, with a 50/50 proportion of cereal/legume. Two varieties for each crop were included. A split-plot experimental design was adopted, with main plots being management (two levels: conventional, i.e., applying the normal levels of fertilizers and pesticides; and low-level, with limited application of fertilizers and pesticides), and subplots being crop combination (including all possible combinations of the cereal and legumes varieties, as well as monocrops). Plot size was 3 × 4 m. Evaluations will include a wide number of parameters, including germination rates, vegetative biomass, weed biomass, plant heights, ground coverage, yields and disease incidence.

This research was supported by DIVERSify project of European Union’s Horizon 2020 research and innovation program, under agreement No. 727284.
Alternative seed treatments as a substitute for chemical seed treatments to control common bunt of wheat. M. NOCENTINI, T. CINELLI, C. COMPARINI, S. BENEDETTELLI, L. MUGNAI. Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: marconoce@gmail.com

In recent years, the cultivation of ancient wheat varieties has been relaunched, due to their high nutrient and protein composition linked to low gluten content, and good organoleptic characteristics, mainly through the work of organic small-scale farmers. For organic production the seed cannot be treated with synthetic chemicals. This created a serious issue, due to the difficulty to obtain organic seed free from common blunt spores, and all the issues related to “homemade” treatments. For the above reasons, common bunt (caused by Tilletia sp.) became a major seed- and soil-borne disease for organic wheat producers. This study investigated alternative control measures to chemical seed treatments, that are environmentally friendly to support cultivar resistance, easy to use and can be applied on small farms. Following good results obtained in vitro, against the germination of Tilletia teliospores, several organic products were used in vivo on seed of an ancient bread wheat cv. Sieve, artificially inoculated with Tilletia teliospores. The products being tested are: monoglycerides, Sinapis alba flour, Pseudomonas chlororaphis, copper complexed with a carrier, copper and zinc mixture complexed with citric acid, and peracetic acid. These products were compared with a traditional copper formulation registered for seed treatment, and two synthetic chemical products, one based on fludioxonil and the other a mixture of sedaxane, fludioxonil and difenoconazole. The results obtained in in vitro and in vivo trials will be presented.

Development of integrated disease management of fire blight using biocontrol agents and plant defense activators. S. AIT BAHADOU, M.A. BOUKHARI, A. OUIJJA, I.2, R. TEJ1, 3. Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. 2 Multidisciplinary Center of Technological Research (CEMIT), General Direction of Scientific and Technological Research (DGICT), National University of Asunción (UNA), Mecal. Estigarribia Km 10,5, 2169 San Lorenzo, Paraguay. 3 Physiology and Biochem-

The bacterial antagonists Bacillus subtilis GB03, B. subtilis QST713, B. subtilis Y1336 and Pantoaea agglomerans P10c, and plant defense activators acibenzolar-S-methyl (ASM), fosetyl aluminium (F-Al), potassium phosphites (PH) and prohexadione-Ca (ProCa) were evaluated individually and in combinations for control of fire blight in Morocco. Under laboratory conditions, on detached blossoms of apple and pear, only biocontrol treatments based on P. agglomerans P10c and its mixture with B. subtilis QST713 showed reduced the incidence of the disease when compared to other treatments. Under field conditions, the above mixture of biocontrol agents, as well as all other strains, were tested alone or combined with plant defense activators, using a split-split-plot trial design. The treatments were applied on trees at timings based on their respective modes of action. Results showed that P. agglomerans P10c reduced blossom infection by 66%, B. subtilis QST713 by 64%, their 1:1 mixture by 62%, B. subtilis GB03 by 64%, and B. subtilis Y1336 by 53%. For the plant defense activators this reduction was 62% for ASM, 57% for ProCa, 50% for F-Al, and 49% for PH. On shoots, disease reductions ranged from 40% to 80% for the biocontrol agents, and 46% to 97% for the plant defense activators. Two applications of ProCa was the most effective treatment for reducing shoot blight incidence. The combination of plant defense activators and biocontrol agents allowed the greatest protection against blossom and shoot blight, ranging from 76% to 98%. The greatest protection was resulted from B. subtilis QST713, P. agglomerans P10c or their mixture combined with ASM or ProCa.

Harpophora maydis affecting maize in Southern Europe: different growth media, long-term storage and in vitro effects of extracts of Lycium europaeum L. C.M. ORTIZ-BUSTOS, Y. MONGELÓS, R. TEJ1, 3, L. MOLINERO-RUIZ. 1 Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. 2 Multidisciplinary Center of Technological Research (CEMIT), General Direction of Scientific and Technological Research (DGICT), National University of Asunción (UNA), Mecal. Estigarribia Km 10,5, 2169 San Lorenzo, Paraguay. 3 Physiology and Biochem-
Late wilt of maize is a vascular disease caused by the soilborne fungus *Harpophora maydis*. The disease is frequent in warm climates where high temperatures occur at maize crop flowering and tasseling stages. Genetic resistance is the most effective control method, but intermediate reactions of resistance highly dependent upon environmental conditions are frequent. Complementary and alternative control strategies are needed within integrated pest management programmes. The genus *Lycium* (Solanaceae) is well known as a herbal medicine with broad biological activities including antimicrobial effects. We selected the most appropriate culture media for *H. maydis* and evaluated different methods for its long-term storage. We also assessed in vitro effects of water and/or methanol extracts from *L. europaeum* on the pathogen. *Harpophora maydis* did not grow on acidified corn meal agar (CMAa). Greatest growth was recorded on lactic acid-potato dextrose agar (PDAa) and CMA, but fungal sporulation on CMA was less than on both PDA or PDAa. Glycerol and a sterilized soil mixture were appropriate to maintain viability of the fungus for at least one year. Maintenance at room temperature favoured mycelial growth. Four methanol extracts from *L. europaeum* (two from leaves and two from stems) reduced growth of *H. maydis*. The greatest antifungal effect was from an extract with the greatest total phenolic content, particularly with a large content of chlorogenic acid.

This research was supported by the Grant P12-AGRI281 (Andalusian Government, Spain) and the European Regional Development Fund (ERDF). Y. Mongelós was supported by Consejo Nacional de Ciencia y Tecnología, CONACYT (Paraguay), and R. Tej by the Ministry of Higher Education and Scientific Research (Tunisia).

Chemical composition and antifungal activity of essential oils from two *Labiatae* species. N. ISSIAKHEM-TAMDA1, F. DAVIS2, M. HAZZIT3, M. AMIALI4, N. ZERMANE1, 1Agricultural National High School - El-Harrach-Algeria-Laboratory of Plant Physiology, Department of Botany, Avenue Hassan Badi, El Harrach-16004, Algiers, Algeria. 2School of Chemistry, Food Biosciences & Pharmacy, University of Reading, UK. 3Agricultural National High School, El-Harrach, Algeria, Laboratory of Chemistry, Department of Technology. E-mail: issiakhem.nadia@gmail.com

The excessive use of synthetic chemicals to reduce the severity of pests and diseases in agriculture has generated pesticide resistance to some of the active ingredients, from selection pressure due to high doses and continuous applications, causing important economic losses. Consequently, it is important to examine alternative control strategies, such as using natural products from plant origin which are unlikely to adversely affect the environment and human health. Analysis and identification were undertaken of essential oils hydrodistilled from aerial parts of the Lamiaceae aromatic plants, origanum (*Origanum floribundum Munby*) and spearmint (*Mentha spicata* L.), using gas chromatography and mass spectroscopy. Their antagonistic activity was evaluated against pathogenic fungi isolated from legume crops, including *Botrytis* sp., *Fusarium* sp., *Alternaria* sp., and *Ascochyta* sp.. The major components of the oregano oil were carvacrol, thymol and p-cymene, and of the spearmint oil were carvone, limonene and eucalyptol. Mycelia discs taken from the margins of 7-d-old cultures were placed in the middle of PDA plates together with 1, 3 and 5 μL of essential oils (applied individually) added on 5 mm sterile Whatman paper discs placed in the middle of each Petri dish cover. The Petri dishes were sealed with parafilm and incubated in darkness at 25°C for 8 d. Control plates were treated with the same amount of sterile distilled water. Oregano essential oils showed the greatest inhibition activity, and causing greater than 70% reduction of the mycelial growth for the three concentrations of essential oil. The spearmint oil was less active with the greatest inhibition activity against mycelia growth obtained with 5μL (about 32% of growth reduction).

Evaluation of microbial antagonists and siderophore production in the tomato phyllosphere as bio-control agents. F. DIÁNEZ1, J. YAU2, M. SANTOS1. 1Departamento de Agronomía, Universidad de Almería, Carretera Sacramento s/n, Almería 04120, Spain. 2National Agriculture Research Institute of Panamá, Buildings 161, 162, Knowledge City, Clayton., Carlos R. Lara Street, Panama Republic. E-mail: msantos@ual.es

Microbial antagonists from phyllospheres of healthy greenhouse-grown tomatoes were isolated and
evaluated for their effectiveness in inhibiting mycelial growth of pathogenic fungi in dual culture and detached leaf assays, and for their siderophore production. A total of 63 bacterial and 68 fungal isolates were isolated from the tomato leaves and their antagonistic activity was evaluated in dual culture assay. The isolates inhibited mycelial growth against Botrytis cinerea (41% reduction), Fusarium oxysporum f. sp. lycopersici (66%), Fusarium oxysporum f. sp. radicis-lycopersici (33%), Mycosphaerella pinodes (53%), Phytophthora parasitica (47%), Pythium aphanidermatum (37%) and Verticillium dahliae (83%). To evaluate the antagonistic activity against B. cinerea, a total of 22 fungal and three bacterial isolates were tested in a detached leaf assay. Five of 22 fungus isolates prevented mycelial growth when compared to untreated controls when these were observed at ×40 magnification. Nineteen 21 fungus isolates produced siderophores, as indicated by CAS assays. These results indicate that organically-grown plants could be a good source of potential biocontrol agents. The future use of biological–chemical combinations, of endophytes in combination with commercial pesticides applied to the seeds or seedlings, could give synergistic effects on one or multiple disease-causing agents.

Evaluation of E.M., Zeolite and Agri-fos 600® to control Verticillium wilt. C. LAGOGIANNI¹, K. SOTIROPOULOS¹, A. KOULOUVARI¹, G. ZAKYNTHINOS², D.I. TSITISGIANNIS². ¹Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. ²Department of Food Technology Educational Institute (A.T.E.I.) of Kalamata, Kalamata, Greece. E-mail: dimtsi@aua.gr

Induced resistance is part of plant immune systems developed after the stimulation of resistance mechanisms, resulting from non-pathogenic microorganisms or chemical inducers. This study evaluated the non-pathogenic beneficial microorganism formulation E.M. and the chemical inducers zeolite and Agri-fos 600®, to induce resistance mechanisms and control Verticillium wilt. E.M. is a formulation based on Effective Microorganisms, and is used to improve the quality-fertility of soil and the growth-quality of crops. Zeolite is a microporous, aluminosilicate mineral used as a commercial adsorbent and catalyst, and as a soil improvement substance. Agri-fos 600® is a formulation of potassium phosphonate anions that induce plant defense mechanisms. Pathogenicity experiments were performed in Arabidopsis thaliana, tomato and eggplants infected with Verticillium dahliae. Zeolite, E.M. and Agri-fos 600® were applied as root drenches. Virulence assays in greenhouse experiments showed that only zeolite and Agri-fos 600® reduced Verticillium wilt in tomato and A. thaliana, by 5-20%, but did not have any effect on eggplant. In contrast, E.M. reduced the disease only in eggplants by 20%. E.M. and zeolite were also evaluated in field experiments in naturally infested soil, where zeolite reduced Verticillium wilt in tomato plants by 25%, and E.M. by 45%. Quantification of Verticillium microclerotia in soil showed that their numbers were reduced by 15% after treatment with E.M. compared to untreated control plants.

Plant pathology and food safety

Identification and characterization of Acidovorax citrulli strains from Serbia. N. ZLATKOVIĆ¹, A. PROKIĆ¹, K. GAŠIĆ², N. KUZMANOVIĆ³, M. IVANOVIĆ³, Ž. PAVLOVIĆ³, A. OBRADOVIĆ³. ¹University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Department of Plant Pathology, Nemanjina 6, 11080 Belgrade, Serbia. ²Institute for Plant Protection and Environment, Department of Plant Pathology Teodora Dražera 9, 11000 Belgrade, Serbia. ³Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany. E-mail: nevena_blagojevic@yahoo.com

In August 2014, typical bacterial fruit blotch symptoms were observed on mature watermelon fruit originating from fields in the Vojvodina province of Serbia. In the summer 2015 and 2016 we registered two more occurrences of the disease, in, respectively, the east and west areas of the country. White, glistening, convex and circular colonies with regular edges were predominantly isolated from diseased watermelon fruit collected from the affected fields. A total of 33 bacterial strains were subjected to further analyses. They were Gram-negative, aerobic, oxidase and catalase positive, nonfluorescent, and did not produce potato soft rot. All but two strains (KFB
whether the pathogen will survive from season to season in Serbian climatic conditions.

This study was supported by the project III46008, financed by the Ministry of Education, Science and Technological Development, Republic of Serbia.

Identification of pathotypes and analysis of the genetic structure of *Fusarium oxysporum* f. sp. *lentis* populations. H.R. POURALIBABA1, Z. SATOVIC2, M.J. COBOS3, D. RUBIALES3, S. FONDEVILLA3. 1 Dryland Agricultural Research Institute, Education and Extension Organization (AREEO), Maragheh 119, Iran. 2 Faculty of Agriculture, Department of Seed Science and Technology, Svetosimunska 25, 10000 Zagreb, Croatia. 3 Institute for Sustainable Agriculture, CSIC, 14004 Córdoba, Spain. E-mail: hpouralibaba@ias.csic.es

Lentil cultivation is threatened worldwide by Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lentis* (Fol). Knowledge on pathogenic diversity and genetic structure of Fol populations is fundamental for breeding for resistance and managing the disease. We therefore studied virulence diversity within a collection of Fol isolates. Twenty-eight resistant lentil accessions were inoculated with six Fol isolates from different geographical origins. The lentil accession × Fol isolate effect was highly significant, which allowed four accessions to be selected as a differential set. Inoculation of this set with 48 Fol isolates from Iran, Syria and Algeria, allowed the identification of seven different virulence patterns, designated pathotypes 1 to 7. In addition, the genetic structure of this Fol collection was analyzed using twelve SSR markers, eight of which were designed in this study.

Screening of mycotoxin profile and mycotoxicin gene clusters in toxigenic fungi of food crop plants reveals phenotypic and genetic variability at intraspecific levels. A. SUSCA, A. LOGRIECO, M. HAIDUKOWSKI, A. VILLANI, A. MORETTI. Institute of Sciences of Food Production, Italian National Research Council (ISPA-CNR), Via Amendola 122/O, 70126, Bari, Italy. E-mail: antonio.moretti@ispa.cnr.it

There is concern regarding occurrence of toxigenic fungi on food and feed crops, since mycotoxin accumulation in the final products represents serious risks for human and animal health. Among the plant pathogens that produce mycotoxins in planta, *Aspergillus* and *Fusarium* spp. are the most common, and show great variability of their mycotoxin profiles, even in closely related species or at intraspecific levels. We report here results from studies conducted using HPLC/FLD and LC-MS/MS measurements and whole genome sequencing: i) variability of Ochratoxin A (OTA) production related to the occurrence of the ota gene cluster in the *Aspergillus niger* clade, where both the intact and deleted clusters coexist; ii) variability of beauvericin (BEA) production and occurrence of the BEA gene cluster in *Fusarium subglutinans* and *F. temperatum*, two phylogenetic sister species where toxigenic potential is not related to real production capacity in vitro; iii) variability of fumonisin production and FUM gene cluster occurrences in *F. proliferatum* isolated from fig and maize, two populations with the same toxigenic potential but different fumonisin production capacity; iv) variability of trichothecene production in the *F. equiseti* *incarnatum* species complex and related variability in the trichothecene gene cluster. Taken together, these data show that mycotoxin gene clusters can differ even within a single species, or among very closely related species. The lack of a given mycotoxin production, at least in in vitro conditions, is frequently, but not always, related to the absence of gene clusters.

This research was supported by the Project MycoKey.
AMOVA showed that there was large molecular variation within groups but also between groups, showing that the Iranian populations were different from non-Iranian populations. STRUCTURE and Fitch-Margoliash tree analyses concluded the presence of two ancestral Fol lineages, one distributed in all regions while the other was only present in Iran. Our results suggest that Iran could be the origin of the diversity demonstrated in this study.

This research was supported by the PhD educational mission No. 3972/200-28/1/1389 of first author, from AREEO and AGL2014-52871 co-financed by FEDER.

Comparison of microbial quality of lettuce grown under three crop systems in Cyprus. C. MENEL-AOS CHRISTODOULOU, E. SAVVA, D. TSALTAS. Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology. E-mail: dimitris.tsaltas@cut.ac.cy

Leafy greens, which are usually consumed raw, are increasingly recognized as important vehicles for transmission of foodborne pathogens. Contamination with pathogenic bacteria can occur at different stages of the production and distribution chain, making food safety of vegetables an important priority. Lettuce is the most commonly consumed vegetable worldwide, growing in close proximity to soil, while rain and irrigation water facilitate microbial movement and contamination. It is of scientific and public interest to explore how different production systems affect the presence of foodborne pathogens, since there is skepticism about safety of organic produce, hydroponics is increasing, aquaponics is a new appealing production system, probably due to specific practices.

Genetic diversity of Botrytis cinerea between tomato greenhouses in Northern Algeria. A. ADJEBLI1, C. LEYRONAS2, K. AISSAT3, P.C. NICOT2. 1Laboratoire d’écologie Microbienne, Faculté des Sciences de la Nature et de la Vie, Université Abderrahmane Mira, Bejaia 06000, Algérie. 2INRA, UR407 Pathologie Végétale, Domaine St Maurice CS 60094, F-84143 Montfavet Cedex, France. E-mail: ahmed.adjebli@univ-bejaia.dz

To estimate the genetic diversity for a better understanding of the spread of Botrytis cinerea, we genotyped with nine microsatellite markers 174 isolates collected from four greenhouses during three growing seasons in the region of Bejaia. Four of these isolates were identified as Botrytis pseudocinerea according to the allele size at locus Bc6. For all other isolates further studied, all loci were polymorphic, with the mean number of alleles per locus ranging from 2.77 to 5.22. Considerable genetic variability was detected in all subpopulations (D* > 0.87; Hnb > 0.40). Based on standardized index of association analysis, significant but low levels of clonality occurred, not excluding the possibility of recombination (Rd = 0.07, P < 0.001). A total of 109 haplotypes were characterized among the isolates, few of which were shared between subpopulations. This, together with moderate genetic differentiation among subpopulations according to the geographical origin (0.080 < Fs < 0.167), suggested a low level of inoculum exchange among greenhouses, and little carry-over of inoculum from one sampling season to the next. The importance of genetic structure of B. cinerea populations should be taken into consideration for management of grey mould in tomato greenhouses.

Postharvest fungal diseases of loquat cv. ‘Algerie’ in Spain. L. PALOU, P. SÁNCHEZ-TORRES, C. MONTESINOS-HERRERO, V. TABERNER. Laboratori de Patologia, Centre de Tecnologia Postcollita (CTP), Institut Valencià d’Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain. E-mail: palou_llu@gva.es
Spain is the second largest producer and the greatest exporter of Japanese loquat (Eriobotrya japonica (Thunb.) Lindl.) for fresh consumption. More than 50% of the cultivated area is in Alacant province (SE of Spain), where approx 98% of total production is of loquat cv. ‘Algerie’, which is mainly exported to European Union (EU) markets. For two consecutive seasons, commercially grown ‘Algerie’ loquats from two orchards were assessed for disease caused by latent and wound pathogens. Selected healthy fruit were either surface-disinfected or artificially wounded in the rind and incubated in humid chambers at 20°C for up to 5 weeks. Additionally, disease was also assessed on commercially handled fruit (manually selected and packaged) stored at 5°C for up to 12 weeks; no loquat postharvest treatments are currently authorized in the EU. Isolated fungi were incubated on potato dextrose agar (PDA) plates at 25°C for purification and subsequent morphological and molecular identification. Pathogenicity of common isolates was demonstrated by fulfilling Koch’s postulates. Disease development was assessed on artificially inoculated loquats stored at either 20 or 5°C. Regardless of the type of infection and postharvest fruit management, the most frequent postharvest diseases were black spot, caused by Alternaria alternata, and blue mold caused by Penicillium expansum. In addition, gray mold, caused by Botrytis cinerea, was frequently observed on both artificially wounded and commercially handled fruit, whereas anthracnose, caused by Colletotrichum gloeosporioides, was frequently observed on surface-disinfected loquats. Other minor pathogens that were found causing latent infections, especially in the fruit stem-end, were Pestalotiopsis clavispora and Diplodia seriata.

This research was supported by the Project AGL 2004-05271/AGR funded by the Spanish MICINN and the European Union (FEDER Program).

The severe threat for sweet cherry production in Turkey: identification of causal agents of bacterial canker. H. ÖZAKTAN1, M. AKBABA1 1University of Ege, Faculty of Agriculture, Department of Plant protection, Bornova, İzmir, TURKEY. E-mail: hatice.ozaktan@ege.edu.tr

Turkey is the world’s greatest producer of sweet cherries (Prunus avium L.), with production of 400,000 metric tons per year. An old but suitable for export variety, ‘0900 Ziraat-Salihli’, played the main role in this industry. Cherry production in Turkey has been threatened by emerging and increasingly severe losses due to bacterial canker, caused by Pseudomonas syringae pathovars. This study identified fluorescent Pseudomonas isolates originating from different symptomatic tissues of sweet cherry trees from the Aegean Region in Turkey. Identification of bacterial canker causal agents was on isolation on microbiological media, phenotypic features of bacteria, including pathogenicity tests on immature fruit, and molecular diagnosis of toxins produced (yercinibactine, coronatine, syringomycin). Eleven of 16 fluorescent Pseudomonas isolates were identified as Pseudomonas syringae pv. syringae (Pss), four as P. syringae pv. morsprunorum race 2 (Psm2), and one as Pseudomonas viridiflava. The pathogenicity tests divided the tested strains into two groups: one including isolates causing black-brown necrosis, and the second of isolates inducing water-soaked superficial lesions. Phenotypic and genetic studies on toxin production showed that all Pss isolates produced syringomycin (syrb gene). However, Psm2 isolates did not show evidence for the coronatine production gene (cfl gene). All pathogenic isolates were also subjected pathogenicity test on micropropagated sweet cherry plantlets, using different inoculation methods. The best inoculation technique for production of typical disease symptoms was dipping non-wounded plantlets in bacterial suspensions.

Diversity of culturable bacteria in Spanish Pleurotus eryngii crops. A.J. GONZÁLEC1, E. TRAPIEL-LO1, M.J. NAVARRO2, F.J. GEA1. 1Laboratorio de Fitopatología, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Carretera de Oviedo s/n, 33300 Villaviciosa, Asturias, Spain. 2Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain.

Pleurotus eryngii, king oyster mushroom, is one of the most valuable cultivated mushroom species in Spain, where it is grown on sterilized substrates. Several bacterial species have been described as causing diseases on edible mushrooms, such as brown blotch or internal stipe necrosis. This study identified bacteria present on P. eryngii fruit bodies harvested from several mushroom growing farms located in Castilla-La Mancha.
Mancha (Spain), and assessed their diversity. From the ten batches of samples analyzed, 39 isolates were obtained. Classical and molecular techniques were used for identification. The phenotypic tests carried out were: Gram, fluorescence under UV light, oxidation/fermentation of glucose and the presence of cytochrome c-oxidase. The LOPAT scheme (levan, oxidase, pectinolysis of the potato, hydrolysis of arginine, hypersensitivity in tobacco leaves) was applied to isolates of the genus *Pseudomonas*. In addition, the 16S rDNA was amplified and sequenced in at least one of the directions and the sequences obtained were compared to those deposited in databases by BLAST. Only 18% of the isolates were Gram positive. Among Gram negative bacteria, *Pseudomonas* was the best represented with 22 isolates, only one of which was non-fluorescent. Among the identified *Pseudomonas* spp., the most relevant were *P. tolaasii* and *P. azotoformans*. Other genera present were *Acinetobacter, Providence, Brochotrix, Myroides, Lactococcus*, and *Wautersiella*.

This research was supported by the Regional Government of the Principado de Asturias and Diputación de Cuenca.

Use of natural products to control key pathogens of typical Mediterranean crops. A. LA TORRE, G. PASCALI, L. RIGHI, S. BERTIN, V. BATTAGLIA. Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria - Centro di ricerca Difesa e Certificazione (CREA-DC), Via C. G. Bertero 22, 00156-Rome, Italy.

This study evaluated the use of essential oils for control of *Plasmopara viticola* and *Fusarium oxysporum* f. sp. *lycopersici*, two important pathogens of, respectively, grapevine and tomato. Clove oil (*Eugenia caryophyllata*) and BIOXEDA formulation containing 20% (w/w) of clove oil (Xeda International S.A.) were tested *in vitro* and *in vivo*. The *in vitro* tests consisted of evaluating the development of *P. viticola* on grapevine leaf discs, or *F. oxysporum* f. sp. *lycopersici* on agar medium supplemented with the tested products at various concentrations. In addition, spore germination was determined after using the products at different concentrations. The *in vivo* tests evaluated activity of the products against grape downy mildew in open field, and *F. oxysporum* f. sp. *lycopersici* on tomato plants in a greenhouse. *In vitro* tests showed inhibitory activity of the products on mycelial growth and spore germination. *In vivo* tests revealed effectiveness of products although they were not as effective as a reference product. This study suggests that application of essential oils could to reduce the use of synthetic pesticides in agriculture, in accordance with European laws, and avoid the environmental pollution.

Seasonal distribution of *Apple mosaic virus* in infected apple and hazelnut tissues. A.BALTACI¹, F.ERTUNC². ¹Blacksea Agricultural Research Institute, Samsun, Turkey, ²Ankara University, Faculty of Agriculture, Department of Plant Protection 06110 Ankara, Turkey. E-mail: ertunc@agri.ankara.edu.tr

This research was carried out to detect the seasonal variation of *Apple mosaic virus* (ApMV) on apple and hazelnut tissues by DAS-ELISA and RT-PCR which the coat protein region was targeted. Tissues were sampled between March – January 2011 and five ApMV infected apple trees (all were Granny Smith variety) and three local hazelnut varieties (Foşa, Yassı badem and Mincane) were selected as five replicates in Hazelnut germplasm culture collection in Giresun. Shoots, leaves, male and female flowers, fruits and husk tissues were collected from infected hazelnut trees, shoots, leaves, flowers and fruits were collected from apple trees according to their set. *Apple mosaic virus* was detected in the shoots and leaves of apple trees collected in May, June and July by DAS-ELISA and similar results were also obtained by RT-PCR. The virus was present in shoots, leaves and female flowers of hazelnut especially collected in April, May, June and very reduced amount in July detected by DAS-ELISA. When the hazelnut tissues analyzed by RT-PCR, the tissues collected in March were also positive for the presence of ApMV. The remaining collected tissues were all negative in RT-PCR and DAS-ELISA, therefore, according to our results, May was the most suitable time for apple sampling while the April was found the most suitable period for hazelnut collection for the detection of ApMV.

Distribution, molecular detection and characterization of corn viruses in Turkey. K. DEGIRMENCİ¹, F. ERTUNC². ¹Ankara University Natural and Applied Sciences Institute. Ankara University, Faculty of Agri-
Leaf samples bearing virus-like symptoms of systemic mosaic, and asymptomatic leaves, were collected in 2010 and 2011 from Bartın, Düzce, Sakarya and Zonguldak provinces of the Blacksea region, and were assessed, using DAS-ELISA tests, for virus infections. The samples were tested for *Maize dwarf mosaic potyvirus* (MDMV), *Sugar cane mosaic potyvirus* (SCMV), *Barley yellow dwarf luteovirus* (BYDV and MAV strains) (ByYDV), *Maize stripe tenuivirus* (MSTV), *Maize mosaic rhabdovirus* (MMV), *Maize whiteline mosaic aureusvirus* (MWLMV), *Johnson grass mosaic potyvirus* (JGMV), *Wheat streak mosaic tritinnovirus* (WSMV), *Barley stripe mosaic hordeiviruses* (BSMV), *Maize chlorotic mottle machlomovirus* (MCMV) and *Cucumber mosaic virus* (CMV), A total of 424 plant samples were collected from the research area. Of the samples, 171 were infected by one of the above viruses and 60 had double or triple virus infections. No viruses were detected in 193 samples. MDMV was widespread, followed by BYDV-MAV, BYDV-PAV and MMV. Infection rates were 43% in Bartın, 52% in Düzce, 38% in Sakarya and 27% in Zonguldak. The other virus infections were rare in the research area. The main infection in maize cobs and seeds were MDMV and MMV. RNAs of infected leaves were isolated and subjected to RT-PCR amplification for MDMV, MMV, BYDV-PAV and MAV, and 336 bp, 457 bp, 320 bp, 320bp amplified products were obtained. Thirty-five MDMV isolates were sequenced and the sequences were deposited in the NCBI database, and phylogenetic analysis were performed. Our isolates were closely related to European MDMV isolates.

This research was supported by the Tagem Project funded by Ministry of Food, Agriculture and Animal Husbandry of Turkey.

Detection of resistance in corn varieties by molecular markers. K. DEGIRMENCI¹, F. ERTUNC². ¹Ankara University Natural and Applied Sciences Institute. ²Ankara University, Faculty of Agriculture, Department of Plant Protection, 06110 Ankara, Turkey. E-mail: ertunc@agri.ankara.edu.tr

*Maize dwarf mosaic virus* (MDMV) is the most important and widespead virus infection of maize in Turkey. In order to determine the genetic susceptibility of our corn varieties, one isolate of MDMV was selected according to symptom expression and virulence, and tested on 88 local maize varieties, ten commercial varieties, two resistant(D21 and D32) and two susceptible (Dcmv 1145 and D408) for resistance against MDMV. Field trials were organized as randomised block designs with three replicates and six plants in each plot. DNA was isolated from the infected plant leaves. Two CAPS markers (Pic 13L2 and Pic 19LX) and one indel marker (M12) were used for the detection of Scmv1 and Scmv2 resistance genes. Amplified products of Pic 13L2 CAP marker were digested with RsaI and the others were digested with NlaIII. From symptom expression of the plants and results of the molecular amplifications, four self-pollinated maize lines, M112, M100, M108 and M 96 were shown to be resistant to MDMV. The Scmv1 gene was present in nine samples and the Scmv2 gene was detected in five samples.

This research was supported by the Tagem Project, funded by Ministry of Food, Agriculture and Animal Husbandry of Turkey.

Development of a biocontrol agent against *Fusarium* spp. using culture media fermented by lactic acid bacteria. C. LUZ¹, F.B. LUCIANO², J. MAÑES³, G. MECA¹. ¹Laboratorio de Química de los Alimentos y Toxicología de la Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, España. ²Departamento de ciencia animal, Escola de Ciències de la Vida, Pontificia Universidad Católica do Paraná, Rua Imaculada Conceição 1155, 80901-215 Curitiba, Paraná, Brasil.

Bioconservation is a biotechnological application that promotes shelf-life extension and food safety using microorganisms or their metabolic products. Some LAB strains produce low molecular weight compounds related to phenolic acids, with important antifungal activities. Provided LAB are food grade organisms and comply with the “Qualified Presumption of Safety” (QPS) introduced by the “European Food Safety Agency” (EFSA), they have considerable potential as biopreservatives in food applications. Reduction of food spoilage caused by mycotoxinogenic fungi is one of the main problems in food security. Seven strains of lactic acid bacte-
YERBES 
Alicante s/n - 46460 Silla, Valencia, España. 
Fitopatológico-Virología, Generalitat Valenciana, Av/ de 
ráneo, Universitat Politècnica de València, Cno. Vera s/n.

GARCÍA 
Laguna, Tenerife, España. 
Carretera del Boqueron s/n Valle de Guerra, 38270 La 
rio de Sanidad Vegetal Dirección General de Agricultura

BLANCO 
Ctra, General del Norte, km 7,2, Cardones, 35415 Aru

SO 
BLANCO, 35415 Aru

RUBIO 
RUBIO, 35415 Aru

M.I. FONT-SAN-AMBROSIO 
Detection of mycotoxin management in the food and feed chain” GA

This study was supported by the Project for Emerging Re
searchers of the Generalitat Valenciana (GV-2016-106), and the European Project H2020-Research and Innovation Ac
This research was supported by the Project INIA E-
RTA2014-00010-C02-02 (Instituto Nacional de Investi
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Effects of the co-infection of Pepino mosaic virus and Southern tomato virus on tomato plants. E.
SANAHUJA1, A. ALFARO-FERNÁNDEZ1, L. EL-

Southern tomato virus (STV) is a member of the new genus Almalgavirus (family Almalgaviridae), with a 3.5 kb double stranded RNA (dsRNA) genome containing two partially overlapping open reading frames (ORFs), coding for the putative coat protein gene and with typical motifs of the RNA-dependent RNA-polymerase (RdRp). This virus is related to families Totiviridae and Partitiviridae. STV is efficiently

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SANAHUJA1, A. ALFARO-FERNÁNDEZ1, L. EL-
Southern tomato virus (STV) was detected in tomato plants showing stunting, fruit discolouration and size reduction, and also in symptomless plants. This virus is efficiently seed transmitted and its role in the tomato infected plants is currently unknown. We evaluated effects of STV in single and mixed infections with Pepino mosaic virus (PepMV) in affected tomato plants. The assay consisted of four different combinations: single STV and PepMV infected plants, plants co-infected with PepMV and STV and non-infected plants (four plants of each treatment). Plants were grown a growth chamber, and different parameters were evaluated, including; time for symptom development, symptom severity index, virus concentration and plant biomass. All of the plants infected with PepMV developed symptoms. However, in plants co-infected with STV symptoms appeared 15 d later. Plants infected only with STV did not develop any symptoms during the assay. The co-infected plants presented greater biomass at the end of the assay than those with single infections of either PepMV or STV, and were similar to those of the uninfe cted plants. The concentration of STV remained almost constant during the assay, and PepMV concentration was greater at 15 d after infection and decreased in following evaluations, regardless of the single or mixed infections in the plants. These results indicate that co-infection of PepMV and STV could improve the development of tomato plants compared to those only infected with PepMV or STV, with similar biomass to the non-infected plants. Further studies are being undertaken to confirm these results.

This research was supported by the Project INIA E-RTA2014-00010-C02-02 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain).

Identification and mycotoxigenic ability of Aspergillus spp. associated with black rot of pomegranate fruit. A. SUSCA¹, L. KANETIS², M. HAIDUKOWSKI¹, A. VILLANI¹, S. TESTEMPASIS³, S. SAMUEL⁴, A. LOGRIECO⁵, G. KARAOGLANIDIS⁶. ¹Istituto di Scienze delle Produzioni Alimentari. Consiglio Nazionale delle Ricerche, via Amendola 122/O, 70126 Bari, Italy. ²Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603, Limassol, Cyprus. ³Aristotle University of Thessaloniki, Department of Agriculture, Plant Pathology Laboratory, 55132, Thessaloniki, Greece. ⁴Department of Agriculture, Rural Development and Environment, 1412, Nicosia, Cyprus. E-mail: antonella.susca@ispa.cnr.it

Due to their nutritional value pomegranate is a rapidly expanding crop with promising prospects, consumed mainly as fresh fruit, juices and jams. Pomegranate fruit rots contribute significantly to crop losses, with black rot (caused by Aspergillus spp.) being a common disease. Black rot damages external fruit surfaces, resulting in fungal invasion of arils that are covered by spore masses of black aspergilli (Aspergillus section Nigri). This fungus group is considered the main source of ochratoxin A (OTA) contamination in numerous food commodities, and species of the section have been reported as fumonisin (FB) producers. Therefore, black rot may not only reduce yield, but also deteriorate products due to mycotoxin production, and compromise consumer health safety. Our purpose was to identify black aspergilli associated with pomegranate fruit rots and investigate their mycotoxin capacities. Thirty-seven Aspergillus spp. isolates from pomegranate fruit showing black rot symptoms were collected from Greece, Cyprus and Italy. Species identification was performed at three genetic loci, beta-tubulin, calmodulin and translation elongation-1a. Thirty-five isolates belonged to A. niger “aggregate”, mostly A. tubingensis, one to A. japonicus and one to A. violaceofuscus. OTA and FB capacity of the isolates was also investigated, with negative results, respectively, on YES and CY205 media. To our knowledge this is the first report of multi-locus characterization of black aspergilli associated with pomegranate black rot. Further studies on an enlarged set of strains, and evaluation of natural occurrence of the toxins, are required to better elucidate the potential mycotoxin risks on pomegranate fruit.

This research was supported by the Cyprus University of Technology Grant 3/319 to LK, and EU project MycoKey Grant 678781.

Population structure of Phytophthora infestans causing to potato late blight in the Çukurova Region of Turkey. H. GÜNAÇTİ¹, T. AY¹, C. CAN². ¹Biological Control Research Institute Koprukoy/Adana, Turkey. ²Gaziantep University, Department of Biology. Gaziantep/Turkey.

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Potato late blight (caused by Phytophthora infestans) is one of the most destructive diseases of potato in Turkey. The pathogen can infect stems, leaves, and tubers. Late blight is becoming increasingly difficult to control, leading to intensified use of fungicides in the potato production. This study explored the molecular and biochemical characters of P. infestans populations in Turkey. Metalaxyl sensitivity, mating type analyses, and phenotypic characterization were carried out in 2013-2014, with surveys in the Çukurova potato cultivation areas, and 186 P. infestans isolates were obtained. Through these analyses metalaxyl resistance profile, mt DNA haplotypes, occurrence of A2 type in the region and genetic differences were determined. These results are the first characterization data on for the P. infestans populations in Turkey.

This research was supported by TUBİTAK (The Scientific and Technological Research Council of Turkey) in project no 112O112.

Survey of distribution of the fire blight pathogen (Erwinia amylovora) on pome fruits in Montenegro. J. LATINOVIC, B. KANDIC and N. LATINOVIC. University of Montenegro, Biotechnical Faculty, Mihaila Lalic 1, 81000 Podgorica, Montenegro. E-mail: jelenalat@ac.me

From 2013 to 2016, a national survey of Erwinia amylovora, the causal agent of fire blight, was carried out in Montenegro, focusing on pome fruits (apple, pear and quince) as the most important host plants. The study included field observations and laboratory identification of the pathogen. The most common symptoms related to fire blight were “shepherd’s crook” on the tips of tree shoots, with brown discolouration under the bark and blighted leaves. Samples from symptomatic plants were examined in the laboratory, and bacteria were isolated nutrient agar. Pathogenicity of obtained isolates was confirmed by artificial inoculation of immature pear fruits, and hypersensitive reaction in tobacco leaves. Identification of isolates was by Gram staining, studying cultural characteristics on nutrient sucrose agar and King’s B medium), and by rapid immunochromatography. Occurrence and distribution of E. amylovora in Montenegro, mostly in northern areas, but also in central areas. The disease was most prevalent in Bijelo Polje and Berane, and quince was the most common host plant. Infection of apple and pear trees was usually noticed if they were in a proximity to old, diseased quince trees. Adequate phytosanitary measures need to be implemented to control the disease.

This research was supported by the Phytosanitary Directorate of Montenegro.

Biological and chemical control of Aspergillus flavus and aflatoxins in maize. C. LAGOGIANNI, D.I. TSITSI-GIANNIS. Laboratory of Phytopathology, Department of Crop Science, School of Agricultural Production, Infrastructure and Environment, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece. E-mail: dimtsi@aua.gr

Aspergillus spp. cause significant rots in maize and produce the carcinogenic aflatoxins. Several chemical and biocontrol formulations and other non-pathogenetic biotic factors were evaluated for the control of A. flavus in maize. In vitro experiments were performed on maize kernels with the following factors: a) Zeolite, a mineral with physicochemical properties; b) Agri-fos 600®, a product based on potassium phosphonate anions that induce plant immune responses; c) Trianum®, a product based on the fungus Trichoderma harzianum that inhibits the infection and colonization by pathogenic fungi; d) Botector®, a product containing the yeast Aureobasidium pullulans whose action is based on inhibition of pathogen colonization; e) Paenibacillus alvei K-165, an antagonistic bacterium that induces systemic resistance of plants; f) Serenade Max®, a product that stimulates natural plant defense mechanisms; g) Vacciplant®, a product that contains laminarin, an inducer of the plant immune system; and h) a non-toxigenic strain of Aspergillus flavus. The fungicides Switch®, Geoxe®, Granuflo®, Cantus®, Chorus® and Quadris® were also tested. The experiments demonstrated that the chemical formulations reduced infection A. flavus in in vitro and field experiments. In particular, the fungicide Switch® reduced disease severity and aflatoxin production by 70%. Additionally, the biopesticides Botector® and Mycostop® reduced aflatoxin contamination of maize by 50%. Applying good agricultural practices and combinations of biological agents and fungicides at the maize growth stage of anthesis and silking can significantly reduce aflatoxin contamination.
Effects of the viroids and rootstocks on fruit yield and juice quality of Tunisian citrus variety “Maltese half-blood”. A. NAJAR1, L. HAMROUFI2, R. BOUHLEL3, A. JEMMALI4, B. JAMMOUSSI5, N. DURAN-VILA6. 1National Institute of Agricultural Research of Tunisia, Street Hedi Karray, 1004 El Menzah, Tunis, Tunisia. 2National Research Institute of Rural Engineering, Water and Forests, Street Hedi Karray, 1004, El Menzah, Tunis, Tunisia. 3Higher Institute of Education and Continuing Education, Tunisia. 4Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain. E-mail: asmanajara@yahoo.fr

In Tunisia, citrus varieties are commonly grafted on sour orange rootstocks. Considering the present strategy to prevent damage which could be associated with tristeza disease, the substitution of sour orange rootstocks with symptomless rootstock/scion combinations is a desirable approach. However, some promising rootstocks are known to be sensitive to viroid infection. The performance of Tunisian ‘Maltese half-blood’ sweet orange infected with Citrus exocortis viroid (CEVd), Hop stunt viroid (HSVd), Citrus bark cracking viroid (CBCVd), Citrus bent leaf viroid (CBLVd) and Citrus dwarfing viroid in single or mixed infections was evaluated on eight rootstocks [sour orange (SO), ‘Carrizo’ citrange (CC), volkamer lemon (CV), ‘Cleopatra’ mandarin (MCL), ‘Swingle’ citrumelo (Citru), ‘Rangpur’ lime alemow (LR) and trifoliate orange (PT)], at the INRAT station in Cap Bon region. The trees were planted in 2005 and size, fruit production and fruit quality were evaluated every year from 2008. Mixed viroid infections decreased the canopies of Maltese grafted on CC by 41%, Citru by 40%, MCL by 39%, LR by 50%, CV by 46% and PT by 60%. The cumulative yield of Maltese grafted on CM and inoculated with HSVd was 76% less than the control. Mixed infections decreased production from the rootstocks Citru by 30% and PT by 60%. The only viroid effect on fruit quality was increased vitamin C content. This was more pronounced from mixed infections where the greatest amounts of vitamin C were recorded for fruit juice of Maltese grafted on CM, CV, LR or PT.

This research was supported by the National Institute of Agricultural research of Tunisia (INRAT, Tunisia).

Effect of temperature on Lactuca sativa cultivars infected naturally with Sclerotinia sclerotiorum - a field study. P. KRÓLIKIEWICZ, V.K. MACIOSZEK, T. JĘCZ, A.K. KONONOWICZ. Department of Genetics, Plant Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Bana- nacha 12, 16, 90-237 Lodz, Poland. E-mail: andrzej.kon- onowicz@biol.uni.lodz.pl

Sclerotinia sclerotiorum is one of the most destructive fungi of cultivated Lactuca sativa (lettuce), causing lettuce drop. Four lettuce cultivars (iceberg lettuce cvs Diamentinas and Templin, green lettuce cv. Lollo Bionda and red lettuce cv. Lollo Rossa) were grown in a horticultural holding in the central Poland (Lodz voivodeship), in the field naturally infested by S. sclerotiorum. In parallel, control lettuces were grown in a non-infested field. The experiment was conducted in 2016, in three yields/repetitions. Lettuce seeds used were purchased by Rijk Zwaan and Nunhems Companies. Seedlings were prepared by Schwanteland GmbH, Jungpflanzen, Germany. Each of lettuce cultivar was grown in 8 × 1 m plots, and each plot was divided into five sectors, each of 20 lettuce heads (100 heads per plot. Fertilizers (5 kg each of ammonium nitrate, potassium sulphate and triple superphosphate) were applied to each plot. Temperature was measured daily, and numbers of infected lettuce heads were counted at 4 and 8 weeks. Severity of lettuce drop was assessed using 5 point scale, and survival of individual plants was estimated. total phenolic and flavonoid content was also assessed. The most resistant cultivar to low temperature as well as to S. sclerotiorum infection was Lollo Rosa. This cultivar also had the greatest phenolic and flavonoid contents.

This research was supported by the University of Lodz grant no. B161100000211.01.

The effects of sulfur dioxide pads on postharvest grey mold and quality of sultana table grapes. P. KINAY TEKSUR, F. SEN1, H.B. ÜNAL1, A.K. SELVI2, A.KALIN3, A.M. AGHDAM1, B. CENBERCI COŞKUN3. 1Ege University Faculty of Agriculture Department of Plant Protection, 35100 Bornova, Izmir, Turkey. 2Ege University Faculty of Agriculture Department of Horticulture, 35100 Bornova, Izmir, Turkey.

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Sultana seedless table grapes are widely grown in Aegean Region. One of the most important problems in the storage of table grapes is latent infections by Botrytis cinerea originating from vineyards. SO₂ generators are used on grapes during the storage to control grey mold. However, these pads, produced by different companies, vary in their activity, which results both from the SO₂ content and different B. cinerea infection levels on grapes. The activities were investigated of SO₂ pads from different companies against different B. cinerea loads, and on fruit quality. Studies were carried out in cold storage rooms (± 0.5°C, 90% relative humidity) at two separate periods (2012-2013 and 2013-2014) for 3 months of storage. Effects were measured on development of decay, microbial populations, and quality parameters on grapes inoculated with B. cinerea (at 10⁵ and 10⁶ spores mL⁻¹) and untreated grapes that had been harvested from two different vineyards. Decay rate was 56% in the second month of storage for the grapes taken from the first vineyard, where different spraying programs were applied, whereas this rate was 92% for grapes taken from the other vineyard, where another control programme was applied. In both year trials, Fresca and Uvas SO₂ generators showed similar success in preventing decay development, especially during the first 2 months of storage. There were no differences in decay development for different SO₂ generators on grapes where B. cinerea was artificially inoculated.

Network of Mediterranean Culture Collections for preserving biodiversity of phytopathogenic and toxigenic microorganisms. A.F. LOGRIECO¹, G. PERRONE¹, L. MUGNAI², R.R.M. PATERSON³, A. VENÂNCIO³, L. LÓPEZ³, M.C. MACIAN⁴, R. AZNAR⁵, N. LIMA⁶. ¹Institute of Sciences of Food Production, National Research Council, Via Amendola 122/O, 70126, Bari, Italy. ²Dipartimento di Scienze Produzioni Agritalmentari e dell’Ambiente, University of Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. ³CEB-Centre of Biological Engineering, Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal. ⁴CECT-Spanish Type Culture Collection, University of Valencia, 46980 Paterna, Spain.

In recent years, there has been increasing concern for the importance of biodiversity. Various actions and initiatives have been applied at the European level to preserve the biodiversity of life (B4Life project), and in particular of microorganisms, such as European Consortium of Microbial Resources Centres (EMbaRC) and Microbial Resource Research Infrastructure (MIRRI) projects. These aimed to establish a self-sustainable community of European Microbial Resource Centres, representing a large biodiversity and offering a wide range of bioresources, experts and services. The particular aspect relevant to preservation and identification of plant pathogenic microorganisms (fungi, bacteria and viruses) is crucial to face the new challenges related to climate change and emerging plant diseases in the Mediterranean region (e.g. Xylella fastidiosa on olive trees, ToLCNDV virus on Solanaceae and Cucurbitaceae). In addition, some fungal strains can produce mycotoxins with severe effects on human and animal health (e.g. Aspergillus flavus on maize; Fusarium spp. on wheat), and can be human pathogens. Biodiversity is changing in relation to global temperature increases, and the relevant plant pathogen issues will be closely dependent to the pathogen species (i.e. thermophiles or others) dominating in the new climate scenarios. Currently, many studies on species characterisation at genetic and biochemical levels are generating a large and numerous datasets, and the scientific community can gain from organising and sharing biological resources and related information. A network on Mediterranean Culture Collections represents an important and strategic initiative to strengthen research activities, to face emerging phytopathogenic and toxigenic microorganisms and to assist food security/safety.

This initiative was supported by Mediterranean Phytopathological Union.

Comparative genomic analysis of secondary metabolite gene clusters in Fusarium species. V.C. LIUZZI¹, F. FANELLI¹, M. CHIARA², J. F. LESLIE³, A. F. LOGRIECO¹, G. MULÈ¹. ¹Institute of Sciences of Food Production, National Research Council, Bari, Italy. ²Dipartimento di Bioscienze, Università degli Studi di
The lack of standard analysis workflows next generation sequencing (NGS) data for toxigenic fungi prevents systematic comparative genomic studies. We have developed guidelines for the assembly and annotation of NGS data derived from genomic projects on phytopathogenic/toxigenic fungi. To demonstrate the potential of our workflow, we have carried out a comparative genomic analysis of different *Fusarium* species, focusing on secondary metabolite (SM) biosynthetic gene clusters. The workflow was structured as follow: 1) retrieval of *Fusarium* genome sequences available from public databases; 2) sequencing of new *Fusarium* genomes; 3) assembling genomes using Spades v5.0; 4) annotation of genomes using Augustus v3.1; 5) retrieval of information concerning known/unknown-SM clusters; 6) annotation of Pfam domains and SM biosynthetic gene clusters prediction; 7) calculation of clusters of orthologous genes (COGs) and gene families; 8) studying the presence/absence, order, orientation of each gene within clusters and the distribution of clusters among the isolates; 9) construction of “pancluster” phylogenetic trees; and 10) collecting all the data obtained in a *Fusarium* SM cluster database. Preliminary data show that phylogenies and SM cluster distribution among the isolates included in the current study are coherent with published data, and recapitulate the discontinuous distribution of SM clusters and thus in the genetic potential of species to produce secondary metabolites. Detailed analysis of individual clusters enabled the identification and the study of different mechanism of inheritance of SM clusters, showing the advantages of systematic strategies for the analysis and annotation of these genomes.

This work was supported by H2020-MycoKey- (E.U.3.2-678781)

Identification and characterization of *Acidovorax citrulli* strains from Serbia. N. ZLATKOVIĆ1, A. PROKIĆ3, K. GAŠIĆ2, N. KUZMANOVIĆ3, M. IVANOVIC1, Ž. PAVLOVIĆ1, A. OBRADOVIĆ1.
1University of Belgrade, Faculty of Agriculture, Institute of Phytotherapy, Department of Pathology, Nemanjina 6, 11080 Belgrade, Serbia. 2Institute for Plant Protection and Environment, Department of Plant Pathology Teodora Dražera 9, 11000 Belgrade, Serbia. 3Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (IKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany. E-mail: nevena_bлагojevic@yahoo.com

In Algeria, cereal products are very important for local food and in the national economy. Barley (*Hordeum vulgare*) is the second most cultivated cereal after wheat. This crop is affected by several foliar diseases. Net blotch is one of the most common diseases in Algeria, caused by *Pyrenophora teres*. The pathogen occurs in two forms, *P. teres* f. *teres* (*Pth*) causing the net form (NFNB) disease and producing longitudinal or transversal necrotic bands, and *P. teres* f. *maculata* (*Ptm*) causing the spot form (SFNB) and producing dark brown circular or elliptical lesions. Since the two pathogen forms are morphologically similar but genetically distinct, PCR primer sets have been developed to allow their differentiation, without symptom or morphological analyses. Since correct identification of pathogens is important for effective disease management and epidemiological study, we to explored the occurrence of *P. teres* and populations in Algeria, using two specific primer pairs. *Pth* is the more prevalent among all the prospected provinces. This evidence will help characterization of the causal agent populations, and assist epidemiological research, to improve control of the net blotch disease in this country.

This research was supported by Ecole Nationale Supérieure d’Agronomie (ENSA), Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1, Avenue Pasteur, Hassen Badi, Algiers, Algeria.

Occurrence of spot and net forms of net blotch of barley in Algeria. I.H. LAMMARI1, Z.E.A FELLA-HH1, A. BENBELKACEM3, H.BENSLIMANE1. 1Ecole Nationale Supérieure d’Agronomie (ENSA), Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, 1, Avenue Pasteur, Hassen Badi, Algiers, Algeria. 2Université El Bachir El Ibrahim, Bordj Bou Arreridj. 3Institut National de la Recherche Agronomique d’Algérie, Unité de Recherche de Constantine, Station ITGC, Elkhroub, Algérie. E-mail: h.lammari@st.ensa.dz

In August 2014, typical bacterial fruit blotch symptoms were observed on mature watermelon fruits
originating from fields in the Vojvodina province. In the summers of 2015 and 2016 we registered two more occurrences in, respectively, the east and west of the country. White, glistening, convex and circular colonies with regular edges were predominantly isolated from diseased watermelon fruits collected in the affected fields. A total of 33 bacterial strains were subjected to further analysis. They were Gram-negative, aerobic, oxidase and catalase positive, non-fluorescent and did not produce potato soft rot. All but two strains (KFB 359 and KFB 363) induced hypersensitive reactions in tobacco leaves. They grew at 41°C and produced beige to tan-coloured, non-mucoid, convex colonies on yeast extract-dextrose-CaCO₃ agar. All the strains utilized L-arabinose, did not reduce nitrate, nor utilized sucrose. Conventional PCR was performed using A. citrulli-specific primers BX-L1/BX-S-R2. The 16S rRNA gene sequence from two strains (KFB 343 and KFB 344) showed 100% identity to strains of A. citrulli from China, Thailand and the USA. According to physiological and biochemical tests, PCR assay and 16s rRNA gene sequencing analysis, the causal organism was confirmed as A. citrulli. Genetic relatedness among strains was investigated by rep-PCR, using BOX and REP primers. All tested strains except one (KFB 358), showed the same BOX-PCR profiles. In addition, all strains were assigned to the same REP-PCR group. These results show that A. citrulli strains isolated in Serbia during 3 years belong to an homogeneous population. These isolated occurrences are considered to originate from seed-borne inoculum, but it remains unknown whether the pathogen will survive from season to season in Serbian climatic conditions.

This study was supported by the project III46008 financed by Ministry of Education, Science and Technological Development, Republic of Serbia.

Phenotypic and genetic characterization of Bacillus amyloliquefaciens strains active against fungal pathogens of wheat. R. CARACCILO1,2, J. CABREFIGA2, I. MORA2, A. FABI2, R. D’OVIDIO2, E. MONTESINOS2, L. VARVARO1. 1 Department Of Agriculture and Forestry Science (DAFNE), University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy. 2 Center for Innovation and Development in Plant Health (CIDSAV)-Institute of Food and Agricultural Technology, University of Girona, Maria Aurèlia Capmany, 61, 17003 Girona, Spain. E-mail: caracciolo@unitus.it

Wheat is one of the most important world food crops and its widespread cultivation constantly exposes this host to many abiotic and biotic stresses. Pathogenic fungi represent a serious threat to the cultivation of this cereal. The use of biocontrol agents (BCAs) is one of the most successful strategies for prevention and control of plant diseases. This study dealt with (i) the in-vitro evaluation of the antifungal activity of three strains of B. amyloliquefaciens (Ba) against Fusarium graminearum, Fusarium culmorum, Bipolaris sorokiniana and Septoria tritici, and (ii) the molecular and chemical characterization of the compounds involved in the activity. Determination of the inhibitory actions of filtrates and extracts from Ba growth broths against all the tested pathogens allowed us to understand the nature of the exoproduced, liposoluble and thermostable molecules involved. Analysis of the presence of ituA, fenD, surA, bmyB and mycA genes confirmed the potential production of cyclic lipopeptides (CLPs), such as Iturin, Fengycin, Surfactin, Bacillomycin and Mycosubtilin, that are reported as antifungal compounds. The production of CLPs, was confirmed with the characterization of the culture filtrates by FPLC, and the antagonistic assays performed with the fractions obtained. Ifengycins were mainly responsible of the antifungal activity. These results confirm that B. amyloliquefaciens could be a valid BCA against the most important pathogens of wheat, and its use in the formulation of commercial bio-pesticides can be recommended.
From 2010 to 2014, Pseudomonas-like bacterial strains were recovered from symptomatic hazelnut plants in different locations in Serbia. To confirm their identity, ten strains were characterized by morphological, biochemical, physiological and molecular tests (PCR detection of syringomycin production, repetitive-sequence PCR and rpoD housekeeping gene analysis). The strains were fluorescent, HR positive, oxidase negative, negative for arginine dihydrolase and pectinase activity, and variable in levan production. All strains showed uniform biochemical characteristics typical for P. syringae pv. syringae: they hydrolysed gelatin and aesculin, produced acid from glucose, sucrose and inositol, and grew at 36°C and in 5% NaCl. All but one strain had positive ice nucleation activity. Pathogenicity of the strains was tested by inoculation of immature sweet cherry fruits. Dark necrotic lesions varying in size developed 2 weeks after inoculation, indicating different levels of virulence of investigated strains. Genetic profiles generated with BOX primers were polymorphic for each strain revealing high genetic diversity. The syrB gene encoding syringomycin production, characteristic for pv. syringae, was detected by PCR analysis in all the strains. RpoD gene sequence analysis also indicated high similarity of the strains and P. s. pv. syringae. Results obtained in this study provided new information about the first detection of P. syringae pv. syringae on hazelnut in Serbia.

This research was supported by the project III46008 financed by Ministry of Education, Science and Technological Development, Republic of Serbia.

Cross inoculation assays confirms grass pea/pea phylogeny proximity at the Fusarium oxysporum host range level. A.M. SAMPAIO1, N.F. ALMEIDA1, N. RISPAI2, D. RUBIALES3, M.C. VAZ PATTO1. 1Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Avenida da Republica, 2781-157 Oeiras, Portugal. 2Instituto de Agricultura Sostenible, CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain. Email: ansampaio@itqb.unl.pt

Grass pea (Lathyrus sativus) has considerable potential as legume crop in dryland farming systems. It is superior in yield, protein value, nitrogen fixation, drought, flood and salinity tolerance when compared to other legume crops. However, yield inconsistency due to sensitivity to specific diseases strongly limits its cultivation. Fusarium wilt, caused by the soil borne fungus Fusarium oxysporum, is one of the most important diseases affecting grain legumes worldwide, and is becoming a threat for grass pea production in Portugal. Understanding its host specificity is important for epidemiology and disease management. No information is available in grass pea, so the host range of two new F. oxysporum isolates collected from grass pea was analysed in several other important legume crops, including pea (Pisum sativum), lentil (Lens culinaris), chickpea (Cicer arietinum) and barrel medic (Medicago truncatula). The responses were also studied of grass pea to F. oxysporum f. sp. infecting these crops, including F. oxysporum f. sp. pisi races 1 and 2, F. oxysporum f. sp. lentis, F. oxysporum f. sp. ciceris and F. oxysporum f. sp. medicaginis. There were very similar responses of pea and grass pea accessions to both of these new grass pea isolates, and to F. oxysporum f. sp. pisi, suggesting little specialization of F. oxysporum to L. sativus. This is not surprizing since grass pea is phylogenetically close to pea. However, we do not exclude that specialization may occur but remains undetected. More isolates should be sampled from grass pea fields to ratify this.

This research was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through the grant PD/BD/114418/2016, the IF/01337/2014 FCT Investigator contract and the Research unit GREEN-it “Bioresources for Sustainability” (UID/Multi/04551/2013), the QualA project (PTDC/AGR-TEC/0992/2014) and by the European Community Seventh Framework Programme (FP7/2007-2013) through the LEGATO project (grant agreement nºFP7-613551).

Identification of pathotypes and analysis of the genetic structure of Fusarium oxysporum f. sp. lentis populations. H.R. POURALIBABA1, Z. SATOVIC2, M.J. COBOS3, D. RUBIALES3, S. FONDEVILLA3. 1Dryland Agricultural Research Institute, Education and Extension Organization (AREEO), Maraghe 119, Iran. 2Faculty of Agriculture, Department of Seed Science and Technology, Svetosimunska 25, 10000 Zagreb, Croatia. 3Institute for Sustainable Agriculture, CSIC, 14004 Córdoba, Spain. E-mail: hpouralibaba@ias.csic.es

Lentil cultivation is threatened worldwide by Fusarium wilt caused by Fusarium oxysporum f. sp. lentis.

**Genomic screens to identify next-generation MAMPs and their cognate pattern recognition receptors.** A. G. Mott, S. Thakur, E. Smakowska, P. W. Wang, Y. Belkhadir, D. S. Guttman, D. Desveaux. 1Department of Cell & Systems Biology, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada. 2Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr Bohr Gasse 3, Vienna 1030, Austria. 3Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada. E-mail: darrell.desveaux@utoronto.ca

The front line of plant defence against pathogens depends on the action of extracellular leucine-rich repeat, receptor-like kinases (LRR-RLKs). These serve as Pattern Recognition Receptors (PRRs) to recognize essential, evolutionarily conserved, features of pathogens called Microbe-Associated Molecular Patterns (MAMPs). MAMP recognition by PRRs activates PRR-triggered immunity (PTI), which suppresses the growth of nearly all “non-host” microbes, as well as many potential pathogens. Next generation sequencing of *Pseudomonas syringae* pathogens has allowed the successful *in silico* prediction of MAMPs, through identification of positive selection signatures on proteins of the core genome. Although these “next-generation MAMPs” induce the hallmark responses of PTI, including virulence suppression, the
Primary metabolism modulation caused by *Onion yellow dwarf virus* infection in ‘Rossa di Tropea’ onion. A. TIBERINI, F. ARANITI, A. CIAMPA, S.B. GRANDE, A. TAGLIENI, M.R. ABENAVOLO, M.T. DELL’ABATE, L. TOMASSOLI, G. ALBANESE. 1Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di AGRARIA, Località Feo di Vito - 89122 Reggio Calabria, Italy. 2Consiglio per la Ricerca in Agricoltura e l’Analisi dell’Economia Agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22 - 00156 Roma, Italy. 3Consiglio per la Ricerca in Agricoltura e l’Analisi dell’Economia Agraria, Centro di Ricerca per la Studio delle Relazioni Pianta Suolo, Via della Navicella 2/4 - 00184 Roma, Italy. E-mail: antonio.tiberini@unirc.it

*Onion yellow dwarf virus* (OYDV, genus *Potyvirus*), an aphid transmitted virus, is one of the most limiting pathogens for onion (*Allium cepa* L.) production worldwide, with infection rates in field up to 100% during the biennial host life cycle. The virus causes severe leaf symptoms and plant stunting, reduced bulb size and seed yield. Recently, OYDV has been found responsible for an severe agronomic decline of ‘Rossa di Tropea’ onion, a cultivar of southern Italy (Calabria), granted by European Union with the Protected Designation Origin (IGP) trademark. This cultivar is known for its mild to sweet flavour but also richness in flavonols and anthocyanins. A research project SLORTO (SIR-MIUR grant – SIORTO-RB-SI149LD5) has been activated to study the effects of
OYDV on accumulation of nutraceutical compounds in ‘Rossa di Tropea’. Gas Chromatography-Mass Spectrometry (GC-MS) and primary metabolism profiling has compared onion bulb samples, healthy versus OYDV-infected, collected at three infection times (bulb harvesting, leaves drying and after bulb storage) during a trial conducted in Calabria. Several metabolites connected to sugar and amino acid metabolism, and the TCA cycle decreased in OYDV-infected bulbs compared to control plants at the first testing time. A pronounced increase of theses metabolites then occurred. Magnetic resonance microimaging (MRI) on whole bulbs highlighted structural alteration of OYDV-infected bulbs as compared to healthy bulbs. This research is the first study on metabolic modulation in the onion/virus pathosystem ‘Rossa di Tropea’/OYDV.

**Downregulation of violaxanthin cycle metabolites is associated with the lesions observed in mlo resistant barleys.** G. MONTILLA-BASCÓN\(^1\), M. ROCA\(^2\), L.A.J. MUR\(^3\), E. PRATS\(^4\). \(^1\)Department of Plant Breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain. \(^2\)Food Phytochemistry Department, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), University Campus Pablo de Olavide, Building 46, Sevilla 41013, Spain \(^3\)Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, UK.

In many temperate regions cereal powdery mildew, caused by *Blumeria graminis* ff. spp., seriously constrains crop production. A durable host resistance mechanism that prevents cell penetration by the pathogen is the formation of papillae, which are localized cell wall appositions at attack sites. Papillae provide race non-specific defense, conferring broad-spectrum resistance. Barley genotypes carrying the *mlo* gene display highly effective papilla-based penetration resistance to powdery mildew, that has been durable for over 30 years. However, the *mlo* gene shows adverse pleiotropic effects such as large necrotic/chlorotic flecks on leaves, accelerated leaf senescence and reduced grain yield, and these adverse effects are particularly dramatic under stress conditions. For this reason, *mlo* cannot be used in winter barley varieties. Despite its importance for crop production, the mechanism(s) leading to these pleiotropic effects are still not understood, nor are its molecular and cellular bases. We have previously observed that the damage was associated with particular genetic backgrounds and was linked to stomatal and photosynthetic dysfunctions. We have investigated changes in xanthophyll cycle metabolite profiles and the chlorophyll degradation pathway in two sets of *mlo*-isogenic lines with different genetic backgrounds. A decrease in chlorophyll a and b occurred in the resistant isoline, characterized by necrotic flecking but accumulation of chlorophyllide or pheophorbide were not detected. Overall, xanthophyll metabolites increased following pathogen inoculation in the resistant *mlo* line lacking lesions. Furthermore, anteroxanthin responded to inoculation with increases in all genotypes but greater differences compared with healthy plants in the resistant *mlo* genotype lacking lesions.

This research was supported by the Project AGL2016-78965-R (Spanish Ministry of Economy and Competitiveness) and the European Regional Development Funds (ERDF).

**Early and late transcriptome changes in a tomato cultivar carrying the Sw5 resistance gene upon infection by a resistance-breaking strain of Tomato spotted wilt virus.** G. BUBICI, F. CILLO, M.I. PRIGIGALLO, R. MONFREDA. Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, 70126, Italy. E-mail: giovanninicola.bubici@cnr.it

We analyzed the transcriptome (RNA-Seq) of leaf samples collected from a field crop of tomato cv. Docet (*Sw5* resistance gene) in Apulia, southern Italy, with different symptom severity and accumulation levels of a resistance-breaking strain of *Tomato spotted wilt virus* (TSWV). Four groups of samples were assumed to be different stages of plant tissue colonization by the virus: plants without symptoms and a null virus titre (group A) or 1 × 10\(^2\) TSWV reads per million (rpm; B), and plants with symptoms and 1 × 10\(^4\) rpm (C) or 2 × 10\(^5\) rpm (D). Transcriptome sequencing revealed that plant response to TSWV infection is profoundly related to its accumulation level in the tissues. At an early stage of infection (B vs. A comparison), genes related to photosystem I were down-regulated, and oxidoreductase activity increased. Considerable virus colonization (C
vs. B) activated defense-related mechanisms such as cell surface receptor signalling, phenylpropanoid biosynthesis and transcription factor activity. In contrast, photosynthesis, transmembrane transporter activity, and biosynthesis of monosaccharides and peptides were down-regulated. This scenario increased at an advanced stage of colonization (D vs. C), with attenuation of response to stimuli (e.g., surface receptor signaling and protein kinase activity) and an increase of catalytic activities such as ubiquitin-protein transferase and ribonuclease. TSWV infection constantly injured tomato cell metabolism (e.g., photosynthesis, monosaccharide and peptide biosynthesis, ion transporter activity) while plant defense (e.g., cell surface receptor signaling, phenylpropanoid pathway), clearly ineffective in such compatible plant-virus interaction, occurred late and disappeared soon after.

Photosynthetic efficiency differs between *Brassica napus* cultivars that are susceptible or resistant to *Alternaria brassicicola*. V.K. MACIOSZEK¹, T. JĘCZ¹, P. KRÓLIKIEWICZ², H. SCHOOLBEK², C. RIDOUT²& A.K. KONONOWICZ². ¹Department of Genetics, Plant Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12, 16, 90-237 Lodz, Poland. ²John Innes Centre, Norwich Research Park, UK. E-mail: violetta.macioszek@biol.uni.lodz.pl

*Brassica napus* (oilseed rape, OSR) cultivars, winter and spring types, have been phenotyped for their changes in photosynthetic efficiency and resistance/susceptibility to the necrotrophic fungus *Alternaria brassicicola*, which causes black spot on all *Brassica* species worldwide. 160 OSR cultivars were grown under laboratory conditions in triplicate. Leaf inoculation (3.5 × 10⁵ conidia mL⁻¹) was performed. Win_DIASS3 system necrosis formation, and FluorCam7 system Kaukaskey effect, and photosynthetic dyes and spectroscopy were used to analyse the third leaf of each plant at 5 d post inoculation, when symptoms developed. Analysis of necroses showed that 111 of the cultivars were susceptible or highly susceptible to *A. brassicicola*, and 49 were resistant or highly resistant. Photosynthetic efficiency expressed as FV/Fm (QYmax) indicated whether *A. brassicicola* infection affected photosystem II in a dark-adapted state. Uninfected (control) leaves showed QYmax values of 0.63 to 0.87, whereas infected leaves were 0.50 to 0.86. The most spectacular decrease in QYmax was observed in two susceptible cultivars Zairai Chousenshu and Ningyou7, with differences between control and infected leaves at 0.29. Resistant OSR cultivars showed no changes in QYmax values (0.01 to 0.05). Two the most susceptible spring cultivars (MONTY-028DH and Zairai Chousenshu) and the two most resistant winter ones cultivars (Savannah and Askari) were selected for further analyses such as changes in levels of photosynthesis-related proteins (RUBISCO, LHCa and LHCb) by Western blot analyses.

This research was supported by a National Centre for Research and Development grant (MAQBAT ERA-CAPS-II/1/2015) and BBfRC BB/N005007/1 in the ERA-CAPS programme.

The role of the necrosis and ethylene inducing gene VdNEP in virulence of *Verticillium dahliae*. A. TRIANTAFYLLOPOULOU¹, A.K. TZIMA¹, S. KANG², E.I. PAPLOMATAS¹. ¹Laboratory of Phytopathology, Agricultural University of Athens, Iera Odos 75, Athens, Greece. ²Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, Pennsylvania, USA. E-mail: alextriantafyl@gmail.com

The VdNEP protein from the fungus *Verticillium dahliae* is a member of the Nep1-Like proteins (NLPs), that have been isolated from plant pathogenic fungi and induce necrosis and ethylene production in dicotyledonous plants, functioning as toxins or effectors. Over-expression of the VdNEP gene under the control of a strong promoter in three races of *V. dahliae* (the tomato race 1, the cotton non-defoliating and defoliating strains) resulted in elevated transcript levels of the VdNEP transgene in transformed strains compared to wild type strains. VdNEP over-expressing strains caused increased disease symptoms on different hosts, compared to the respective wild type strains. Differentiation between the defoliating and non-defoliating races of the pathogen was possible when the VdNEP gene was used as a molecular marker. Furthermore, a VdNEP-EGFP fusion construct was generated and inserted in the three races of *V. dahliae*, to observe localization and/or secretion of the protein. Observation of the fusion protein within fungal cells is currently in progress.
Developmental processes regulated by small RNAs during Arabidopsis-Root knot nematode interaction. F.E. DíAZ-MANZANO1, J. CABRERA1, M. BARCALA1, R. OLMO1, A.C. SILVA2, M.F. ANDRÉS2, I. MARTÍNEZ3, V. RUIZ-FERRER1, C. FENOLL1, C. ESCOBAR1. 1Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha, Avenida de Carlos III, s/n, 45071, Toledo, Spain. 2Departamento Protección Vegetal, Instituto Ciencias Agrarias-CSIC, Calle Serrano, 115, 28006, Madrid, Spain. E-mail: Fernando.Diaz@uclm.es

Root knot nematodes (Meloidogyne spp.) currently cause major agricultural losses. They infect plants in the root elongation zone and penetrate intracellularly into the vascular cylinders, inducing galls containing nematode feeding cells, the giant cells (GCs). We studied the differential transcriptome of Arabidopsis GCs and galls after Meloidogyne spp. Infection, as compared to vascular cells, revealing high-repressed genes probably due to gene expression reprogramming during their differentiation. Sequencing of small RNAs (sRNAs) showed profiles consistent with a role of sRNAs in gene silencing. The 24 nt-sRNAs, known to be involved in epigenetic regulation, were highly induced in early formed galls (3 d post infection), and together with differentially regulated miRNAs could be mediating the large gene repression that occurs during early development of GCs/galls. We have studied the roles of miR390 and miR172, accumulated in galls at early infection stages. Loss of function Arabidopsis lines for both miRNAs showed reduced numbers of galls after nematode infection. The two miRNAs participate in plant developmental processes in different ways. TAS3 precursor is cleaved by miR390 triggering tasiRNAs biogenesis that inhibits ARF2-4, releasing repression of lateral root growth. In contrast, miR172 downregulates the AP2-like genes during flowering via a translational mechanism rather than by mRNA cleavage. We discuss the putative molecular networks induced by plant-nematodes in this biotic interaction through miR172 and miR390.

Type 2c Ser/Thr phosphatases (PTCs) are a class of protein phosphatases, conserved in eukaryotes. The PP2C proteins are involved in the regulation of many cellular functional processes, addressed by their role on MAPK cascades. Seven putative PTC proteins have been identified in Fusarium oxysporum f. sp lycopersici 4287, using the BLAST algorithm, with PTCs from Saccharomyces cerevisiae and Fusarium graminearum. The expression of these genes in different stress conditions and plant infection was evaluated by RT-qPCR. Upregulation of ptc1 was observed after cell wall stress, osmotic stress and plant infection, while downregulation was detected after invasive growth. A mutant strain, Δptc1, was obtained by the split marker strategy. The Δptc1 strain was more sensitive to SDS (0.125%) and menadione (20 μg mL−1) than the wild type, indicating possible roles of PTC1 in, respectively, cell wall/membrane stress (MPK1 pathway) and oxidative stress (HOG1 pathway). In addition, the Δptc1 strain showed greater tolerance to LiCl (0.15M and 0.30M) on different media pH (5, 7 and 8.5) than wild type, suggesting a role of PTC1 in lithium efflux mediated by the Nha1 (Na+/K+/? Li+/Rb+ antipporter). The phosphorylation level of HOG1, MPK1 and FMK1 proteins was evaluated by western-blot; the Δptc1 strain showed increased phosphorylation level of HOG1 compared to the wild type. These results suggest an important role of PTC1 on the HOG1 pathway of Fusarium oxysporum f. sp lycopersici.

This research was supported by the Project BIO2016-78923-R (Ministerio de Economía y Competitividad, Spain). PPFL received a PhD fellowship supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Ministério de Educação, Brasil).

Transientsilencing of the FaWRKY1 strawberry gene (Fragaria x ananassa) in fruit induces resistance to Colletotrichum acutatum infection. J.J. HIGUERA-SOBRINO1, F.J. MOLINA-HIDALGO1, I. ARJONA-GIRONA2, F. AMIL-RUIZ1, J. GARRIDO-GALA1, A. LEKHBOU1, J.A. MERCADO2, F. PLIEGO-ALFARO3, J. MUÑOZ-BLANCO1, C.J. LÓPEZ-HERRERA2, J.L. CABALLERO1. 1Departamento de Bioquímica y Biología Molecular, Edif. Severo Ochoa-C6, Planta Baja-Ala Norte. Campus de Rabanales s/n. Universidad de Córdoba-14071, Córdoba, Spain. 2Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible, C.S.I.C. C/Alameda del Obispo s/n, Apartado 4084, Córdoba, Spain. 3Departamento
mento de Biología Vegetal, Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora”, IHSM-UMA-CSIC, Universidad de Málaga-29071, Málaga, ES. E-mail: b92hiso@uco.es

Anthracnose, caused by Colletotrichum acutatum, is responsible for significant yield losses in commercial strawberry production worldwide. For this reason, it is of interest to uncover the molecular basis underlying this strawberry/pathogen interaction. Previously, FaWRKY1 was identified as an important element mediating defence responses. This gene encodes an AtWRKY75-like transcription factor (type IIc), which is upregulated in strawberry following C. acutatum infection. In this study, Agrobacterium-mediated transient transformation was used to both silence and overexpress the FaWRKY1 gene in fruit, with the aim to clarify its function in the strawberry defense mechanism. Analyses of FaWRKY1-RNAi strawberry fruits showed resistance to C. acutatum infection, 5 d after inoculation with this pathogen. Overexpression of this gene in strawberry fruit showed increased susceptibility to C. acutatum. Molecular analysis is being carried out with these fruit samples to elucidate candidate genes transcriptionally regulated by FaWRKY1. Furthermore, in vitro DNA-binding assays have revealed a tentative consensus sequence [G/T][T/C]TGAC[T/C], containing the core sequence TGAC (W box), as the likely target sequence for FaWRKY1 binding. These analyses will strengthen genome-wide promoter target site prediction for FaWRKY1.

This research was supported by the Project P12-AGR-2174 (Junta de Andalucía, Spain).

Evidence that the putative movement protein (MP2) of Broad bean wilt virus 1 is a pathogenicity determinant. C. CARPINO1,2, I. FERRIOL3, L. ELVIRA-GONZÁLEZ1, L. RUBIO1,3, E. PERÍ2, S. DAVINO2,4, L. GALIPIENSO1,3,4. 1Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. CV-315, 46113 Moncada, Valencia, Spain. 2Department of Agricultural and Forestry Science, University of Palermo, Piazza Marina 61, 90133 Palermo, Italy. 3Euro-Mediterranean Institute of Science and Technology (IEMEST), Vía Michele Miraglia 20, 90139 Palermo, Italy. 4Department of Biotechnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.

Broad bean wilt virus 1 (BBWV-1, genus Fabavirus, family Secoviridae) infects crops of economic importance, such as broad bean, pepper, tomato, spinach, and ornamental plants. The virus genome is constituted by two molecules of positive single stranded RNA, each encoding a polyprotein which is further processed by proteolytic cleavage. RNA1 encodes the proteins involved in viral replication and expression, while RNA2 encodes the movement protein (MP) and two coat proteins (LCP and SCP). RNA2 contains an alternative second start codon rendering a smaller putative movement protein, called MP2. To date, the BBWV-1 proteins related to pathogenicity are unknown. The roles of MP2 in symptom determination, post-transcriptional gene silencing (PTGS) and elicitation of hypersensitive response (HR) were examined. Expression of MP2 in Nicotiana benthamiana through Potato virus X (PVX) caused necrotic lesions, indicating that MP2 is a symptom determinant. Analysis of O$_3$ accumulation and necrosis staining revealed that this protein elicited the cellular HR. Transient expression of MP2 in N. benthamiana 16C, that constitutively expresses Green Fluorescent Protein (GFP), and a complementation assay with a vector based on Turnip crinkle virus sequence (TCV-sGFP) showed that this protein acts as a suppressor of PTGS.

Chlorophyll degradation pathway is linked to stomatal and photosynthetic dysfunctions observed in oats resistant to powdery mildew. G. MONTIL-LA-BASCÓN1, M. ROCA2, L.A.J. MUR3, PRATS E1. 1Department of Plant breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain. 2Food Phytochemistry Department, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), University Campus Pablo de Olavide, Building 46, Sevilla 41013, Spain. 3Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, UK.

Cost of resistance is usually associated with the energy and nutritional penalties linked to induction of defenses. Currently, a mechanistic understanding of the sources of these costs is lacking, other than vague
suggestions of the energy “lost” in inducing the defense. We have shown that penetration resistance or hypersensitive response (HR) provoke stomatal and photosynthetic dysfunctions, which could be important components of the disease resistance cost. More importantly, the stomatal dysfunctions (lock-up) are genotype, but not response-type, dependent, since genotypes with similar resistance responses show very different locking patterns when assessed histologically. We have assessed the content of several photosynthetic pigments including chlorophyll \( a \) and \( b \), several metabolites of the xanthophyll cycle, and metabolites of the chlorophyll degradation pathway in healthy and powdery mildew (Blumeria graminis f. sp. avenae) inoculated oat seedlings. Resistant genotypes associated with stomatal and photosynthetic dysfunctions activate the chlorophyll degradation pathway early after pathogen inoculation, increasing the level of pheophytin \( a \) content. These genotypes also showed a reduction in chlorophyll \( a \) and \( b \) contents, whereas the resistant genotypes lacking physiological dysfunctions showed no variation in the level of these compounds.

This research was supported by the Project AGL2016-78965-R (Spanish Ministry of Economy and Competitiveness) and the European Regional Development Funds (ERDF).

Early signalling during mlo-based papilla resistance involve a subtle crosstalk between jasmonate, salicylic acid and abscisic acid pathways. F. CANALES-CASTILLA\(^1\), G. MONTILLA-BASCÓN\(^2\), N. RISPAIL\(^3\), A. GÓMEZ-CADENAS\(^2\), V. ARBONA\(^3\), PRATS E\(^1\). \(^1\)Department of Plant breeding, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14004 Córdoba, Spain. \(^2\)Ecofisiología i Biotecnologia, Dpt. Ciències Agràries i del Medi Natural. Universitat Jaume I - Campus Riu Sec. E-12071 Castelló de la Plana, Spain. \(^3\)Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (IHSUM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: sandratienda@uma.es

Powdery mildew is one of the most widespread and damaging crop diseases. One of the most efficient and durable powdery mildew resistance mechanisms was originally found in barley lines carrying homozygous recessive alleles at the Mlo locus. These lines show efficient resistance to pathogen penetration based on formation of papillae, which are localised cell wall appositions at attack sites. The Mlo gene encodes a protein considered a negative regulator of the defence response so that its loss leads to more rapid and/or enhanced papilla formation. Although it is known that host plants sense powdery mildew fungi and start to activate defenses as early as 30 min following pathogen challenge, very little is known of the signaling that leads to the efficient papillae formation of mlo genotypes. We have explored the profile of several signaling molecules in two sets of mlo-isogenic lines with different genetic background, over a time course ranging from 30 min to 24 h. Abscisic acid decreased following inoculation in all susceptible and resistant genotypes whereas salicylic acid increased only in the resistant mlo genotypes from 2 h post inoculation, with a maximum at 24 h. Jasmonic acid and its derivative, Ile-jasmonic increased in resistant genotypes at 10-12 h after inoculation, whereas its biosynthetic intermediate 12-OPDA accumulated in resistant genotypes as early as 4 h and following. These data, showing a subtle and very early regulation of these signaling pathways, will shed light on the mechanisms of papilla formation.

This research was supported by the Project AGL2013-48687-R and AGL2016-78965-R (Spanish Ministry of Economy and Competitiveness) and the European Regional Development Funds (ERDF).

**Biocontrol, natural compounds and plant defense stimulants**

**Characteristics of the biocontrol rhizobacterium Pseudomonas chlororaphis PCL1606.** S. TIENDA, C. VIDA, A. DE VICENTE, F.M. CAZORLA. Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (IHSUM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: sandratienda@uma.es

The major disease affecting avocado crops in the Mediterranean area is avocado white root rot, caused by Rosellinia necatrix. The biocontrol rhizobacterium Pseudomonas chlororaphis PCL1606 has been isolated from rhizosphere of healthy avocado trees, growing in an area affected by white root rot. As a main characteristic, PCL1606 showed strong in vitro antagonism against R. necatrix and other important soil-borne pathogens. This is mainly due to produc-
tion of the antimicrobial compound 2-hexyl, 5-propyl resorcinol (HPR). Production of other antifungal compounds by PCL1606 has also been detected. PCL1606 has the ability to persist on and colonize avocado roots, where the bacterium interacts closely and colonizes R. necatrix hyphae, leading to negative effects on the fungus. Those phenotypes, acting together, allowed PCL1606 to display biocontrol activity towards R. necatrix in avocado plants. We have observed that PCL1606 shows no plant growth promoting activities. The availability of the complete genome sequence of PCL1606 will allow identification of additional features of the strain involved in biocontrol.

This work was supported by National plan I+D+I MINECO (AGL2014-52518-C2-1-R; MINECO, Spain), and with FEDER (EU). S. Tienda has received a grant from FPI program MINECO.

Biological characterisation of Pochonia chlamydosporia isolates associated with root-knot nematodes. J. HORTA, I. ABRANTES, I.L. CONCEIÇÃO Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Universidade de Coimbra, P-3000 456 Coimbra, Portugal. E-mail: isabelluci@gmail.com

The nematophagous fungus Pochonia chlamydospora (Pc) is a ubiquitous facultative parasite of eggs of potato cyst nematode (PCN; Globodera spp.), and root-knot nematode (RKN; Meloidogyne spp.). This study assessed the potential of Portuguese Pc isolates as biocontrol agents (BCA) of nematodes. Four isolates associated with Meloidogyne spp. eggs (PcI, PcII, PcIV and PcV), three associated with tomato infected roots (PcVI, PcVIII and PcIX), one (Pc2) from PCN eggs, as well as an exotic isolate (Vc10), were evaluated, using in vitro assays, for their abilities to produce chlamydospores, colonise tomato rhizospheres, and parasitise G. rostochiensis and M. incognita eggs. The isolates had marked differences in performance. Isolate PcI colonised the host rhizosphere extensively (89%), whereas PcIX was a poor coloniser (<50%). Isolates PcII and Pc2 produced greatest numbers of chlamydospores on solid medium (>20 × 10^5 chlamydospores g^-1). The proportion of RKN eggs parasitised was low (<60%) for all isolates. PcI and PcVIII were the best parasites against RKN eggs (>50%), and PcV and Pc2 parasitised more than 50% of the PCN eggs. These results suggest that PcI is the native isolate with the greatest potential as a BCA, since this isolate revealed desirable traits such as good rhizosphere competence and prolific chlamydospore production, thus having a greater potential than the other isolates for exploitation as a BCA.

This research was supported by FEDER – “Fundo Europeu de Desenvolvimento Regional”, through the COMPETE 2020 – “Operacional Programme for Competitiveness and Internationalisation” (POCI), and by Portuguese funds through FCT – “Fundação para a Ciência e a Tecnologia” in the framework of the project POCI-01-0145-FEDER-016611 (PTDC/AGR-PRO/3438/2014).

The efficacy of plant-derived protein hydrolysates against zucchini powdery mildew is affected by their biochemical characteristics. M. CAPPELLETTI1,2, M. PERAZZOLLI1, A. NESLER1,3, O. GIOVANNINI1, I. PERTOT1,4. 1Department of Sustainable Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all’Adige, Italy. 2DI4A, Department of Agrifood, Environmental and Animal Sciences, University of Udine, 33100 Udine, Italy. 3Bi-PA - Biological Products for Agriculture, B-1840 Londerzeel, Belgium. 4Center Agriculture, Food and Environment, University of Trento, 38010 San Michele all’Adige, Italy. E-mail: martina.cappelletti@fmach.it

The substitution of pesticides has become a priority in agriculture, and induction of plant resistance by protein hydrolysates may offer a sustainable solution. Peptide fragments can act as elicitors of plant immunity, and a protein extract was shown to reduce powdery mildew symptoms by stimulating grapevine defense responses under field conditions. The present research investigated potential correlations between the efficacy of plant-derived hydrolysates against the zucchini powdery mildew (caused by Podosphaera xanthii) and their biochemical features, such as peptide and amino acid composition, in order to clarify their modes of action. Under greenhouse conditions, soybean, rapeseed and guar hydrolysates were tested. These were obtained by enzymatic and acid hydrolysis of low-cost protein meals. Preventative foliar treatments with guar hydrolysates produced with Alcalase and 6N sulfuric acid demonstrated disease reduction compared with
the non-hydrolysed protein source. A positive correlation was found between efficacy of guar acid hydrolysates and degree of hydrolysis, suggesting that this hydrolysis method may enhance the functional properties of the original protein source. Positive correlations were also found between efficacy and concentrations of specific peptides and amino acids, which may contribute to the induction of resistance. Different from acid hydrolysates, no toxic effect on P. xanthii conidium germination was observed for the enzymatic hydrolysates, suggesting the activation of plant defense responses. Our results confirmed that the biocontrol activity of protein hydrolysates is affected by the original source, and the method and degree of hydrolysis. Further research is required to fully clarify the mode of action of these compounds.

This project was supported by the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 324416 (project INNOVA, theme FP7-PEOPLE-2012-IAPP), and the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 722642 (project INTERFUTURE).

Endo- and epi-phytic fungal communities of olive twigs is influenced by cultivar and olive knot infection. T. GOMES1,2, J.A. PEREIRA1, T. LINO-NETO2, A. BENNET3, P. BAPTISTA1. 1CIMO/ Polytechnic Institute of Bragança, School of Agriculture, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. phaptista@ipb.pt. 2Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. 3Ecological Sciences, The James Hutton Institute, Errol Road, Invergowrie, Dundee, DD2 5DA UK. E-mail: teresa.mdg@gmail.com

Olive tree phyllospheres are colonized by a diverse microbial assemblage that may interact with pathogenic fungi, making them potential candidates for disease suppression. Olive knot (OK) is caused by the bacterium Pseudomonas savastanoi pv. savastanoi (Psv.), causing significant economic losses in olive tree (Olea europaea). We evaluated the effects of cultivar and OK infection on endo- and epi-phytic fungal communities inhabiting olive twig tissues. For this, fungal composition and diversity was assessed in asymptomatic and OK-symptomatic twigs co-occurring olive cultivars with different susceptibilities to OK disease. Isolated species were identified using ITS rDNA sequencing. The cultivar and OK infection were important in shaping the endophytic and epiphytic fungal communities. Fungal community composition differed (P = 0.005) between olive tree cultivars, being Nectriaceae - the dominant family in cvs Cobrançosa and Verdeal Transmontana, whereas Pleosporaceae was dominant in the cv. Madural. Epiphytic and endophytic fungal communities also differed in size and composition in asymptomatic and OK-symptomatic twigs, for the three cultivars. In general, asymptomatic twigs had more diverse and rich populations (up to 1.4-fold) when com-
pared to OK-symptomatic twigs. Among the species identified in the asymptomatic tissues, *Cladosporium cladosporioides* was most frequently isolated within the epiphytic community, and *Chromelosporium carneum* within the endophytic community. In the OK-symptomatic tissues, *Cladosporium* sp. was the most frequently isolated within epiphytic community and *Fusarium lateritium* within the endophytic community. According to indicator species analysis, *C. carneum, Pyronema domesticum* and *Phoma aloeis* (IndVal up to 0.56) may be promising species for the OK suppression. Better acknowledgement should be developed to uncover their roles on olive tree health.

This work was supported by FEDER funds through COMPETE (Programa Operacional Factores de Competitividade), and with national funds from FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project EXCL/AGR-PRO/0591/2012. T. Gomes thanks FCT, POPH-QREN and MINO s/n, Córdoba, Spain. AGRH-PRO/0591/2012. T. Gomes thanks FCT, POPH-QREN and MINO

Constitutive secretion of pisatin in root exudates participates in pea defence against *Fusarium oxysporum* f. sp. *pisi*. N. RISPAIL, M. BANI, A. Ciminino, A. EVIDENTE, D. RUBIALES. 1 Instituto de Agricultura Sostenible, CSIC, Avenida Menendez Pidal s/n, Córdoba, Spain. 2 Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte. S. Angelo, Via Cintia 4, 80126 Napoli, Italy. 3 Ecole Nationale Supérieure de Biotechnologie, Ville universitaire Ali Mendjeli, BP E66 25100 Constantine, Algeria. E-mail: nrispail@ias.csic.es

Root exudates are important regulators of plant rhizospheres. They can modulate the composition and dynamics of soil micro-organisms and participate in the dialogue between plants and micro-organisms. They are known to modulate germination and growth of soil microorganisms. As such, they may contribute to crop resistance to soil-borne pathogens. To determine whether root exudates can contribute to defense against the pea root pathogen *Fusarium oxysporum* f. sp. *pisi* (*Fop*), and to identify the active metabolites, we studied the effects of the root exudates of 12 pea accessions with differential responses to the disease. Most root exudates stimulated the germination of *Fop* conidia in liquid bioassays. The root exudates of three accessions, by contrast, inhibited germination, indicating the presence of inhibitory substances in these root exudates. Ethyl acetate extraction of root exudates indicated that the inhibiting substances were contained in the apolar fraction, that contains most secondary metabolites. Further fractioning and analysis identified the pea phytoalexin pisatin as the most active metabolite, and pisatin was identified in the active fraction of pea root exudate extracts. This compound to inhibit *Fop* germination in liquid bioassay and its amount in root exudates was negatively correlated with the extent of *Fop* germination. These results indicate the existence of a pre-penetration mechanism in pea that can delay the build-up of pathogenic populations in soil. Our results also suggest an important role of pisatin in the constitutive defense of pea against *Fop*.

This research was supported by the European KBBE project LEGATO (FP7-KBBE2013.1.2-02-613551), and the Spanish national project AGL2014-52871-R from the Spanish Ministry of Economy and Competitiveness (MINECO), and was co-financed by the European fund for regional development (FEDER). NR holds a Ramón y Cajal post-doctoral position from MINECO.

Control of bacterial plant diseases using licorice extract with a new controlled release formulation. S. Jacobs1, H. Özaktan2, M. Akbab2, A. Akköprü3, I. Kleeberg3, A. Schikora3. 1 Trifolio-M GmbH, Dr.-Hans-Wilhelmi-Weg 1, 35633 Lahnau, Germany. 2 Department of Plant Protection, Faculty of Agriculture, University of Ege, 35100 Bornova Izmir, Turkey. 3 Federal Research Centre for Cultivated Plants (JKI), Institute of Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany. E-mails: Sophie.Jacobs@trifolio-m.de; Adam.Schikora@julius-kuehn.de

The lack of active substances for the control of bacterial diseases in plants causes serious problems. Only a few agrobactericides are available, but all have distinct shortcomings. The European Union banned the agricultural use of antibiotics in 2004 fearing antibiotic multi-resistant strains, which occurred in fire blight control/prevention in orchards in USA, New Zealand and Israel. In Europe, permission for antibiotic use is limited to cases of “clear and present danger”. Alternatively, different copper-based products are authorized as bactericides, but natural resistances in pathogenic *Pseudomonas* and *Xanthomonas* strains have become prevalent, and intensive copper sprays used to control the diseases resulted in
Assessment of biological control agents against Gnomoniopsis smithogilvyi (syn. castanea), the fungus causing chestnut brown rot and canker. M. CONTI1, J. CROVADORE1, B. COCHARD1, R. CHABLAIS1, M. JERMINI2, F. LEFORT1. 1Plants and pathogens Group, Institute Land Nature Environment, hepia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland. 2Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch

In vitro challenge tests were carried out between Gnomoniopsis smithogilvyi and seven antagonistic bacterial strains and nine fungal strains. Two genotypes of G. smithogilvyi from Geneva (GE1) and Ticino (TI1) were used during these tests. These in vitro challenge tests allowed selection of five fungal and three bacterial strains, which demonstrated strong inhibitory activity on growth of G. smithogilvyi. These were: Trichoderma harzianum B05, T. harzianum F1, T. hamatum, T. aureoviride and T. asperellum; and Pseudomonas putida, Bacillus amyloliquefaciens Ba4 and B. amyloliquefaciens Ba2. These organisms were retained for biological control experiments on chestnut scions. Batches of eight chestnut scions were inoculated with each fungal or bacterial antagonists by soaking them for 48 h at room temperature in bacterial or fungal suspensions in water. The scions were transferred individually into glass culture tubes and placed in a climatic chamber for 3 weeks. to allow a uniform endophytic growth of the antagonists. A suspension of G. smithogilvyi conidia was then applied to the scions of all modalities, and half of the control scions. The development of fructifications on bark of the scions, and the condition of the scions, were observed before and after inoculation, for a total duration of 6 weeks. Most of the organisms did not reduce disease in vivo, but the bacterial strain P. putida UASWS0946 and the fungal strain T. hamatum UASWS1405 totally inhibited the growth of G. smithogilvyi and C. parasitica.

This research was supported by the strategic research fund of the University of Applied Sciences and Arts Western Switzerland.

Preliminary characterization of the bioactive metabolites produced by Ascochyta lentis var. lathyri, responsible for a grasspea disease. A. BOARI1, A. CIMITINO, A. EVIDENTE2, A. INFANTINO3, M. MASI, M.C. ZONNO1, and M. VURRO1. 1Institute of Sciences of Food Production, National Research Council, via Amendola 122/O, 70126 Bari, Italy. 2Department of Chemical Sciences, University of Naples “Federico II”, Complesso Universitario Montesant’Angelo, via Cintia 4, 80126, Naples, Italy. 3Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria, Centro di ricerca per la patologia vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. E-mail: evidente@unina.it

Ascochyta lentis var. lathyri causes necrotic lesions on leaves and stems of grasspea (Lathyrus sativus L.) plants, recently described for the first time in Italy. This fungus was not pathogenic to seedlings of nine other leguminous species, including lentil (Lens culinaris Medik.). For this reason, and in consideration of its morphological characteristics, the fungus was considered a pathogenic, and morphological variant, of Ascochyta lentis (pathogenic to lentil), despite genetic similarities. Considering, (a) the increasing interest for the cultivation of grasspea as a source of protein and genetic resistance to diseases; (b) the known capability of the genus Ascochyta to produce biologically active secondary metabolites; and (c) the potential of comparative metabolic analysis to
provide valuable information on fungal ecology and evolution, studies were instigated to investigate the production of bioactive metabolites by A. lentis and A. lentis var. lathyrri. Two strains of each pathogen were grown on a defined liquid medium. Culture filtrates were extracted with organic solvents, and tested for bioactivity using different assays. The successive steps of the purification, bioassay guided, were performed by CC and TLC. This provided different bioactive fractions and metabolites, which are being characterized using spectroscopic and physical methods. Preliminary results have confirmed differences in the metabolic profiles of the strains, in agreement with other differences previously described.

Inhibition of early development stages of rusts and powdery mildew by fungal and plant metabolites. E. BARILLI¹, A. CIMMINO², M.J. GONZALEZ BERNAL¹, M. MASI, D. RUBIALES¹, A. EVIDENTE². ¹Institute for Sustainable Agriculture, CSIC, Córdoba, 14004, Spain. ²Department of Chemical Sciences, University of Naples “Federico II”, Complesso Universitario Montesant’Angelo, via Cintia 4, 80126, Naples, Italy. E-mail: evidente@unina.it

Foliar diseases caused by biotrophic pathogens, such as rusts and powdery mildews, are major limiting factors in legume and cereal production worldwide. Crop protection is largely based on chemical control, although there is renewed interest in the discovery of natural products as alternatives to synthetic fungicides. A plant and a fungal metabolite (respectively, inuloxin A and sphaeropsidin A), belonging to different classes of naturally occurring compounds, have been evaluated, together with a synthetic fungicide, at different concentrations on pea and oat plants for their potential to inhibit spore germination and subsequent fungal growth. Pathogens responsible of rust (Uromyces pisi) and powdery mildew (Erysiphe pisi) on pea, and these diseases on oat (P. coronata f. sp. avenae and Blumeria graminis f. sp. avenae), were artificially inoculated on their susceptible hosts under controlled conditions. Spore germination, plant penetration and fungal development were microscopically scored on detached leaves at 24 and 48 h after inoculation. Inuloxin A and sphaeropsidin A both reduced spore germination and fungal development of all the pathogens, at values comparable to those obtained by the synthetic fungicide, offering potential as natural fungicides for management of these diseases.

This research was supported by FP7-ARIMNet-MEDILEG and AGL2014-52871 projects and Programme STAR (A.C.) financially supported by UniNa and Compagnia di San Paolo, Italy.

Secondary bioactive metabolites produced by emerging forest pathogens in Sardinia. M. MASI¹, B. T. LINALDEDDU², A. CIMMINO², P. NOCERA¹, L. MADD AU³, A. EVIDENTE¹. ¹Department of Chemical Sciences, University of Naples “Federico II”, Complesso Universitario Montesant’Angelo, via Cintia 4, 80126, Naples, Italy. ²Università degli Studi di Padova - Dipartimento di Territorio e sistemi agro-forestali (TESAF), vialle dell’Università 16, 35020 Legnaro (PD). ³Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100, Sassari, Italy. E-mail: evidente@unina.it

Several studies have demonstrated the important role of fungal species in the aetiology of serious disease affecting forest trees in Sardinia (Italy). Among them, Diplodia corticola has serious impacts on oak ecosystems, adversely affecting vitality and productivity of trees. Recently, a severe trunk and branch disease caused by a newly described species, Dia- portella cryptica, was observed in several hazelnut groves in Sardinia. The considerable ecological relevance of forests, and severe damage caused by these fungi, requires increased knowledge of the bio-ecology of these invasive species, and particularly identifying the virulence factors involved in the pathogenesis processes. Extensive studies on liquid cultures of D. corticola have indicated the potential of this fungus to produce an array of bioactive secondary metabolites in vitro, some of which have shown potential in application studies. Sphaeropsidin A has been studied as novel therapeutic strategy to combat drug-resistant cancer. Other metabolites, such as diorcinol and diplopyrone B, have shown activity against important plant pathogens belonging to different phyla, especially Phytophthora spp. which are effectively controlled with few synthetic fungicides in forestry. Investigations carried out with organic extracts of D. cryptica have shown the ability of this pathogen to produce bioactive compounds. Organic extracts and column chromatographic fractions were
phytotoxic on tomato leaves at 2 mg mL\(^{-1}\) in a leaf puncture assay.

**Biofumigant action of *Brassica* seed meals against *Phytophthora cinnamomi* in dehesa ecosystems.**

M. GONZÁLEZ\(^1\), P. RÍOS\(^2\), P. FERNÁNDEZ\(^1\), A. DE HARO\(^2\), M.S. SERRANO\(^1\), M.E. SÁNCHEZ\(^1\). \(^1\)ETSIAM, Universidad de Córdoba, Ctra. Madrid-Cádiz km 396, 14014-Córdoba, Spain. \(^2\)Instituto de Agricultura Sostenible (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. E-mail: ag1sahem@uco.es

*Phytophthora cinnamomi* causes a highly destructive root rot that seriously affects oaks in seminatural woodlands (dehesa systems). Disease management using soil biofumigation is promising, but requires further validation. The effectiveness of ground seeds from *Brassica carinata* and *B. juncea* to inhibit mycelial growth and reduce chlamydospore viability of *P. cinnamomi* in soil was established, in contrast with the inability shown by *B. napus* seed meals. Reduced root necrosis in inoculated *Lupinus luteus* plants was also achieved when infested soils were treated with *B. carinata* or *B. juncea* seed meals. Glucosinolate content analyses of these seed meals indicated that effectiveness was related to large content of sinigrin (2-propenyl glucosinolate). Biofumigation with seed meals rich in sinigrin should be considered as an effective measure to be incorporated in the integrated control of the oak disease caused by *P. cinnamomi* in dehesa ecosystems. Aas host seed production levels are low, this approach should be best applied on agricultural lands.

This research was supported by the Project RTA2014-00063-C04-03 (INIA, Spain) and the European Union (LIFE11 BIO/ES/726).

**Control of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae:Bruchinae) adults through trichodene produced by *Trichoderma harzianum*.**

A. RODRÍGUEZ-GONZÁLEZ\(^1\), V. SUÁREZ-VILLANUEVA\(^1\), S. MAYO\(^1\), G. CARRO-HUERGA\(^1\), S. ÁLVAREZ-GARCÍA\(^2\), P.A. CASQUERO\(^1\), S. GUTIÉRREZ\(^2\).

\(^1\)Grup de Investigación en Ingeniería y Agricultura Sostenible, Instituto de Recursos Naturales, Medio Ambiente y Biodiversidad, Escuela Superior y Técnica de Ingeniería Agraria, Universidad de León, Avenida de Portugal 41, 24071-León, Spain. \(^2\)Área de Microbiología, Universidad de León, Avenida de Astorga s/n, 24401-Ponferrada, Spain. E-mail: alrog@unileon.es

Control of *Phytophthora* root rot on Mediterranean *Quercus* spp. Using fosetyl-Al trunk injections. M. GONZÁLEZ\(^3\), M.A. ROMERO\(^3\), C. RAMO\(^3\), M.S. SERRANO\(^3\), M.E. SÁNCHEZ\(^3\). \(^3\)ETSIAM, Universidad de Córdoba, Ctra. Madrid-Cádiz km 396, 14014-Córdoba, Spain. \(^4\)Estación Biológica de Doñana (CSIC), Américo Vespucio 26, 41092-Sevilla, Spain. E-mail: ag1sahem@uco.es

Potassium phosphite (PP) is the most used plant defense stimulant against *Phytophthora* diseases, but PP formulations are prohibited in Spain when registered as fertilizers. In previous pot experiments, fosetyl-aluminium (fos-Al) demonstrated better efficacy than PP for prevention of root disease caused by *Phytophthora cinnamomi* on *Quercus suber* and *Q. ilex*. In November 2014, 4% fos-Al was applied by trunk injection (one 20 mL-injector at each 20 cm-perimeter) to trees in two different *Quercus* woodlands affected by *P. cinnamomi*. For each woodland, 20 asymptomatic trees (defoliation class, DC = 0), 20 trees with low crown symptoms (10-25% defoliation, DC = 1), and 20 with moderate crown symptoms (26-50% defoliation, DC = 2), were randomly chosen. For each DC, ten trees were injected with fos-Al and ten with water only. All trees were sampled (rhizosphere soil and rootlets) before treatments. At 1 year intervals, all trees were evaluated for defoliation class, re-sampled (roots and soil). Samples were checked for *P. cinnamomi* presence (roots) and *P. cinnamomi* inoculum density (chlamydospores g\(^{-1}\) dry soil). Disease severity (variation in DC with time) decreased in treated trees when compared with untreated trees, mainly because the treated trees had decreased DCs, but also because untreated trees had increased DCs. Detection of *P. cinnamomi* in roots increased in the water-treated trees, although soil inoculum densities decreased with time, not differing between treated or untreated trees. This trial has demonstrated the effectiveness of fos-Al for prevention of *P. cinnamomi* oak disease in the field, decreasing crown symptoms and pathogen detection in roots.

This research was supported by the BBVA Foundation (Spain) and the European Union (LIFE11 BIO/ES/726).
Acanthoscelides obtectus (Coleoptera: Chrisomelidae: Bruchidae) of an emerging grape pest, Xylotrechus arundinaceum (a trichothecene producer) has been expressed in the wild isolate of T. harzianum CECT 2413 (a trichothecene non-producing strain), a potential biocontrol agent (BCA) of phytopathogens, to determine if trichodiene (a volatile sesquiterpene precursor) had ability to control different stages of insect pests. Trichoderma harzianum (T34) and its trichodiene producer transformant (T34-tri5.27) were used to determine, under laboratory conditions, their insecticidal activity against A. obtectus adults. The susceptibility of A. obtectus adults against T. harzianum (T34 and T34-tri5.27) was evaluated with a spray tower. One mL of spore suspension (1 × 10^5 spores mL^-1) of Trichoderma strains was applied directly to the insects. Mortality monitoring was carried out every 2 d after treatment for 14 d. T34 accumulated a mortality of 59%, whereas T34-tri5.27 reached 66%. The effects of both fungi were different (P < 0.05) to the control treatment. The ability of T. harzianum to control A. obtectus adults was increased by the production of trichodiene. Production of non-toxic volatile terpenes by BCAs could be an effective tool to ameliorate their potential to control insect pests, and hence make them more appealing than conventional insecticides.

Evaluation of five essential oils from Mediterranean aromatic plants for use as bio-fungicides in the control of Cladobotryum mycophilum in cultivated button mushroom. F.J. GEA¹, M.J. NAVARRO¹, M. SANTOS², F. DIÁNEZ², G. ORTÍZ-DE-ELGUEA³, R. SANCHEZ⁴, D. HERRAIZ². ¹Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain. ²Departamento de Agronomía, Escuela Politécnica Superior, Universidad de Almería, Almería, Spain. ³Departamento de Ciencia y Tecnología Agroforestal y Genética, ETSIAM- IDR (UCLM), Albacete, Spain. ⁴Centro de Investigación Agroforestal de Albaladejito (CIAF), Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF), Cuenca, Spain. ⁵Parque Científico-Tecnológico de Castilla-La Mancha, Albacete, Spain. E-mail: fjiea.cies@dipucuenca.es

Cobweb, caused by the mycoparasite Cladobotryum mycophilum, is one of the most serious diseases that affect cultures of white button mushroom (Agaricus bisporus) worldwide. Effective control of this disease includes the application of fungicides (metrafenone or prochloraz) and strict hygiene measures. However, biological control plays an important role. The essential oils (EOs) from five typically Mediterranean aromatic plant species belonging to the Labiatae family (Lavandula x intermedia, Thymus mastichina, Thymus vulgaris, Salvia lavandulifolia and Satureja montana) were tested against this pathogen. The plant material was cultivated in the experimental fields of CIAF Albaladejito – IRIAF. Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus, and chemically characterized by Gas Chromatography (GC-FID). Essential oils were assayed in vitro using a macrodilution test for antifungal activity against six isolates of C. mycophilum. The sensitivity of C. mycophilum was estimated from EC90 values (mg L^-1 of EO inhibiting radial mycelial growth by 50%). The most effective EOs for inhibiting in vitro growth of C. mycophilum were T. vulgaris (mean EC90 = 3.9 - 41.7 mg L^-1) and S. montana (EC90 = 9.4 - 27.8 mg L^-1). The main compounds in T. vulgaris EO were p-cymene (29.7%) and thymol (25.8%), while in S. montana EO carvacrol (17.2%), p-cymene (11.5%) and camphor (10.1%) predominated. Thymus vulgaris and S. montana EOs and their compounds, especially thymol and carvacrol, have potential alternatives to the synthetic fungicides that are applied in mushroom cultivation to prevent cobweb disease.

This research was supported by Project E-RTA2014-00004-C02-01 (Instituto Nacional de Investigación y Tecnología Agropecuaria, INIA, Spain) and the European Regional Development Fund (ERDF).

Assessment of specific traits of Pseudomonas fluorescens PICF7 for involvement in endophytic lifestyle, rhizosphere survival and biocontrol of Verticillium wilt of olive. N. MONTES-OSUNA, J. MERCADO-BLANCO. Department of Crop Protection, Institute for Sustainable Agriculture (CISC), Avenida Menéndez Pidal s/n, Campus “Alameda del Obispo”, 14004 Córdoba, Spain. E-mail: nuriamontes@ias.csic.es

Pseudomonas fluorescens PICF7 is a natural colonizer of olive rhizospheres, able to endophytically colonize the root tissues and act as an effective biocon-
trol agent against Verticillium wilt of olive. This disease is difficult to manage, and single control measures are mostly ineffective. An integrated management strategy is therefore recommended. Within this framework, biocontrol approaches represent an excellent option, particularly if they are combined with other disease management tools. We aim to identify and characterize genes of strain PICF7 implicated in phenotypes such as rhizosphere/soil persistence (copper resistance, 1-aminocyclopropane-1-carboxylate deaminase activity, ACC), root colonization (biofilm formation) and plant growth promotion (phytase activity). Previously, presence of a putative ACC gene (involved in degradation of the ethylene precursor) was suggested in the genome of PICF7. However, ACC deaminase activity was not demonstrated in this strain, whereas the presence of a putative D-cysteine desulhydrase coding gene was found. Approx. 4,000 tetracycline-resistant colonies from an available Tn5 random insertion mutant bank were screened for phenotypes defective in some of the traits mentioned above. A collection of 80 mutants was selected, including 34 showing reduced (or no growth) or colour change in medium supplemented with copper, ten with impaired biofilm formation, 18 unable to grow or with altered morphology in YEM medium, and 18 displaying reduced or no production of phytase. Molecular characterization of these mutants is currently being performed, to identify the affected genes and determine their involvement in (endophytic) colonization, biocontrol performance, and rhizosphere survival of strain PICF7.

This research is supported by grant P12-AGR667 (Junta de Andalucía, Spain), co-funded by the ERDF of the UE. The authors thank Antonio Valverde for excellent technical assistance.

Using endophytic bacteria to improve tomato growth and control bacterial spot caused by Xanthomonas euvesicatoria. A. AKKÖPRÜ. Yüzüncü Yıl University, Faculty of Agriculture Department of Plant Protection, Van, Turkey. E-mail: ahmetakkopru@yyu.edu.tr

This study evaluates effects of endophytic bacteria (EB) isolates (Ochrobactrum sp. CB36/1, Pantoea agglomerans CC37/2, Bacillus thuringiensis CA41/1, Pseudomonas fluorescens CC44) on bacterial spot disease of tomato caused by Xanthomonas euvesicatoria (Xe), and on tomato (cv. Marmande) growth parameters. The EB strains were applied twice to seedlings as drenches at the second leaf stage, and 4 d before inoculation of Xe. Suspensions of Xe (10⁶ cfu mL⁻¹) were applied by spraying on 4-week-old seedlings. Disease severity was evaluated using a 0-4 scale, 3 weeks after Xe inoculation. The seedlings were grown in peat medium at 24(±2)°C, 60% humidity, 14/10h day/night photoperiods in a climate chamber. Although the EB strains did not significantly affect plant length, all the strains increased fresh and dry weights of shoots by approx. of 6.5%. Root fresh weights were increased by 9.5% to 52% by the isolates, and Bacillus thuringiensis CA41/1 was the most effective isolate. Root dry weights were increased by 31% by the Pantoea agglomerans CC37/2, even under the disease pressure. Although all EB isolates except B. thuringiensis CA41/1 decreased the disease severity compared with positive controls, the most effective isolate was Ochrobactrum sp. CB36/1 which decreased severity by 32%.

Assessment of water extracts and essential oils from Mediterranean plants against Verticillium dahliae in olive. A. MULERO-APARICIO, A. VARO, M. ADEM, L.F. ROCA, M.C. RAYA-ORTEGA, FJ. LÓPEZ-ESCUDERO, A. TRAPERO. Departamento de Agronomía (Patología Agroforestal), Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Celestino Mutis (C4), 14071 Córdoba, Spain. E-mail: z32muapa@uco.es

Verticillium wilt of olive is considered the most concerning disease in all olive growing areas. In recent years, biological control within integrated disease management has gained importance, due to the lack of an effective treatment against this disease. There are several plant substances, including water extracts, essential oils or biological products, that possess antifungal activity, are easily degraded, and are safe for human use and the environment. This study describes the potential effect of 44 plant extracts and 20 essential oils against Verticillium dahliae. The results demonstrate the in vitro and in planta effectiveness of essential oil from Thymus, in particular Thymus sp. 04 (prepared in the laboratory) and the commercial product Thymus sp. 01, against V. dahliae. Inhibition of mycelial growth and microsclerotia reached 100% for both treatments. Disease re-
durable and stable product formulation, for assess the use as natural fungicides.

Effectiveness of a non-pathogenic strain of Fusarium oxysporum (FO12) against Verticillium dahliae. A. MULERO-APARICIO, A. VARO, A. TRAPERO. Departamento de Agronomía (Patología Agroforestal), Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Celestino Mutis (C4), 14071 Córdoba, Spain. E-mail: z32muapa@uco.es

The strain FO12 of Fusarium oxysporum, isolated from cork, was one of the most effective treatments against Verticillium dahliae, from more than 200 natural products evaluated in previous studies. The present study evaluated potential of FO12 as biocontrol agent (BCA) against Verticillium wilt of olive (VWO). In vitro and in vivo studies were carried out to determine the mode of action of FO12. These included: dual cultures and mycelial growth of V. dahliae in potato dextrose agar amended with serial amounts of sterilized crude FO12 extract; efficacy of crude extract, conidia, supernatant and chlamydospores against VWO in controlled conditions and on inoculum reduction of V. dahliae in naturally infested soils; and possible induction of resistance in olive plants through foliar or irrigation treatments in controlled conditions. In addition, FO12 was evaluated under partially controlled and field conditions with irrigation treatments. Antibiosis and parasitism mechanisms were not observed in dual culture essays, but mycelial growth of V. dahliae was reduced in the presence of FO12. The treatments with the crude extract and chlamydospores were effective both for controlling VWO and reducing V. dahliae inoculum in naturally infested soil under in vitro, partially controlled and field conditions. FO12 can easily produce chlamydospores, favouring a durable and stable product formulation, for assessment of potential of FO12 as BCA against VWO in field conditions.

Biological control of quarantine bacterial diseases with selected strains of Bacillus amyloliquefaciens. J. CABREFIGA, I. MORA, E. MONTESINOS. Center for Innovation and Development in Plant Health (CIDSAV), Institute of Food and Agricultural Technology, University of Girona, Maria Aurèlia Capmany, 61, 17003 Girona, Spain. E-mail: jordi.cabrefiga@udg.edu

Emerging quarantine bacterial plant diseases are important problems in crop production in the European Union. Some of the more relevant are caused by Erwinia amylovora, Pseudomonas syringae pv. actinidiae, Xanthomonas arboricola pv. pruni and Xanthomonas fragariae. There are considerable difficulties in control of fruit production losses and preventing the spread of these pathogens. Chemical control in EU countries is limited to copper compounds, because antibiotics are not authorized. Therefore, there is urgent need for alternative or complementary control methods. An extensive collection of strains of Bacillus subtilis and B. amyloliquefaciens, obtained from Mediterranean crops and forest, that have been screened for the control of these bacterial diseases. The first step was to build a collection of strains having the biosynthetic genes related with to production of antimicrobial compounds, the actual production of these compounds, and the range of antagonistic in vitro activity against these plant pathogenic bacteria. The best strains were tested for control of infections in the corresponding plant hosts (pear, peach, strawberry, kiwifruit), under controlled environment conditions in a greenhouse, and under semi-field assays. Promising results were obtained in the efficacy of control in comparison with commercial biological products.

Study of diversity of endophytic communities from Posidonia oceanica and their in vitro antagonistic activities against Pythium aphanidermatum. M. SANTOS, F. DIÁNEZ, J.M. PERALCS, F.J. GEA, M.J. NAVARRO. Agronomy Department. University of Almeria. Carretera Sacramento s/n. Almería 04120. Spain.msantos@ual.es Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintero del Rey, Cuenca, Spain. E-mail: msantos@ual.es

Most of the Mediterranean sublittoral area occupied by seagrasses is dominated by the endemic plant Posi-
donia oceanica. Despite its ubiquity and dominance in the Mediterranean Sea, the mycoflora of *P. oceanica* has been rarely studied. We isolated and identified fungal endophytes in the roots of this plant. In addition, we selected fungal strains able to produce antagonistic substances in vitro against *Pythium aphanidermatum*. In summer 2016 at three sampling points were investigated along Parque Natural de Cabo de Gata-Nijar, Almería (Spain). Roots of *P. oceanica* were collected using scuba diving. Roots of 45 plants were carefully excavated from the substrate, separated from the shoots, and placed in 100 mL capacity beakers filled with seawater. These were stored in the dark at 4°C. The rhizomes were carefully washed in tapwater. Roots were surface-sterilised by sequential washing in 5% NaOCl for 5 min, 95% EtOH for 1 min and 5% H2O2 for 3 min, and then rinsed three times in distilled sterile water. Root fragments were aseptically excised and placed on malt-extract agar (MEA, Oxoid) in Petri dishes (10-cm diam.) for preliminary morphological identification on the basis of macroscopic and microscopic characters. Fifteen fungal taxa (mainly Ascomycota, and in the Pleosporales), were identified. Antagonistic capacity of *Gliomastix* sp., *Papulaspora* sp. Cladosporium sphaerospermum had greater higher antagonistic activity against *P. aphanidermatum* than the other isolated fungi.

**Antifungal activity of plant essential oils against Trichoderma aggressivum f. sp. europaeum.** F. DÍANEZ1, C. PARRA1, M.J. NAVARRO2, F.J. GEA2, M. SANTOS3. Departamento de Agronomía. Universidad de Almería. Carretera Sacramento s/n. Almería 04120. Spain. 2Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), 16220 Quintanar del Rey, Cuenca, Spain. E-mail: msantos@ual.es

This study investigated the fungicidal activity against *Trichoderma aggressivum* f. sp. *europaeum* of essential oils (EOs) from: *Szegyium aromaticum*, *Pelargonium graveolens*, *Lavandula angustifolia*, Cupressus sempervirens, Mentha piperita, Santolina chamaecyparissus, Citrus sinensis, *Podostemon patchouli*, Thymus mastichina, *Thymus vulgaris*, *Eucalyptus globulus* and *Rotundifolia* officinalis. The disc diffusion method was used to evaluate the inhibition of hyphal growth. Test discs were prepared with 8 μL of each oil at concentrations of 5, 10, 15, 20 and 30%, and control discs with 1% Tween-80. These discs (5 mm diam.) were arranged around the *T. aggressivum* colony on each agar plate, at a distance of 4 cm, and incubated at 28°C for 1 week. The inhibition of hyphal growth was visually evaluated and photographed. EOs of rosemary (5%, growth inhibition >50%), mentha (15%, >70%), *Patchouli* and *Lavandula* (15%, >50%) inhibited hyphal growth of *T. aggressivum*. The other EOs did not reduce growth of hyphae at the concentrations tested. This study has indicated that rosemary, which is available and cost effective, is an attractive option for further investigations as an alternative to synthetic fungicides for the control of green mold caused by *T. aggressivum*.

This research was supported by the Project E-RTA2014-00004-C02-O1 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain) and the European Regional Development Fund (ERDF).

**Screening of potential biocontrol bacteria against Pseudomonas savastanoi pv. Savastanoi, and elucidation of their modes of action.** D. MINA1, J. PEREIRA1, T. LINO-NETO2, P. BAPTISTA1, 1CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. 2BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt

Over the last decades, the olive knot disease, caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv), has been responsible for severe damage in olive orchards. Reduced vigour and stem dryness caused by the pathogen lead to decreased olive fruit production, and severe losses for farmers. Bacterial endophytes and epiphytes from olive tree phyllospheres were screened for the suppression of Psv. Several mechanisms for this activity were also studied by evaluating indoleacetic acid (IAA), siderophore and lytic enzyme production. Interspecific interactions were assessed on solid media with agar overlays. IAA was estimated spectrophotometrically, and siderophores and lytic enzymes were evaluated qualitatively. Several tested bacterial species reduced Psv growth by up to 70%, as well as its viability. The greatest inhibition was observed for *Frondihabitans* sp. and *Paenibacillus* sp. Reduced production of IAA and siderophores by Psv, which are associated with knot development, was detected in the presence of the most efficient bacteria. Produc-
tion of lytic enzymes by antagonists, such as lipase, chitinase, protease and amylase, was also identified. These results indicate that some of the bacteria tested have potential as biocontrol agents, due to their capacity to produce metabolites/lytic enzymes that can interfere with PsV growth and/or development of knots. These potential biological agents should be further evaluated under natural conditions.

This work is supported by FEDER funds through COMPETE (Programa Operacional Factores de Competitividade) and by national funds by FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project EXCL/AGR-PRO/0591/2012. D. MINA thanks the Fundação para a Ciência e Tecnologia (FCT), Portugal for the Ph.D. grant SFRH/BD/105341/2014.

Molecular characterisation of Pochonia chlamydosporia isolates associated with root-knot nematodes. J. HORTA, I. ABRANTES, M.C. VIEIRA dos SANTOS. Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Universidade de Coimbra, P-3000 456 Coimbra, Portugal. E-mail: mcvs@sapo.pt

Root-knot nematodes (RKN; Meloidogyne spp.) are among the most economically damaging soil-dwelling parasites of agricultural crops. Exploitation of natural enemies of nematodes could lead to successful pest management strategies. Pochonia chlamydosporia is a widespread facultative parasite of nematode eggs that has been developed as a biocontrol agent. However, knowledge of the genetic diversity of naturally-occurring of P. chlamydosporia populations is still limited. This study identified and characterised Portuguese P. chlamydosporia isolates associated with RKN. Three tomato root samples infected with Meloidogyne spp. from three plots of a greenhouse in Setúbal, Portugal, were screened for the presence of P. chlamydosporia. Before screening, RKN females were identified by esterase phenotyping. Three phenotypes were detected: Hi4 (M. hispanica), I2 (M. incognita) and J3 (M. javanica). Pochonia chlamydosporia isolation was carried out by plating nematode eggs and roots on a semi-selective medium. Ten isolates were obtained and their identities confirmed by PCR using specific diagnostic primers derived from the β-tubulin gene. Intra-specific variation was evaluated by enterobacterial repetitive intergenic consensus (ERIC) PCR and restriction fragment-length polymorphism (RFLP) of the ITS region. A Portuguese isolate from Globodera rostochiensis eggs and two non-native isolates, Vc10 (IMI 331547) from Brazil and Pc3922 (IMI SD 187) from Cuba, both originally obtained from M. incognita eggs, were also analysed. Clustering analysis of ERIC-PCR profiles revealed similarities related to the geographic origin of the isolates, and there seems to be no relation between the clusters and the host nematode species.

This work was supported by FEDER – “Fundo Europeu de Desenvolvimento Regional” funds through the COMPETE 2020 – Operacional Programme for Competitiveness and Internationalisation (POCI), and by Portuguese funds through FCT – “Fundação para a Ciência e a Tecnologia” in the framework of the project POCI-01-0145-FEDER-016611 (PTDC/AGR-PRO/3438/2014). A grant was also made to M.C. Vieira dos Santos ((SFRH/BPD/92308/2013) supported by national funds FCT /MCETS and the European Social Fund through the “Programa Operacional do Capital Humano” – POCH of the National Strategic Reference Framework.

Assessment of specific traits of Pseudomonas fluorescens PICF7 for their involvement in endophytic lifestyle, rhizosphere survival and biocontrol of Verticillium wilt of olive. N. MONTES-OSUNA, J. MERCADO-BLANCO. Department of Crop Protection, Institute for Sustainable Agriculture (CSIC), Avenida Menéndez Pidal s/n, Campus “Alameda del Obispo”, 14004 Córdoba, Spain. E-mail: nuriamontes@ias.csic.es

Pseudomonas fluorescens PICF7 is a natural colonizer of olive rhizospheres, able to endophytically colonize root tissues and act as an effective biocontrol agent against Verticillium wilt of olive. This disease is difficult to manage, and single control measures are mostly ineffective. An integrated management strategy is therefore recommended. Biocontrol approaches represent an excellent option, particularly if they are combined with other disease control methods. We identified and characterized genes of strain PICF7 implicated in phenotypes such as rhizosphere/soil persistence (copper resistance, 1-aminocyclopropane-1-carboxylate deaminase activity, ACC), root colonization (biofilm formation), and plant growth promotion (phytase activity). Presence, in the genome of PICF7, of a putative ACC gene (involved in degradation of the ethylene precursor), was previously suggested. However, ACC deaminase activity was not demonstrated in PICF7,
Characteristics of the biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606. S. TIENDA, C. VIDA, A. DE VICENTE, F.M. CAZORLA. *Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora”* (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: sandratienda@uma.es

The major disease affecting avocado crops in the Mediterranean area is white root rot, caused by *R. necatrix*. The biocontrol rhizobacterium *Pseudomonas chlororaphis* PCL1606 has been isolated from rhizospheres of healthy avocado trees, growing in an area affected by white root rot. As a main characteristic, PCL1606 showed strong in vitro antagonism against *R. necatrix* and other important soil-borne pathogens, mainly due to the production of the antimicrobial compound 2-hexyl, 5-propyl resorcinol (HPR). Production of other antifungal compounds has also been detected. PCL1606 persists and colonizes avocado roots, closely interacting and colonizing hyphae of *R. necatrix*, leading to negative effect on the fungus. These phenotypes, acting together, allowed PCL1606 to display biocontrol activity towards *R. necatrix* in avocado plants. We have observed that PCL1606 shows no plant growth promoting activities. The availability of the complete genome sequence of PCL1606 will allow identification of additional features involved in biocontrol by this bacterium.

This work is supported by National plan I+D+I MINECO (AGL2014-52518-C2-1-R; MINECO, Spain), and co-funded by FEDER (EU). S. Tienda is funded by a grant from FPI program MINECO.


Mycoviruses that cause hypovirulence are potential biocontrol agents of their fungal hosts. In previous research, we characterized FodV1, a chryso-like mycovirus found in isolate Fod116 of *Fusarium oxysporum* f. sp. *dianthi* (Fod). The transference of FodV1 to a new Fod recipient isolate evidenced the induction of hypovirulence in the fungal host. We have analysed the prevalence of FodV1 as well as the incidence and diversity of mycoviral dsRNAs in a collection of 300 Fod isolates. RT-PCR using total RNA extracts and specific primers for the RdRp segment of FodV1, and subsequent sequence analysis, showed that mycovirus FodV1 was present in only three additional Fod isolates. Cellulose column chromatography analysis showed the presence of other dsRNA molecules in 40 isolates. These dsRNAs corresponded to at least five banding patterns, characteristic of different viral families, and three of them were selected for further characterization. Partial sequence data indicated that a monopartite 2.5 kb mycovirus corresponds to a mitovirus, and that a quartipartite mycovirus shows high homology with Aspergillus foetidus dsRNA mycovirus, and probably corresponds to a new member of the family Alphavirus. A third monopartite 9.5 kb mycovirus (FodV2) has been almost fully sequenced. This shows high homology with a number of previously described hypoviruses. To determine the putative hypovirulent nature of...
FodV2, we transferred it by hyphal anastomosis to a new hygR-tagged recipient isolate, and analysed its effect on some hypovirulence-associated phenotypic traits. Results obtained indicated that FodV2 does not induce hypovirulence in its fungal host.

This research was supported by the Project AGL 2013-48980-R, from the Spanish Ministry of Economy and Competitiveness, co-funded by the European Union (FEDER funds).

Role of the gluconic acid production by the rhizobacterium Rahnella aquatilis in pH regulation and biocontrol of the vascular wilt fungus Fusarium oxysporum. D. PALMIERI1, F. DE CURTIS1, D. VITULLO1, A. DI PIETRO2, G. LIMA1, D. TURRA2. 1Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc - 86100 Campobasso, Italy. 2Department of Genetics, University of Cordoba, Campus Rabanales, Ed. Gregor Mendel - 14071 Cordoba, Spain. E-mail: davide.palmieri@studenti.unimol.it

pH affects all aspects of life. Microbes have evolved efficient mechanisms of ambient pH adaptation and modification. In plant rhizospheres, secretions from roots promote the proliferation of microbes, which can alter the pH of this ecological niche. Previous research revealed that rhizosphere pH acts a key factor during infection of the vascular wilt fungus F. oxysporum f. sp. lycopersici (Fol) on its host plant tomato (Solanum lycopersicum). While non-infected roots acidify the extracellular environment, infection by Fol results in marked root alkalization, which promotes fungal pathogenicity. We studied the role of pH modification by the soil-inhabiting Gram-negative bacterium Rahnella aquatilis (Ra) in its interaction with Fol in the tomato rhizosphere. Co-inoculation of tomato roots with Ra provided efficient protection from vascular wilt caused by Fol. Ra produced strong extracellular acidification, both in artificial media and in the tomato rhizosphere, most likely through production of gluconic acid from glucose through the enzyme glucose dehydrogenase (Gcd). Preventing rhizosphere acidification by Ra, either through application of a buffer solution or by targeted deletion of the bacterial Gcd gene, led to loss of the biocontrol activity against Fol. These results suggest that extracellular pH regulation plays a key role in the interaction between bacteria and fungi in the rhizosphere, with important consequences for plant health.

This research was supported through project BIO2013-47870-R from the Spanish Ministerio de Innovación y Competitividad (MINECO).

Effects of farnesol production by Trichoderma on the development of bean (Phaseolus vulgaris). S. MAYO1, A. RODRIGUEZ-GONZÁLEZ2, O. GONZÁLEZ-LÓPEZ1, A. LORENZANA1, G. CARRO-HUERGA1, M.P. CAMPELO3, S. GUTIÉRREZ2, P.A. CASQUERO1. 1Research Group of Engineering and Sustainable Agriculture, Natural Resources Institute, University of León, Av. Portugal 41, 24071 León, Spain. 2Area of Microbiology, Research Group of Engineering and Sustainable Agriculture, University School of Agricultural Engineers, University of León, Ponferrada Campus, Av. Astorga s/n, 24401 Ponferrada, Spain. E-mail: pacasl@unileon.es

Common bean (Phaseolus vulgaris) is the third most important food legume worldwide, surpassed only by soybean and peanut. Trichoderma (Teleomorph: Hypocrea) is a fungal genus found in the soil. These fungi are secondary, fast growing, opportunistic invasive organisms, which produce enzymes that degrade fungal cell walls, and induce production of compounds with antimicrobial activity. We evaluated the effect of farnesol production of T. harzianum (T34) on the development of bean. In vivo assays were performed with this isolate and two transformants (T34dpp1.2 and T34dpp1.3) which were overexpressing the dpp1 gene. Bean seeds were coated with a spore suspension of each Trichoderma isolate. They were sown and maintained with a photoperiod of 16 h light, 25°C/16 °C (day/night), and 60% RH. Plants were removed 45 d after sowing, evaluated for: hypocotyl diameter, root system length,and dry weights of shoots and roots. T34dpp1.3 and control plants (without fungi) were larger than plants inoculated plant with T34, in hypocotyl diameter, root system length, and shoot dry weight. However, T34 did not present differences in comparison with T34dpp1.3 for root system dry weight root system, but T34dpp1.2 did.

This research was supported by the National project (AGL2012-40041-C02-02) (Ministry of Economy and Competitiveness) and by the Regional project (LE228U14) (Junta de Castilla y León).
Biological control of *Pseudomonas savastanoi* pv. *savastanoi* by two bacteria isolated from olive tree phyllospheres. D. MINA, A. SANTOS, J. PEREIRA, T. LINO-NETO, P. BAPTISTA. 1CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. 2BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt

Olive knot disease, caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv), has been responsible for severe crop losses in olive orchards, especially in Mediterranean countries. Olive knot cannot be eradicated once it is established in an orchard, so control is based on preventative measures. Previous laboratory experiments showed the capacity of some bacterial species, isolated from olive tree phyllospheres, to inhibited Psv growth. The two most promising bacterial isolates (*Fronthihabitan* sp. and *Paenibacillus* sp.) were evaluated for the control of Psv in olive plantlets (*Olea europaea*) under greenhouse conditions, to predict their effects in natural conditions. In pot experiments, 2-year-old olive plants (cv. Cobrançosa) were inoculated with the antagonistic bacteria and Psv, individually or in combination. Inoculations were performed in wounds previously made in three different sites of the main stem of each plant. Thirty replicate plants were used per strain. The plants were observed for symptom development and the number of bacteria on the inoculation sites was periodically evaluated, for up to 120 d after inoculation. To quantify the reduction of symptom expression, knots were excised from stems and their weights were compared between treatments. Inoculation with Psv resulted in the formation of knots with greater weights compared to treatments. Inoculation with Psv and both tested bacteria also reduced the amount of Psv in the inoculation sites, suggesting their effectiveness for reducing multiplication of the pathogen. Data presented demonstrate, for the first time, this bacterial potential in suppressing olive knot, and these two species should be considered in the future as potential biocontrol agents against Psv.

This work is supported by FEDER funds through COMPETE (Programa Operacional Factores de Competitividade) and by national funds by FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project EXCL/AGR-PRO/0591/2012. D. Mina thanks the Fundação para a Ciência e Tecnologia (FCT), Portugal for the Ph.D. grant SFRH/BD/105341/2014.

Antimicrobial activity of natural plant compounds against phytopathogenic bacteria and interference with quorum sensing. A. CARUSO, A. ANZALONE, L. GURRIERI, S. PROVENZANO, P. BELLA, R. PALMERI, V. CATARA, G. LICCIARDELLO. 1Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. 2Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze Ed. 4, 90128 Palermo, Italy. E-mail: gralicci@unict.it

Natural plant products have received a great deal of attention as sustainable alternatives for management of plant diseases caused by bacteria. We evaluated the antimicrobial activity of citrus peel components and phenols with relevant antioxidant activity (cathecol, citronellol, esperidin, limonene, quercitin and rutin) against nine phytopathogenic bacteria in the genera *Clavibacter*, *Erwinia*, *Pectobacterium*, *Pseudomonas* and *Xanthomonas*. The greatest inhibitory activity was induced by cathecol against *Xanthomonas* species and *P. syringae* pv. *Tomato*, and by citronellol against *C. michiganensis* subsp. *michiganensis* and *E. amylovora*. Cathecol minimum inhibitory concentrations ranged from 0.5 to 0.0625 mg mL\(^{-1}\), and those for citronellol were 1 to 0.125 mg mL\(^{-1}\). In addition, the ability to inhibit the quorum sensing (QS) cell-to-cell signaling system, which controls the virulence behaviour of a broad spectrum of bacterial pathogens, was evaluated. Using *Chromobacterium violaceum* as a biosensor system, citronellol was active against medium chain N-acyl-homoserine lactones preventing the production of violacein, as indicated by the lack of pigmentation of the indicator organism in vicinity of the treated disks. To determine if this suppression was linked to anti-virulence activity, the effect of citronellol was tested in the QS active phytopathogen *Pseudomonas corrugata* strain CFBP 5454, causal agent of tomato pith necrosis, in which the PcoIR AHL-based signaling system regulates production of phytotoxic cyclic lipopeptides (CLPs). Consistently with QS inhibition, the relative expression of genes contributing to the production of the CLPs cormycin and corpeptins was reduced in a concentration-dependent manner.
in response to non-lethal concentrations of citronellol.

Innovative approaches in plant disease diagnosis and management

Establishment of specific molecular diagnostic tests for *Gnomoniopsis smithogilvyi* (syn. castanea) and *Cryptonectria parasitica*. M. CONTI¹, J. CROVADORE², B. COCHARD³, R. CHABLAIS², J.B. MEYER², M. JERMINI¹, F. LEFORT¹. ¹Plants and Pathogens Group, Institute Land Nature Environment, hepia, University of Applied Sciences and Arts Western Switzerland (HES-SO), 150 route de Presinge, 1254 Jussy, Switzerland. ²Unit Biodiversity and Conservation Biology, Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmendorf, Switzerland. ³Agroscope, Cadenazzo Research Centre, A Ramél 18, 6593 Cadenazzo, Switzerland. E-mail: francois.lefort@hesge.ch

Two fungi cause chestnut tree diseases in Switzerland: *Cryptonectria parasitica*, the endemic chestnut canker agent, and *Gnomoniopsis smithogilvyi*, an endophytic fungus, recently identified in Europe and Switzerland as the main agent of chestnut fruit brown rot, also causing chestnut canker. *Gnomoniopsis smithogilvyi* causes high plant mortality in young chestnut nurseries and orchards. Presence of these fungi was evaluated in plant material used for the multiplication of six of chestnut varieties in Ticino, using specific molecular diagnostic tests developed for both species. All sequences available in GenBank for the internal transcript spacer (ITS) of the ribosomal DNA, the elongation factor 1-alpha (EF1a) gene and the beta-tubulin gene (TUBB), were collected for these two fungi. Significant differences between *G. smithogilvyi*, *Gnomoniopsis ssp.* and *C. parasitica* were sought. After analysing 164 ITS, 90 EF1a and 45 TUBB sequences, only the TUBB gene sequences showed any significant differences between the species. Specific PCR primers for each species were then designed from the TUBB sequences alignment. In silico analyses with BLAST (GenBank) confirmed the strict specificity of these primers. The two primer pairs were then tested with DNA extracted from previously characterised isolates of *G. smithogilvyi* and *C. parasitica* from Ticino, Wallis and Geneva, from roots and stems of germinated chestnuts or leaves of chestnut trees. These tests showed great robustness, and provide a tool to indicate the phytosanitary status of propagation material, especially for the endophyte *G. smithogilvyi*.

This research was supported by the strategic research fund of the University of Applied Sciences and Arts Western Switzerland.

Does resistance to *Plasmopara viticola* in grapevine influence infectivity of sporangia? F. BOVE, T. CAFFI, V. ROSSI. Department of Sustainable Crop Production, Diproves, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy. E-mail: federica.bove@unicatt.it

Partial plant resistance impacts on different epidemiological components of pathogens, which modify dynamics of disease epidemics. In *Plasmopara viticola*, the causal agent of grapevine downy mildew, different morphological characteristics have been observed between sporangia originated from lesions on susceptible and resistant hosts. This study evaluated whether, in addition to morphological modifications, partial host resistance can affect the infectivity of *P. viticola* sporangia, i.e., their ability to cause infection. Artificial inoculation experiments were performed between 2014 and 2016. A population of *P. viticola* sampled from susceptible vineyards was used for artificial inoculations on leaf discs of cv. Merlot and of fifteen grape breeding lines showing partial resistance, conferred by one or more *Rpv* loci. The sporangia produced on lesions originating on the susceptible and resistant varieties were then re-inoculated on leaf discs of cv. Merlot at three different vine growth stages (shoot elongation, full flowering, ripening of berries), and the infection efficiency was evaluated as the proportion of inoculation sites showing disease symptoms. There were no significant differences for the infection efficiency of sporangia produced on the different host varieties.

This research was supported by the European collaborative project InnoVine, from the European Union’s Seventh Framework Programme for research, technological development and demonstration, under grant agreement N° 311775.

Development of DDct Real Time RT-qPCR for the detection of *Onion yellow dwarf virus*. A. TIBER-
Cytogenomic analyses reveal nuclear content variation along the life cycles of the Pucciniales (rust fungi). T. RIBEIRO1, C. FEITEIRA1, S. TAVARES1,2, A.P. RAMOS1, M. MONTEIRO1, M. COELHO5, M.C. SILVA1, J. LOUREIRO6, L. MORAIR-CECILIO6 and P. TALHINHAS1,2. 1LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomía, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. 2Centro de Investigación das Ferrugens do Caféeiro, Instituto Superior de Agronomía, Universidade de Lisboa. Quinta do Marquês, 2780-505 Oeiras, Portugal. 3Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg Copenhagen, Denmark. 4Instituto Gulbenkian de Ciência. R. Quinta Grande, 6. 2780-156 Oeiras, Portugal. 5CREM-Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa. Quinta da Torre, Campus Universitário, 2829-516 Caparica, Portugal. 6CFE, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra. 3001-401 Coimbra, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt

Onion yellow dwarf virus (OYDV, genus Potyvirus), an aphid stylet-borne virus, was identified in Italy in 1993, and in the Italian onion variety ‘Rossa di Tropea’ in 2005. First investigations for OYDV were performed using serology, whereas, more recently, a specific RT-PCR test was used to examine the incidence of the virus in ‘Rossa di Tropea’, in bulb and seed production cycles. The correlation was assessed between OYDV infection and nutraceutical compounds in ‘Rossa di Tropea’, and a specific Real Time RT-qPCR assay was developed for OYDV. Specificity has been evaluated by including no target viruses related to OYDV and/or viruses generally found in onion. Analytical sensitivity was determined using ten-fold dilution series in crude extracts, either from leaf or bulb samples derived from field trials and from surveys carried out in Calabria (Southern Italy). The analytical sensitivity was directly compared with ELISA and end point RT-PCR, and allowed detection of the virus up to the dilution limit of 1 × 10⁶ for leaves and 1 × 10⁵ for bulbs. A DDCt Real Time RT-qPCR assay was performed using the 5.8S rDNA gene as reference to normalize the relative quantification data. This assay allowed investigation of the modulation of virus titre in the OYDV - ‘Rossa di Tropea’ pathosystem.

This research was supported by the SIORTO research project funded by the Italian Ministry of Education, University and Research.

Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens with complex life cycles (up to five spore types). The urediniosporic infection cycle is frequently the most important for pathogen dissemination, as the only stage capable of multiple uninterrupted repetition. The cell nuclear content of rust fungi is thought to follow that of other Basidiomycota, with haploid nuclei throughout the life cycle, only becoming diploid upon karyogamy in telia and immediately returning to the haploid state as meiosis takes place leading to the formation of basidiospores. The presence of 1C, 2C and a low proportion of 4C nuclei was recently detected in different stages of the urediniosporic cycle of several rust fungi, using genome size quantification techniques. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, compatible with the occurrence of karyogamy and meiosis prior to urediniospore formation, although endopolyploidy or other parasexuality phenomena cannot be ruled out. This unexpected phenomenon may be transversal to the Pucciniales, since it has been detected in over 60 rust species, with no apparent phylogenetic structural forms.

This research was financially supported by the Project PTDC/BIA-MIC/1716/2014 (Fundação para a Ciência e a Tecnologia, Portugal).

A diagnostic microarray for the multiplex characterization of strains of the Ralstonia solanacearum species complex. G. CELLIER1, S. ARRIBAT2, F. CHIROLEU3, P. PRIOR1, I. ROBENE2. 1Tropical Pests and Diseases unit, Plant Health Laboratory, ANSES,
Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (Rssc), is one of the most destructive plant diseases worldwide. Rssc affects a wide host range, and includes several ecotypes that represent major constraints and are under strict regulation (e.g. brown rot or Moko strains). The reliable characterization of epidemiological strains at the ecotype level is a challenge because of this complexity, and is generally achieved by combining several diagnostic protocols. We used microarray technology (ArrayTube) to develop a standard protocol that performs a multiplex characterization of RSSC strains in a single reaction, from the phylotype to the ecotype level (17 targeted groups of interest). Based on 27 sequenced genomes of RSSC, probes were designed with a 50-mer length constraint and thoroughly evaluated for any flaws or secondary structures. Validation data performed on 75 target and 12 non-target strains showed strong intra- and inter-repeatability, reproducibility, and good specificity, which allowed for the accurate detection of the 17 groups of interest. This custom microarray represents a significant improvement in the epidemiological monitoring of Rssc strains worldwide, and it has the potential to provide insights for phylogenetic incongruence of Rssc strains, based on the host of isolation. The microarray may be used to indicate potentially emergent strains.

This research was supported by the European Union (POSEIDOM phytosanitaire, 2011/132/UE, 2012/182/UE, 2013/175/UE, C(2014)8353, the Conseil Régional de La Réunion, and the French National Research Institutes ANSES, INRA and CIRAD.

Selection of genetic variants of *Citrus tristeza virus* as a strategy to protect against severe seedling yellows strains. G. SCUDERI1,2, R. FERRARO2, M. RUSSO1,2, M. C. BAZZANO1, A. CATARA2, G. LICCIARDELLO1,2,3. 1Agrobiotech Z.I. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania Italy. 2Parco Scientifico e Tecnologico della Sicilia ZI. Blocco Palma I, Str.le V. Lancia 57- 95121 Catania Italy. 3Dipartimento di Agricoltura Alimentazione e Ambiente, Università degli Studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy. E-mail: gralicci@unict.it

*Citrus tristeza virus* (CTV) is a phenotypically complex virus causing severe economic losses to citrus industries worldwide. In Sicily (Italy) tristeza disease affects more than 5 million trees with devastating effects in some areas and mild symptoms in others, despite the same scion/stock combinations being grown. To investigate either the presence of different CTV strains or a natural cross protection phenomenon, a genetic assessment of the virus population structure has been carried out through an In-Check platform based on Lab-on-chip technology. This has revealed a prevalent diffusion of VT-like genotypes. Two genotypes showed symptomless phenotypes, despite the VT genotype. Appropriate biological tests showed they reduced severe symptoms in pre-inoculated sour orange seedlings challenged with the aggressive CTV-VT isolate SG29 (KC748392) prevalent in Sicily. A study of genetic variants has been undertaken to find genetic differences of the virus (if any) which interfere with the VT aggressive genotype. Deep sequencing of the two potentially cross-protective VT strains revealed they are genetic variants of isolate SG29, which differ for few nucleotides. Comparative analyses have shown eight conserved non-silent mutations in comparison to the VT aggressive strain, including three in the p33 gene, described as involved in cross-protection by the superinfection exclusion mechanism. This technology opens new prospects in the strategy against seedling yellows CTV, and may be suitable for other pathogens.

This research was supported by the Project PON 2007-2013 IT-Citrus Genomics (PON 01_1623), coordinated by Science and Technology Park of Sicily.

CRISPR-Cas for genome-editing of fungi of interest in agriculture. S. SARROCCO1, J. VANG2, I. VICENTE MUÑOZ1, L. MALFATTI1, M. LÜBECK2, G. VANNACCI1. 1Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124, Pisa, Italy. 2Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University of Copenhagen, A.C. Meyers Vænge 15, 2450, Copenhagen, Denmark. E-mail: giovanni.vannacci@unipi.it
Genome editing of filamentous fungi using CRISPR-Cas9 technology has increased in recent years. There are few reports about CRISPR-engineered filamentous fungi related to biocontrol and crop disease. Our goal was to use this technique, as proof of concept of its feasibility, to edit the genome of a Trichoderma afro-harzianum and a T. gamsii isolate, well known as biocontrol and biostimulating fungi, as well as in a mycotoxigenic Fusarium graminearum isolate, the causal agent of Fusarium Head Blight (FHB). A gene encoding a polyketide-synthase, disruption of which can be easily detected phenotypically, was chosen as the target gene in all the three isolates, and used to design the RNA-guide to be included in the RGR-cassette. The cassette was then assembled in a Cas9 expressing plasmid. The resulting vector will be used for fungal transformation by protoplasts. Resulting mutants from all the three fungi will be phenotypically and molecularly analyzed, to verify the knockout of the selected gene. The presence of a shortened AMAl sequence will allow rapid removal of the plasmid from the edited strains, simply by reducing the selection pressure. Edited strains will be checked for the presence of foreign DNA, to contribute to the debate about the inclusion of this type of genetically manipulated microorganisms within GMOs. The ability to manipulate, beneficial and plant pathogenic isolates at a genetic level with these techniques represents a tool to increase knowledge of how these fungi interact with their hosts.

Spore trapping and quantitative PCR for monitoring airborne inoculum of Mycosphaerella nawae in persimmon. M. BERBEGAL, J.L. MIRA, J. ARMENGOL, A. VICENT. 1Instituto Agroforestal Mediterráneo, Universitat Politècnica de València. 46022, Valencia, Spain. 2Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada 46113, Valencia, Spain. E-mail: mobermar@etsia.upv.es

Circular leaf spot of persimmon, caused by Mycosphaerella nawae, includes symptoms of necrotic leaf lesions, defoliation and fruit drop. The disease is widespread in humid regions in Japan and South Korea, and, more recently, also in Mediterranean areas in Spain. The pathogen reproduces in leaf litter through ascospores formed in pseudothecia. Fungicide sprays are scheduled based on ascospore monitoring to define the periods of inoculum availability. Airborne ascospores of M. nawae are routinely quantified by counting using microscopy. This technique is time-consuming, especially for field sampling for rapid decision making. Monitoring airborne inoculum using spore traps combined with real-time PCR assays for quantification can be rapid, specific, reproducible and reliable. A real-time PCR assay for M. nawae quantification (qPCR) was designed and evaluated under laboratory conditions. To validate the technique under field conditions, two Burkard volumetric spore traps were deployed in a 100 m² plot. Soil was covered with persimmon leaf litter severely affected by M. nawae, and overhead sprinkle irrigation was used to enhance ascospore release. The spore traps were operated during May to July in 2016. Tapes from both spore traps were changed weekly, one was used for microscope counting and the other for qPCR analyses. Ascospore counts were correlated against DNA concentration of M. nawae based on Ct qPCR values. Results indicate that monitoring of M. nawae ascospores by qPCR may be a more efficient alternative to conventional inoculum counting, based on microscope examination.

This research was supported by RTA2013-00004-C03-00 INIA-FEDER.

Root colonization of host (Cucumis sativus) and non-host (Solanum lycopersicum) species by a DsRed-fluorescent strain of the specific pathogen Fusarium oxysporum f. sp. radicis-cucumerinum. M. DE CARA-GARCÍÁ, C. LECOMTE, M. FERNÁNDEZ-PLAZA, L. MUELA-JORDÁN, A. BOIX-RIUZ, C. STEINBERG. 1IFAPA Centro La Mojonera, Camino de San Nicolás, 1, 04745, La Mojonera, Spain. 2I.N.R.A. UMR Agroécologie, Rue Sully, 17, 21065, Dijon, France. 3University of Almería. Dept. Agronomía, Ctra. Sacramento s/n., 04120, Almería, Spain. E-mail: francisco.coma@juntadeandalucia.es

A monoconidial Fusarium oxysporum isolate (codified as 14/1Fo3), originally collected from sporodochia of a diseased cucumber plant showing root and stem rot, was identified as F. oxysporum f. sp. radicis-cucumerinum. The isolate was transformed by Agrobacterium tumefaciens, by means of EHA105-DsRed2 strain containing the binary vector pAN-DsRed2, carrying red fluorescent insert DsRed2, and the hgh gene.
One transformant (codified as Forc3T1) was selected for its fitness (growth rate, production of chlamydo-spores and macroconidia), root colonization ability, fluorescence intensity and sporodochium production. Forc3T1 and 14/1Fo3 isolates were inoculated separately on 2-4 true-leaf cucumber ‘Marketer’ and tomato ‘RAF’ plants, by watering each pot with 10⁶ microconidia suspended in water. Twenty-four days post inoculation (dpi), all cucumber plants showed rotten stems and roots, and most died, but no tomato plant was symptomatic (100% roots were healthy). Results were identical for both isolates, so tomato responded as a non-host for the transformant, whereas cucumber behaved as a host. In parallel, the root colonization strategy was studied with epifluorescence microscopy. Roots from tomato and cucumber were excised, washed and directly mounted under the microscope, from 1 to 16 dpi. At 3 dpi, appresoria were detected on epidermal cells and at 5 dpi intercellular hyphae were observed for both plant species. However, intracellular invasion of root cells was present on tomato (as early as 5 dpi), but not on cucumber (even at 16 dpi). Many macro- and micro-conidia were recovered from the supernatant obtained after root washing at 16 dpi for both host plant species.

This research was supported by the European Regional Development Fund (ERDF) and the European Social Fund (ESF) through the research project PP.TRA.TRA201600.9 and the fellowship granted to M. de Cara by IFAPA.

Rapid isothermal detection of Grapevine red blotch-associated virus by recombinase polymerase amplification. R. LI¹, M.F. FUCHS², K.L. PERRY³, T. MEKURIA⁴, S. ZHANG⁴. ¹Agdia, Elkhard, IN, U.S.A. ²Cornell University, Geneva, NY, U.S.A. ³Cornell University, Ithaca, NY, U.S.A. ⁴Vintage Nurseries, Wasco, CA, U.S.A. E-mail: rugang.li@agdia.com

Grapevine red blotch-associated virus (GRBaV) is a newly identified DNA virus in the family Geminiviridae in North America. GRBaV infects red and white grapevine cultivars, and affects fruit quality by delaying fruit ripening and reducing sugar content at harvest. A rapid, sensitive, and user-friendly test is needed to quickly identify GRBaV-infected grapevines, and facilitate their timely removal from vineyards. An isothermal test (AmplifyRP Acceler8) was developed for GRBaV that can be used in laboratories and vineyards. The test consistently detects GRBaV up to a 1:10⁶ dilution of infected grapevine leaf crude extracts diluted in healthy grapevine leaf crude extracts, and up to 11 copies of GRBaV genomic DNA in a matrix of healthy grapevine leaf crude extract. The test has no cross reactivity to host plant tissues and grapevine-infecting pathogens, including Arabis mosaic virus, Grapevine fanleaf virus, Grapevine leafroll-associated virus 1, Grapevine leafroll-associated virus 2, Grapevine leafroll-associated virus 3, Grapevine leafroll-associated virus 4 strain 5, Grapevine fleck virus, Tomato ringspot virus, Tobacco ringspot virus, Xylella fastidiosa, and Botrytis cinerea. The test has been validated using both viral DNA and crude plant extracts as templates.

This research is supported by Agdia, Inc.

Detection of plant pathogenic bacteria by the LAMP based ICGENE mini system. G.R. QUINTERO MACÍAS¹, P. BELLA², V. CATARA², S. DRAGO³. ¹Enbiotech S.r.l., Via Aquileia, 34. 90144 - Palermo, Italy. ²Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Ed. 4, 90128 - Palermo, Italy. ³Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Via S. Sofia 100, 95123 - Catania, Italy. E-mail: patriziabella@unipa.it

The ICGENE mini system includes ready-to-use kits with reagents and a portable device to perform on-site analyses based on Loop mediated isothermal amplification (LAMP) technology in different fields of applications. This system utilizes rapid DNA extraction from a small quantity of sample, isothermal genetic amplification, detection of the fluorescence emitted from the sample and automatic interpretation of the final result using the instrument ICGENE mini. We developed a diagnostic kit for Xylella fastidiosa (Xylella Screen Glow EBT501) that was validated according to EPPO PM 7/98 and PM 7/84, and this is in use in many laboratories. We report the optimization of two additional protocols for the detection and identification of Erwinia amylovora (Ea), which causes fire blight of Rosaceae, and Xanthomonas campestris pathovars (Xc) infecting cultivated Brassica crops. Both kits were able to identify target strains from different plant species and geographical origins with a sensitivity of approximately 10⁶ cells for both bacterial species, and not react with...
non-target strains. Spiked samples and naturally infected plants were tested with ICGENE mini, allowing completion of the test in less than 1 h. Diagnosis was also accomplished by isolation on culture media and/or PCR based techniques. Based on laboratory tests, LAMP with the ICGENE mini system could provide a rapid diagnostic presumptive test and direct bacterial colony identification.

**Early detection of *Citrus tristeza virus* using remote sensing.** F. SANTORO, S. GUALANO, A.M. D’ONGHIA. CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy. E-mail: fsantoro@iamb.it

The early detection of *Citrus tristeza virus* (CTV) is crucial for efficient large-scale virus monitoring and the rapid application of control measures. Remote sensing, supported by GIS and spatial analysis methods (automatic tree counting), was evaluated for the identification of CTV-suspected trees on a large scale. Preliminary trials were conducted in an agroforest and in the field, collecting leaf spectral signatures of CTV-positive and negative plants grafted onto the susceptible rootstock. Spectral reflectance of CTV-positive plants was greater in the visible region and less in the near infrared region. Specific indices (NDVI, mYI, PSRI, NCI, MCARI) were selected for the implementation of a detection algorithm, which was developed for processing GeoEye-1 satellite images. The output synthetic image with all combined indices was effective in discriminating CTV-infected and non-infected trees in the studied groves. The correlation of CTV infection to different canopy stresses was almost 100% in the severe declining trees, while it reached 75% in highly chlorotic trees. However, 52% of correlation was also reported in mild chlorotic or apparently asymptomatic trees. The developed algorithm was validated by processing a multispectral image from an apparently pathogen-free area. The prediction map obtained showed the suspected infected sites as coloured spots ranging from red (high probability to find CTV infection) to green (low probability to find CTV infection). Three red spots were highlighted in the prediction map, the assessment of which showed a new CTV focus, whereas *Phytophthora* disease was observed in the remaining red spots. The new finding of CTV in a free area revealed the potential of this approach for large scale virus monitoring.

The utility of mtDNA and rDNA for barcoding and phylogeny identification of plant-parasitic nematodes from Longidoridae (Nematoda, Enopla). J.E. PALOMARES-RIUS1, C. CANTALAPIEDRA-NAVARRETE1, A. ARCHIDONA-YUSTE3, S.A. SUBBOTIN2, P. CASTILLO1. 1Instituto de Agricultura Sostenible (IAS), Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Avda. Menéndez Pidal s/n, 14004, Córdoba, Spain. 2Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA. 3Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia. E-mail: palomaresje@ias.csic.es

Traditional identification of plant-parasitic nematode species by morphology and morphometric methods is difficult because of the high morphological variability which can lead to considerable overlapping of many characters and ambiguous nematode identification. It is essential to use several approaches to give accurate species identification (integrative taxonomy). DNA barcoding aids identification of species and advances species discovery. We have unravelled the use of the mitochondrial marker cytochrome c oxidase subunit 1 (cox1) for Longidoridae nematode species identification, as barcoding, for determining their molecular diversity and use as phylogenetic marker. Ribosomal markers (ITS region and the D2 and D3 expansion segments of the 28S rRNA gene) have also been explored. This provides molecular markers obtained using voucher specimens identified by integrative taxonomy. The results showed that mitochondrial and ribosomal markers could be used as barcoding markers using several barcoding approaches, with the exception of some species from the *X. americanum*-group. However, some species presented variability in cox1 that need to be further studied. Analysis of the newly provided sequences, deposited in GenBank, showed some misidentifications, and the use of voucher species and topotype specimens is a priority for this group of nematodes. The use of cox1 and the D2 and D3 expansion segments of the 28S rRNA gene did not clarify phylogenies at the species level, but showed important accuracy at the species level.

This research was financially supported by grants P12-AGR 1486 and AGR-136 from ‘Consejería de Economía,
Integrative taxonomic approach and molecular phylogeny for identification of dagger and needle nematode species infesting grapevine soils in Portugal. C. GUTIÉRREZ-GUTIÉRREZ¹, M. TEIXEIRA SANTOS², M. MOTA²¹. ¹NemaLab/ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas & Dept. de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal. ²Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta do Marquês, 2780-159 Oeiras, Portugal. ²¹Dept. Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, C. Grande 376, 1749-024 Lisboa, Portugal. E-mail: carlosg@uevora.pt

“Dagger” (Xiphinema spp.) and “needle” (Longidorus and Paralongidorus spp.) nematodes are economically important parasitic nematode groups in grapevine worldwide. They are polyphagous root ectoparasites causing severe damage to plants by their direct feeding, and in addition some species can transmit plant viruses. Grapevine fanleaf virus (GFLV) is transmitted by Xiphinema index, and is one of the most economically important viral diseases affecting grapevine in many Mediterranean growing regions. Nematode surveys have been conducted from 2015 to 2017 during spring and autumn seasons in the main Portuguese grapevine-growing areas. An integrative taxonomic approach, based on the combination of morphometric and morphological characterizations with molecular analysis using ribosomal DNA (rDNA) sequences from ITS regions and D2–D3 expansion segments of the 28S gene, were used for species delimitation and identification. High biodiversity of longidorid nematode species was found, greater in dagger than needle nematodes. Xiphinema pachtaicum, X. santos and X. index were the most frequently found dagger nematodes in Portuguese vineyards, while L. vineacola was the most common needle nematode. Severe nematode infestations were found in grapevine soils in the oldest vineyard regions, highlighting the importance X. index. Disease symptoms were observed on aboveground plant parts of the grapevines infected with X. index, and these included yellow mosaic pattern in leaves which are characteristic of infections by GFLV.

This research was supported by FCT - Foundation for Science and Technology postdoctoral fellowship SFRH/BPD/95315/2013 and FEDER Funds through the Operational Programme for Competitiveness Factors – COMPETE, and National Funds through FCT under the Strategic Projects PEst-C/AGR/UI0115/2011 and PEst-OE/AGR/UI0115/2014 (Portugal).

Epidemiology and modeling

Modelling yield losses, caused by multiple wheat diseases in France. L. WILLOCQUET S. SAVARY. AGIR, INRA, Université de Toulouse, INPT, INP- E1 PURPAN, Castanet-Tolosan, Centre Inra Occitanie-Toulouse, France. E-mail: laetitia.willoquet@inra.fr

Yield loss quantification is critical to inform tactical and strategic decisions in plant disease management. Yield loss quantification and modelling entails analysis of relationships between disease intensity, and attainable and actual yields, of a crop grown in a given production situation. Yield losses caused by individual and combined wheat diseases were estimated using a process-based simulation model, WHEAT-PEST, together with a dataset from a network of experiments on winter wheat in France where disease intensity and actual yields were measured. The disease-free, attainable yield was not measured. The analysis focused on 70 combinations [year × Region × variety × crop management]. These considered five years (2003 to 2008), four French Regions, two varieties (one high yielding and one hardy variety), and two levels of crop management corresponding to two levels of chemical intensification. Simulated overall yield losses from combined diseases ranged from 0 to 4.2 t ha⁻¹, with a mean of 0.80 t ha⁻¹ and a standard error of the mean of 0.10 t ha⁻¹. Variety and crop management had significant (P < 0.05) effects on yield loss caused by combined diseases. Septoria tritici blotch was associated with greatest simulated yield loss, followed by brown rust, Fusarium head blight, yellow rust and powdery mildew. This approach allows estimation of yield losses caused by individual and combined diseases, and can be ap-

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Identification of TYLCD-associated begomoviruses and ToLCNDV-ES co-infections in Spain.
A. SIMON, L. RUIZ, C. GARCÍA, D. JANSSEN. Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA), Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain. E-mail: dirk.janssen@juntadeandalucia.es

In 2014, leaf samples from 50 tomato plants showing symptoms of tomato yellow leaf curl disease (TYLCD), including leaf curling, chlorosis and vein thickening, were collected from natural infections in commercial greenhouses from southern Spain. None of the sampled tomato cultivars carried resistance genes against begomovirus. The Mld strain of Tomato yellow leaf curl virus (TYLCV-Mld, referred to as TYLCV), Tomato yellow leaf curl Sardinia virus (TYLCSV), Tomato yellow leaf curl Astarquia virus (TYLCAxV), and the ES strain of Tomato leaf curl New Delhi virus (ToLCNDV-ES) were detected, using species-specific primers and conventional PCR. From the sampled tomato plants, 41% had mixed infections of ToLCNDV-ES and one or more TYLCVD-associated species. The most frequent combination of mixed begomovirus infections was ToLCNDV-ES+TYLCV+TYLCSV, although ToLCNDV-ES+TYLCV+TYLCSV+TYLCAxV was also identified in single tomato plants. Many of the mixed infection plants showed more severe symptoms than plants with single infections, expressed as green-bright yellow mosaic, vein thickening and leaf distortion. Despite of differences in degree of symptom expression, qPCR revealed that the titres of genome DNA-A and DNA-B from ToLCNDV-ES were similar in single and mixed infected tomatoes plants ($P > 0.05$). The infections of ToLCNDV-ES and TYLCV-complex begomoviruses were also independent (Fisher’s test, $P > 0.05$). Nevertheless, the frequency of mixed infections of TYLCD-associated begomoviruses and ToLCNDV-ES in tomatoes from southern Spain could pose epidemiological risks, because of genome recombination events which are likely to occur between different begomovirus species.

Virus diseases affecting chickpea crops in Uzbekistan.
S.G. KUMARI1, Z. ZIYAEV2, S.D.A. KEMAL3. 1International Center for Agriculture Research in the Dry Areas (ICARDA), Terbol Station, Beqa’a Valley, Zahla, Lebanon. 2Kashkadarya Scientific Research Institute of Grain Breeding and Seed Production, Beshkent 3km, Karshi, Uzbekistan. 3ICARDA, Rabat, Morocco. E-mail: s.kumari@cgiar.org

A field survey was conducted during the 2012 and 2013 cropping seasons to monitor occurrence of virus diseases affecting chickpea in the major production areas of Uzbekistan (Tashkent, Sirdarya, Jizzah, Samarkand and Surkhandarya regions). Surveyed fields were randomly selected and types of viruses and their incidence were determined based on symptoms observed. In addition, 15-20 symptomatic samples were collected from each field for laboratory testing. Chickpea samples with symptoms suggestive of virus infection (chlorosis, stunting, necrosis, yellowing, reddening, mosaic/mottling) were collected from 23 (386 samples) during 2012 and 19 fields (288 samples) during 2013. All samples collected were tested by tissue blot immunoassay (TBA) using 12 specific polyclonal and monoclonal antibodies. Serological tests showed that Faba bean necrotic yellow virus (FBNYV) was the most common (detected in 20% of tested samples), followed by Bean yellow mosaic virus (BYMV) (6%), Bean leafroll virus (BLRV) (5%) and Chickpea chlorotic stunt virus (CpCSV) (3%). Molecular characterization (PCR and sequencing) indicated that the viruses which infect chickpea crops in Uzbekistan are BYMV, FBNYV, BLRV, CpCSV, Beet western yellow virus (BWYV), Soybean dwarf virus (SbDV) and Cucurbit aphid-borne yellows virus (CABYV). We conclude that a long term research plan is needed to manage the spread of virus diseases, and to minimize yield losses in areas where virus incidence is high and chickpea crops are important for small holder farmers.

This work was partially supported by CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS).
Ecological succession of pathogenic fungi of pines in Italy associated with climate change. L. GHELARDINI¹,², P. CAPRETTI¹, L. BOTELLA³, C. AGLIETTI¹, N. LUCHÉ⁴. ¹Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, I-50144, Firenze, Italy. ²Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy. ³Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic. E-mail: paolo.capretti@unifi.it.

Gremmeniella abietina is an ascomycete causing Scleroderris canker on Pinus species and conifers in the Northern Hemisphere, including Europe from the Boreal to the Mediterranean regions. The disease occasionally caused severe damage in Europe, and is a constant threat in North America and Japan. The pathogen kills buds, young shoots and foliage of hosts, and bark necroses and branch dieback. Whole crowns may be infected, and plants may die after repeated attacks. Seedlings may die quickly. The pathogen is psychrophilic, favoured by wet and cool weather, recurrent late frost and prolonged snow cover. In Italy, Scleroderris canker was historically observed on young and adult pines in the Alps and the Apennines, where conditions were locally favourable. Fungal populations were genetically differentiated between northern and southern sites, and had different optima and host ranges. We surveyed areas where G. abietina had been observed in the past and found that its prevalence decreased over the last 40 years. Especially reduced was the frequency of the thermophilic form of the fungus in southern areas. The pathogen was often replaced by Diplodia sapinea, an opportunistic fungus shifting from an endophytic to pathogenic lifestyle in stressed host plants. Replacement of G. abietina by D. sapinea in the Apennines is likely a bioindicator of current climate change. The incidence of Scleroderris canker has probably decreased in other areas at the southern range edges, and distribution of G. abietina will be further reduced, making way for the emergence of other pathogens driven by climate-change related stressors.

Field studies on the primary inoculum and early infections of almond red leaf blotch (caused by Polystigma amygdalinum) in Spain. E. ZÚÑIGA¹, J. LÚQUE², X. MIARNAU², O. ARQUERO³, M. LOVERA³, A. OLLERO³, L.F. ROCA³, A. TRAPERO³. ¹Pa-tología Vegetal, IRTA, Carretera de Cabrils km 2, 08348 Cabrils, Spain. ²Estació Experimental de Lleida, IRTA, Parc Científic i Tecnològic Agroalimentari de Lleida (PCITAL). Parc de Gardeyn, Edifici Fruitcentre, 25003 Lleida. ³IFAPA “Alameda del Obispo”, Avenida Menéndez Pidal s/n, 14080 Córdoba, Spain. ⁴Departamento de Agronomía (Patología Agroforestal), Universidad de Córdoba. Campus de Rabanales, Edificio C-4, 14071 Córdoba, Spain. E-mail: erick.zuniga@irta.cat.

Red leaf blotch of almond (caused by Polystigma amygdalinum), is a common disease in continental climate areas of Spain and other countries in the Mediterranean region. Early symptoms are yellow discoloured blotches on leaves, which turn red and then become dark necroses. The disease usually causes early defoliation of trees that causes decreased fruit production. Little is known about the biology of the pathogen in Spain and worldwide. Co-ordinated research was carried out in southern (Andalusia) and northeastern (Catalonia) Spain, to monitor the dynamics primary P. amygdalinum inoculum production, and the period of plant infectivity. Monitoring in Catalonia of ascocarp and ascospore development showed optimum maturation of propagules by mid spring (April-May), which was coincident with high ascospore records obtained from leaf samples in the field. In Andalusia, the primary inoculum potential occurred from February to May, a longer period than in Catalonia. The period of maximum ascospore production was less in both areas, and varied greatly between years and areas. The periodical exposure of almond ‘trap’ plants to natural infections in the field showed that the infectivity period in Catalonia extended from April to late June, while in Andalusia it occurred from March to May. These preliminary results on the biology of P. amygdalinum are a first step in the establishment of an integrated disease control strategy against almond red leaf blotch in Spain, and other almond growing regions.

This research was supported by projects RTA2013-00004-C03-01 (INIA, Spain), Transforma de Fruticultura Mediterránea (IFAPA, Spain) and the European Regional Development Fund (ERDF). The first author was supported by a predoctoral grant by CONACYT, Mexico.
Epidemiology and control of Cucumber green mottle mosaic virus in Spain. M.A. ELORRIETA1, L. RUIZ2, D. JANSSEN3. 1LABCOLOR, COEXPHAL, C/ Esteban Murillo, 3. Venta El Viso 04746 La Mojonera, Almería, Spain. 2Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA), Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain. E-mail: dirk.janssen@juntadeandalucia.es

Spain is one of the main producers of cucurbits crops in Europe, and one of the top-ten producers in the world. The tobamovirus Cucumber green mottle mosaic virus (CGMMV) was first described in Spain during the early 1990’s, and has caused periodic outbreaks since then in cucumber and watermelon in greenhouses in the province of Almeria. To improve CGMMV control, we studied the epidemiology of the virus in the southeast of Spain. Between the years 2013 and 2015, 154 protected crops of cucumber (119), melon (21), watermelon (13) and zucchini (1), located in the provinces of Almeria and Granada, were selected randomly and examined. Leaves of plants were collected for analysis of CGMMV, and detailed information was gathered on the location, the greenhouse features, and the management of crops and diseases. CGMMV infections were detected in 23 greenhouses, predominantly of cucumber (20/119). The presence of CGMMV was not dependent on the use of grafted plantlets, the variety and source of seeds and plantlets, or on the origin of the irrigation water (owned or shared water wells). However, infections did depend heavily on the previous infection history of farms and surroundings. The enquiries revealed that the greenhouse structures and the irrigation water reservoirs were not cleaned periodically. Gloves and disinfectants were rarely used during crop manipulation. Successful control of CGMMV through crop management was positively correlated with soil disinfection by solarization and with crop rotation using non-cucurbbit species.

This work was supported by the Project RTA2012-00003-00-00 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain).

Temporal persistence and distribution of Heterobasidion abietinum in a planted forest of silver fir in Central Italy: a contribution to forest management. L. GHELARDINI1,2, P. CAPRETTI1, L. BOTELLA3, C. AGLIETTI1, P.CAPRETTI1. 1 Department of Agrifood production and Environmental sciences (DISPAA), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy. 2 Department of Forest Protection and Wildlife Management, Mendel University in Brno (UOLM). Zemědělská 3, 61300 Brno, Czech Republic. E-mail: luisa.ghelardini@unifi.it

One of the main problems in mature conifer plantations is related to damage by Heterobasidion annosum. Occurrence and persistence of H. abietinum were assessed in a planted forest of silver fir (Abies alba) in Vallombrosa (Florence, Central Italy) over the past 60 years. The presence of the pathogen in the area has been known since the 18th Century, and is related to management choices. For centuries, silver fir in Vallombrosa was regularly managed with a 100 year growth period and artificial replanting. When a modern Forest Management Plan was first compiled in the 1960s, the occurrence of Heterobasidion was reported in the ecological description of all silver fir areas of the forest. Again in 1990, the presence and frequency of Heterobasidion in Vallombrosa was investigated in wood samples from Silver fir stumps left after thinning. At the time, H. abietinum, identified according to Korhonen’s method (paring colonies with testers), was found at over 80% of the intersection points of a square (500 m) sampling grid covering the whole forest, with the greatest frequency (56%) on silver fir stumps. More recently, after a severe wind storm destroyed about 50 ha of the forest in spring 2015, a systematic sampling was carried out, and Heterobasidion species were identified with molecular methods. Taken together these studies define distribution of the pathogen over space and time, providing support for design of an informed recovery plan for the Vallombrosa forest.

Ecological succession of pathogenic fungi of pines in Italy associated with climate change. L. GHELARDINI1,2, P. CAPRETTI1, L. BOTELLA3, C. AGLIETTI1, N. LUCHI2,3. 1 Department of Agrifood Production and Environmental Sciences, University of Florence. Piazzale delle Cascine 28, I-50144, Firenze, Italy. 2 Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy. 3 Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in
Gremmeniella abietina caused Scleroderris canker on Pinus species and conifers in the Northern Hemisphere, including Europe from the Boreal to the Mediterranean regions. The disease occasionally caused severe damage in Europe, and is a constant threat in North America and Japan. The pathogen kills buds, young shoots and foliage, causes bark necroses and branch dieback. Whole crowns of trees may be infected, and plants may die after repeated attacks. Seedlings may die quickly. The pathogen is a psychrophilic fungus favoured by wet and cool weather, recurrent late frost and prolonged snow cover. In Italy, Scleroderris canker was historically observed on young and adult pines in the Alps and the Apennines, where conditions were locally favourable. Fungal populations were genetically differentiated between northern and southern sites, and had different optima and host ranges. We surveyed areas where G. abietina had been observed in the past, and found that its prevalence decreased over the last 40 years. Especially reduced was the frequency of the thermophilic form of the fungus in southern areas. Gremmeniella abietina was often replaced by Diplodia sapinea, an opportunistic fungus shifting from endophytic to pathogenic state in stressed host plants. The replacement of by D. sapinea in the Apennines is likely to be a bioindicator of current climate change. Incidence of Scleroderris canker has probably decreased in other areas at the southern range edges, and the distribution of G. abietina will be further reduced, making way for the emergence of other pathogens driven by climate-change related stressors.

Epidemiology, aetiology and modelling of olive anthracnose. P. TALHINHAS, A. LOUREIRO, A.P. RAMOS, J.P. MELO E ABREU, H. OLIVEIRA. LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: ptalhinhas@isa.ulisboa.pt

Olive anthracnose affects olive fruit at maturity, causing yield losses and poor olive oil quality. Specific agroecological circumstances, combining increased average humidity and rainfall during autumn, widespread use of susceptible varieties and abundance of inoculum reservoirs favour high disease incidence and severity. Disease control using agrochemicals is often the only immediate disease management option, although the presence of pesticide residues is problematic in table olives and olive oil. Olive anthracnose is associated with at least six species of Colletotrichum, with C. nymphaeae being prevalent in some Mediterranean areas and C. godetiae in others, while C. acutatum s.s. is emerging. Colletotrichum nymphaeae and C. acutatum s.s. are more virulent than others, although differential interactions between fungal species and olive varieties have been documented. Modelling anthracnose epidemiology is therefore very important, and this should consider the combination of climatic factors, production systems (super-intensive, intensive or traditional orchards, considering also neglected groves and oleaster patches), prevalence of the crop, cultivar preference and predominant pathogen species, at regional scales. Olive anthracnose will be addressed combining epidemiological, aetiological and agroecology-based modelling approaches. This will better characterize the disease, forecast disease risk scenarios, and assist informed decisions regarding disease control.

LEAF research unit is supported by Fundação para a Ciência e a Tecnologia, Portugal (UID/AGR/04129/2013).

Cryptic species and population genetic structure of Plasmopara viticola in São Paulo State, Brazil. M.P. CAMARGO, C.F. HONG, L. AMORIM, H. SCHERM. 1Department of Plant Pathology, Luiz de Queiroz College of Agriculture, University of São Paulo, CEP 13418-900, Piracicaba, SP, Brazil. 2Department of Plant Pathology, University of Georgia, Athens, GA 30602, USA. E-mail: meyriescamargo@usp.br

Downy mildew (caused by Plasmopara viticola) is one of the most important diseases in grape-growing areas worldwide, including Brazil. Little is known about the pathogen population structure in subtropical areas. To examine pathogen diversity, 516 single lesions of P. viticola were collected during the 2015/16 growing season from 11 locations in São Paulo State, and from nine grapevine cultivars. To allow recognition of cryptic species (clades), a subsample of 130 isolates were analyzed using cleaved amplified polymorphic sequence (CAPS) markers with two re-
striction enzymes (Asel and HpyCH4V). In addition, the ITS1 region of 94 isolates was sequenced to substantiate results. Seven previously reported microsatellite markers were used for genotyping all 516 P. viticola isolates. Results obtained from CAPS analysis and ITS1 sequencing suggest that the population of P. viticola in São Paulo State may a single cryptic species, P. viticola clade aestivalis. Twenty-three alleles and 55 multilocus genotypes (MLGs) were observed among the 516 isolates. Half of the MLGs observed were clonal, and four dominant MLGs represented 66% of the observed genotypes. Most populations showed significant linkage disequilibrium, and excess of heterozygosity was verified in many loci. Principal coordinate analysis revealed no clusters among populations. No significant isolation by distance was found, suggesting high levels of gene flow. These results demonstrate that epidemics result from multiple clonal infections caused by a few genotypes, and that asexual reproduction predominates for P. viticola in São Paulo, Brazil.

This research was supported by the São Paulo Research Foundation (FAPESP Project 2015/26106-5) and the University of Georgia.

Development and verification of a dynamic model for predicting olive scab development. J. ROME-RO1, L.F. ROCA1, C. AGUSTI-BRISACH1, E. GON- ZALEZ-DOMINGUEZ2, V. ROSSI1, A. TRAPERO1.
1Departamento de Agronomía, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain.
2Istituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, 29100 Piacenza, Italy. E-mail: joaquinromrod@gmail.com

Olive scab, caused by Venturia oleaginea, is the main olive leaf disease worldwide. Traditionally, chemical control of this disease was based on a fixed schedule of fungicide applications, mainly using copper products. However, integrated pest management (IPM) should be implemented to rationalize fungicide treatments. A mechanistic model to predict risk of infection and olive scab epidemics was developed, according to the system analyses, and implemented in a computerized system. Hourly data of air temperature, rainfall and relative humidity were used to produce daily olive scab predictions as outputs. Simulations are based on sub-processes of conidial production and dispersal, infection and latent period (i.e., the state variables). Mathematical equations that relate state variables (i.e., the driving variables) were developed using published data on V. oleaginea. The model was able to represent the real system, and assisted understanding of olive scab epidemics in four olive-growing areas, traditionally considered as having different favourable conditions for olive scab development. Model outputs for these areas were generated, agreeing with traditional knowledge. Based on the model outputs, different strategies of fungicide treatments can be suggested in each growing area, reducing the amount of fungicide applied. Weaknesses of the model are discussed, and additional research is advisable. However, this model could be useful for implementing an IPM approach. This is the first olive scab model based on the biological knowledge of the disease. Other disease models will soon be added to complete a decision support system for the main aerial diseases in olive groves.

This research was supported by the project “Validación del modelo epidémico Repilos” funded by the Bayer Crop Science. Carlos AGUSTÍ-BRISACH is the holder of a ‘Juan de la Cierva-Formación’ fellowship from MINECO.

Epidemiology of peach powdery mildew (Podosphaera pannosa) in Catalonia, Spain: towards a degree-day model to initiate fungicide spray programmes. N. MARIMON1, J. LUQUE1, J. MARTÍN- EZ-MINAYA2, D. CONESA2, A. VICENT3. 1Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Carretera de Cabrits km 2, 08348 Cabrits, Spain. 2Departament d’Estadística i Investigació Operativa, Universitat de València, 46100 Burjassot, Spain. 3Centro de Producción Vegetal y Biotecnología, Instituto Valen-ciano de Investigaciones Agrarias (IVIA), 46113 Mon-cada, Spain. E-mail: neus.marimon@irta.cat

Powdery mildew of peach (caused by Podosphaera pannosa) is a common disease in Spain where these fruit trees are grown. The disease is usually managed by calendar-based fungicide spray programmes, commencing at the petal fall host stage. This study monitored powdery mildew progress in untreated trees, in order to: 1) describe overall disease progress in relation to a degree-day scale starting at 50% blossom; and 2) establish a degree-day threshold for the detection of primary infections and thus initiate a more rational weather-based fungicide programme.
Five trees per experimental plot were chosen in each of seven commercial peach orchards located in Catalonia, NE Spain. Disease monitoring was carried out from March to summer (June-July) 2013 to 2015, by recording the incidence and severity of the disease on fruits. An automatic weather station was located in each plot to record the main environmental data. Accumulated degree-days (ADD) from the blossom biofix were calculated for each orchard. Observations indicated that primary infections were detected at 242.0 ± 13.1 ADD, while last infections were at 483.5 ± 42.2 ADD (mean ± standard error, n = 15). Disease progress followed a clear sigmoidal trend, and Beta-regression equations between disease incidence on fruits and ADD were successfully fitted using Bayesian inference with Integrated Nested Laplace Approximation. The model showed good performance when validated against independent data. This preliminary research is a first step towards a decision support system based on epidemiological modelling for the integrated management of peach powdery mildew in Catalonia.

This research was supported by projects RTA2013-00004-C03-00 (INIA, Spain), MTM2016-77501-P (Ministry of Economy and Competitiveness, Spain) and VALi+d ACIF/2016/455 (Generalitat Valenciana), and the European Regional Development Fund (ERDF). The first author was supported by a predoctoral grant by INIA, Spain.

Huanglongbing epidemiology in Brazilian orchards. K. PAZOLINI, J.H. ARRUDA, G.A. CHINE-LATO, A. BERGAMIN FILHO, J. BELASQUE JUNIOR. Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11 – Piracicaba, Brazil. E-mail: pazolinikelly@gmail.com

Huanglongbing (HLB) (caused by ‘Candidatus liberibacter spp.’) is the main citrus disease worldwide. There are still no viable curative measures or varieties with genetic resistance to HLB. Recommended disease management is the use of healthy seedlings, eradication of symptomatic trees and chemical control of the vector, Diaphorina citri. Our aim was to understand the temporal and spatial progress of HLB in an area, with strict management of disease in Brazilian orchards. Temporal (logistic and Gompertz) and spatial (exponential and power law) models were tested, by non-linear regression to orchard data (177 plots for temporal, 12 plots for spatial analyses), on a single farm in São Paulo state. The management of HLB in this property was carried out with four or more inspections per year, for eradication of symptomatic trees and weekly or biweekly sprays with insecticides for vector control. For temporal analyses, the logistic model was adjusted (\( P < 0.05 \)) to 115 of the 177 plots studied (progress rates of 0.2 to 1.5), while the Gompertz model was adjusted to only 29 plots (progress rates from 0.2 to 0.5). For spatial analysis, both models presented a good fit to the 12 plots studied. However, the model inverse power law presented the best residual pattern and greater \( R^2 \) (0.91) than the exponential model (\( R^2 = 0.88 \)). The progress of HLB with time was best described by the logistic, and in space by the inverse power model.

This research was supported by the projects 2016/01796-1(FAPESP) and 161090/2015-0 (CNPq).

Microbiomes and their roles in plant health

New Pseudomonas strains from olive rhizospheres as effective biocontrol agents against Verticillium dahliae. C. GÓMEZ-LAMA CABANÁS\(^1\), G. LEGARDA\(^2\), D. RUANO-ROSA\(^3\), P. PIZARRO-TOBÍAS\(^3\), A. VALVERDE CORREDOR\(^3\), J.L. NIQUI\(^3\), J.C. TRIVIÑO\(^3\), A. ROCA\(^3\), J. MERCADO-BLANCO\(^3\). \(^1\)Department of Crop Protection, Institute for Sustainable Agriculture (CSIC), Avenida Menéndez Pidal s/n Campus ‘Alameda del Obispo’, 14004 Córdoba, Spain. \(^2\)Bioinformatics Department, Sistemas Genómicos Ltd, Valencia, Spain. \(^3\)Bio-Iñiberis Research and Development SL, Granada, Spain. E-mail: cgomezlama@gmail.com

Previous studies have demonstrated that rhizospheres of nursery-produced olive (Olea europaea L.) plants are sources of bacteria with potential as biological control agents (BCA) of Verticillium wilt of olive (VWO), caused by Verticillium dahliae. A collection of 189 bacterial isolates from healthy olive (cv. Picual) plants was generated, based on different morphological and biochemical characteristics and in vitro antagonistic activity against several olive pathogens. Three strains (PIC25, PIC105 and PICF141) showing the greatest potential as BCAs, particularly against V. dahliae, were eventually selected. These were further tested for nutritional requirements and chemical sensitivities. Their effectiveness against VWO

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caused by the defoliating pathotype of *V. dahliae* was also demonstrated. Genotypic and phenotypic traits traditionally associated with plant growth promotion and/or biocontrol abilities were evaluated (e.g. phytase, xylanase, and glucanase activities, and siderophore and HCN production). Phylogenetic analysis revealed that the strains belonged to the *Pseudomonas* genus. Strain PICF141 was affiliated to the ‘*P. mandelli* subgroup’, with *P. lini* as the closest species. Strains PIC25 and PIC105 were affiliated to the ‘*P. aeruginosa* group’, *P. indica* being the closest species. Strain PIC105 was identified as *P. indica*, this being the first reort of the species as a potential BCA. Sequencing and in *silico* analyses of the genomes of these strains enabled the identification of traits involved in plant-bacteria interactions. Seed adhesion and root colonization abilities of the novel BCA were also assessed, providing valuable information for the development of future bioformulations based on these rhizobacteria.

This research was supported by grants P12-AGR667 (Junta de Andalucía) and RECUPERA 2020 (MINECO/CSIC contract), both co-funded by ERDF of the EU.

**New bacterial antagonists for the biocontrol of fire blight caused by Erwinia amylovora.** S. AIT BAHADOU1,2, A. OUIJJA1, A. KARFACH2, A. TAHIRI3.

1Laboratory of Plant Biotechnology and Molecular Biology, Moulay Ismail University, Faculty of Sciences, BP 11201, Ave Zitoune Meknes, Morocco. 2Laboratory of Microbial Biotechnology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Technologies, BP 2202, Route d’Imouzzer FES, Morocco. 3Department of Plant Protection and Environment of the National School of Agriculture-Meknes, Km10, Rte Haj Kaddour, BP 540, Meknès 50001, Morocco. E-mail: s.aiitbahadou@edu.umi.ac.ma

The biocontrol effectiveness of antagonistic bacteria against fire blight (caused by *Erwinia amylovora*) was evaluated under *in vitro* and field conditions. Among 61 bacteria isolated from soil and flowers of fire blight host plants from different Moroccan areas, 20 isolates showed antagonistic activity against the pathogen during agar-diffusion-tests, attached blossoms assays and in a bioassay on immature pear fruits. Effective isolates were identified using biochemical tests and 16S rDNA gene sequencing. These isolates were grouped into the following genera: *Alcaligenes* (ACBC1), *Bacillus* (CPa12, CPa2, HF6, JB2, LMR2, SF14, SF16, SP10, SP13, SP18), *Brevibacterium* (SF3, SF4, SF7, SF15), *Pantoea* (ACBC2, ACBP1, ACBP2), *Pseudomonas* (SP9), and *Serratia* (HC4). The isolates were reported in the NCBI nucleotide sequence database (GenBank) under the accession numbers from KY357285 to KY357304. In a field assay with susceptible apple varieties, spray treatments were carried out with different bacterial antagonists. Their efficacies were evaluated 15 d post-inoculation on blossoms, and ranged from 55 to 95% for 11 strains. Most strains gave efficacies that were better than that obtained with commercial bacterial strains P10c (66%) and QST713 (63%). The strains showed no pathogenicity towards plant tissue (pear fruitlets, pear and apple blossoms, and tobacco leaves), and are, therefore, considered as potential candidates to as microbial biocontrol formulations for fire blight control.

**Qualitative and quantitative impacts of Bactrocera oleae on the fungal microbiota of ripe olive drupes.** D. RUANO-ROSA1, A. ABDELFAH2, M.G. LI DESTRI NICOSIA2, S.O. CACCIOLA2, G.E. AGOSTEO2, L. SCHENA2. 1Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, P.O. Box 4084, 14080 Córdoba, Spain. 2Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito-89122 Reggio Calabria, Italy. 3Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: ruanodavid@gmail.com

The olive fly, *Bactrocera oleae*, is a major key pest of olive drupes, greatly affecting quality and quantity of olive oil production. Fungus species associated with olive drupes can also have important impacts on olive production. However, little is currently known about the interaction between olive fly and fungi. Ripe olive drupes of three olive varieties, either with or without olive fly infestations, were collected in southern Italy. These were pitted and total DNA was extracted and analyzed using real-time quantitative PCR (qPCR) and metabarcoding based on Illumina MiSeq sequencing. Both analyses were performed using fungal universal primers targeting the ITS2 region of the rDNA. QPCR analyses enabled the quantification of the total fungal DNA, and revealed...
Dynamics of fungus endophytes during different phenological stages of olive trees. F. MARTINS1,2, J.A. PEREIRA1, P. BAPTISTA1. 1CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. 2University of Léon, Department of Engineering and Agricultural Sciences, Av. Portugal, nº 41, 24071 Léon, Spain. E-mail: pbaptista@ipb.pt.

Endophytic fungi are a diversified group of microorganisms that reside asymptptomatically in the tissues of most plant species. Despite their known roles in protecting hosts against several diseases, little is known on the sources of established endophytes and how plants select specific microbial communities to establish associations. We used cultivation-dependent approaches to assess the endophytic fungus communities in olive tree floral buds, inflorescences and fruits, to determine differences in different host phenological stages follow the phenological stages from floral buds to fruits. The fungus endophytes were identified by rDNA sequencing. From the floral bud to flower stage, the frequency of colonization and abundance of endophytes increased progressively up to 2.4-fold; and from flower to fruit decreased up to 5.0-fold. Biscogniauxia mediterranea was the most frequent species isolated from the buds and inflorescences (N = 89), whereas at the fruit stage, the most abundant species was Neofabraea vagabunda (N = 38). Endophytic fungus communities also differed in composition over the phenological stages, probably due to variations of weather conditions and the chemical nature of the plant organs. Phomopsis, Venturia and Coniozyma were common in floral buds and inflorescences, but disappeared from fruits, being replaced by genera such as Aspergillus, Coriolopsis and Eutypella. Our results indicate that endophytic fungus communities were distinct and specific to the host phenological stages, raising the question of whether these specific species may induce plant protection against biotic stresses.

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013). The first author also thanks the award of a PhD Scholarship (ref. SFRH / BD / 112234/2015) by FCT.

Fungal endophyte communities in olive fruits: effects of maturation index and anthracnose incidence. F. MARTINS1,2, J.A. PEREIRA1, P. BAPTISTA1. 1CIMO / School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: pbaptista@ipb.pt. 2University of Léon, Department of Engineering and Agricultural Sciences, Av. Portugal, nº 41, 24071 Léon, Spain. E-mail: pbaptista@ipb.pt

Olive anthracnose, caused by different species of Colletotrichum, is one of the most economically harmful fruit diseases of olive crop worldwide. In the Trás-os-Montes region (Northeast of Portugal), although the presence of the pathogen has been reported on olive orchards in almost all areas, lower levels of incidence were observed in specific areas. This study evaluated the diversity of endophytic fungi inhabiting fruits of the anthracnose-susceptible cultivar Madural, in olive groves from areas of high and low anthracnose incidence. Differences in the endophytic community composition were assessed. Fungi were isolated from symptomless olive fruits at three different maturation indices (MI). The isolates were identified by rDNA sequencing. Overall, the frequency of colonization and abundance of endo-
Endophytic and epiphytic fungal communities associated with olive trees differ in antagonistic activity against *Pseudomonas savastanoi* pv. savastanoi. T. GOMES1,2, J. A. PEREIRA1, T. LINO-NETO2, P. BAPTISTA1. 1CIMO/ Polytechnic Institute of Bragança, School of Agriculture, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. 2Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: pbaptista@ipb.pt

Olive knot (OK) caused by the *Pseudomonas savastanoi* pv. savastanoi (Psv) is an important disease, causing severe damage and yield losses in olive trees worldwide. In a previous study, we isolated this bacterium from the phyllospheres of olive trees, together with many fungal species. In these complex communities, microorganisms compete for space and resources, promoting survival of the best-adapted individuals. This has prompted interest in the exploitation of these microorganisms for OK control. In this study, 48 fungal species from the endo- and epiphytic communities of olive twigs were screened for growth inhibition of Psv under *in vitro* conditions. The time course of interspecific interactions (24, 48, 72 and 144 h) was studied on potato dextrose agar and olive leaf + twig extract (OLTE) media, by assessing clear zones of bacterial growth inhibition around fungus colonies. The epiphytic community was the main reservoir for antagonistic fungi. Almost 70% of the tested epiphytes inhibited Psv growth, with *Dothiorella iberica*, *Aspergillus felis* and *Aspergillus brasiliensis* the most prominent species. The proportion of antagonists within endophytic communities was less (46%), with the most efficient being *Epicoccum nigrum* and *Rinocladiella similis*. Antibacterial activity was observed to be affected (*P* < 0.01) by growth medium and period of interaction. Greater growth inhibition was found with the OLTE culture medium, showing that inhibition of these endophytic and epiphytic fungi was specifically enhanced by the host plant extracts. Most of the fungi tested (up to 64%) from both microenvironments showed greatest antibacterial activity in the first 24 h of interaction, whereas only 16% strongly inhibited Psv after 48 h and 19% after 144 h. These results indicate that *D. iberica*, *E. nigrum* and *A. felis* are the best candidates for biocontrol of olive knot, and these should be further evaluated under natural conditions.

This work is supported by FEDER funds through COMPETE (Programa Operacional Factores de Competitividade), and by national funds by FCT (Fundaçao para a Ciência e a Tecnologia) in the framework of the project EXCL/AGR-PRO/0591/2012. T. Gomes thanks FCT, POPH-QREN and FSE for the PhD grant SFRH/BD/98127/2013.

New bacterial antagonists for biocontrol of fire blight, caused by *Erwinia amylovora*. S. AIT BAHADOU1,3, A. OUIJJA1, A. KARFACH2, A. TAHIR3. 1Laboratory of Plant Biotechnology and Molecular Biology, Moulay Ismail University, Faculty of Sciences; BP 11201, Ave Zitoune Meknes, Morocco. 2Laboratory of Microbial Biotechnology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Technologies; BP 2202, Route d’Imouzzer FES, Morocco. 3Department of Plant Protection and Environment of the National School of Agriculture-Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknès 50001, Morocco. E-mail: s.atanbhadou@edu.um.ac.ma

The biocontrol effectiveness of antagonistic bacteria against fire blight (caused by *Erwinia amylovora*) was evaluated under *in vitro* and field conditions. Among 61 bacteria isolated from soil and flowers of fire blight host plants from different Moroccan
areas, 20 isolates showed greatest antagonistic activity against the pathogen in agar diffusion tests, attached blossoms assays and in a bioassay on immature pear fruits. Effective isolates were identified using biochemical tests and 16S rDNA gene sequencing. These isolates were grouped in the following genera: 

- Alcaligenes (ACBC1), Bacillus (CPa12, CPa2, HP6, JB2, LMR2, SF14, SF16, SP10, SP13, SP18), Brevibacterium (SF3, SF4, SF7, SF15), Pantoea (ACBC2, ACBP1, ACBP2), Pseudomonas (SP9), and Serratia (HC4). The isolates were reported in the NCBI nucleotide sequence database (GenBank) under the accession numbers KY357285 to KY357304. In a field assay with the susceptible varieties of apple, spray treatments were carried out with different genera of bacterial antagonists. Their efficacies were evaluated 15 days post-inoculation on blossoms, and ranged from 54.6 to 95.0% for 11 strains, most of which gave better reductions than that obtained with commercial bacterial strains P10c (66%) and Q5ST713 (63%). The strains showed no pathogenicity towards plant tissues (pear fruitlets, pear and apple blossoms, tobacco leaves), and are candidates for microbial formulations for fire blight control.

**Diversity of fungal endophytic community in Quercus suber L. and detection of opportunistic phytopathogenic fungi.** D. COSTA1,*, J. CUNHA1,*, R. M. TAVARES1, P. BAPTISTA2, T. LINO-NETO1.

1BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. 2CIMO/School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: tlneto@bio.uminho.pt

Cork oak (Quercus suber) is of high ecological importance in the Mediterranean region, and has high relevance for the Portuguese economy, due to cork production and processing. The sustainability of cork oak is currently being threatened by reduction of water availability that would increase the occurrence of diseases. Charcoal disease, caused by Biscogniauxia mediterranea, leads to death of the cork oak trees. Diplodia corticola is involved in various diseases considered responsible for the decline of cork oak in the Mediterranean region. To identify endophytic fungi in cork oak, including opportunistic pathogens, four sites of continental Portugal (Bragança, Gerês, Alcobaça and Grândola), with differences in water availability, were selected for collection of biological material. Fungal endophytes from leaves, stems and roots were evaluated. Roots had more diverse fungal communities than the aboveground organs. Although no disease symptoms were detected on the studied trees, the pathogenic fungi were essentially affecting stems and leaves. In general, greatest endophyt colonization frequency and diversity occurred in Grândola, and least in Alcobaça. From all studied sites, cork oaks from Gerês showed the most distinct community and did not present the pathogens. Diplodia corticola only infects trees from southern regions, while B. mediterranea also infected trees in Bragança. The exclusive presence of both pathogens in aboveground organs and the absence of visible disease symptoms in all studied cork oaks, encourage the search for adequate biocontrol agents from the endophytic communities for restricting these cork oak diseases.

This research was supported by National Funds from FCT – Portuguese Foundation for Science and Technology, under the project UID/Multi/04046/2013. Daniela Costa was supported by FCT, grant reference SFRH/BD/120516/2016, and the Doctoral Programme “Agricultural Production Chains – from fork to farm” (PD/00122/2012).

**Xylella fastidiosa research in Europe**

Natural competence and recombination in vitro occurs frequently among Xylella fastidiosa isolates from subsp. fastidiosa and multiplex. P.P. KANDEL1, L. DE LA FUENTE1. 1Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA, 36849. E-mail: lzd0005@auburn.edu

*Xylella fastidiosa* (Xf) is a plant pathogenic bacterium that causes incurable diseases in economically important crops such as grapevine and citrus. For more than a century, Xf-caused diseases were restricted to the Americas, but recent reports in new locations (e.g. Italy, France, Spain), and new hosts (e.g. olive, Polygala myrtifolia) exemplifies the ability of this pathogen to adapt to new environmental conditions. Based on genetic diversity studies, inter-subspecific recombination (ISR) was proposed to contribute to host shifts. Natural competence, as a mode of recombination, was shown to occur at high frequen-
cies in model systems mimicking natural habitats; viz. microfluidic chambers with xylem sap. However, little is known about the variability of recombination potential among Xf isolates. Therefore, we compared recombination frequencies of thirteen Xf isolates belonging to two subspecies (fastidiosa and multiplex), using five different plasmids containing antibiotic resistance markers flanked on either side by Xf homologous regions. Recombination frequency varied greatly among isolates (3.14 × 10^2 to 2.3 × 10^8 recombinants per parental cell), and was not correlated with the sequence identity of the homologous regions. Nevertheless, the ability to recombine was correlated with twitching motility (r = 0.71, P = 0.006). When combination of marker-tagged, heat-killed donor and live recipient cells from two subspecies were mixed, ISR occurred within a genomic region of ~10kb. This study demonstrates that recombination, and therefore evolutionary potential, differ among Xf isolates, which is a serious threat in those cases where isolates can co-exist in the same environment.

This research was supported by the HATCH AAES (Alabama Agricultural Experiment Station) program, and Agriculture and Food Research Initiative competitive grant no. 2015-67014-23085 from the USDA National Institute of Food and Agriculture

Photointerpretation of high resolution aerial images for large scale monitoring of the olive quick decline syndrome associated to Xylella fastidiosa. S. GUALANO, F. SANTORO, F. VALENTINI, A.M. D’ONGHIA. CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy. E-mail: donghia@iamb.it

Xylella fastidiosa (Xf) is the main cause of the olive quick decline syndrome (OQDS), a serious threat for the olive trees in the EU-Mediterranean regions. After the first outbreak in 2013, the rapid identification of Xf on territorial basis was crucial in Apulia, Italy. For this purpose, the photointerpretation of high resolution aerial images was successfully applied to identify OQDS-like trees in a buffer area of Lecce, with 20% correlation between OQDS and ELISA-positive trees. The same technique was evaluated in the buffer/container zones (apparently Xf-free) of the demarcated area in Apulia. High geometrical resolution aerial images from three regions of interest (ROI), ranging from about 0.5 to 1.6 km^2 each, were processed in the visible (VIS) and near infrared (NIR) in a GIS environment. Analyses were oriented to identification of phototypes, morphologically associated to the OQDS. Results of the recognition process have provided the classification of 637 OQDS-like trees (3.7%) out of 17,220 photointerpreted trees. Following field inspections, 462 trees (73%) showed OQDS while the remaining could not be inspected (pruned trees or inaccessible groves), and few were altered by other factors. All OQDS trees were serologically tested for Xf, which was found in two ROI with different infection rates: 12% (20 infected trees out of 165 OQDS trees) and 3.5% (four infected trees out of 112 OQDS trees). The method was effective for identifying new foci of infection in the buffer and containment zones, orienting inspections in the official monitoring for rapid identification of infected trees and allowing immediate modification of the demarcated areas.

Genetic diversity of Xylella fastidiosa assessed in imported ornamental Coffea arabica plants. M. BERGŠMA-VLAMI*, J.L.J. VAN DE BILT, N.N.A. TJOU-TAM-SIN, C.M. HELDERMAN, P.P.M.A. GORKINK-SMITS, N.M. LANDMAN, J.G.W. VAN NIEUWBUG, E.J. VAN VEEEN, M. WESTENBERG. Dutch National Plant Protection Organization (NPPONL), P.O. Box. 9102, 6700 HC Wageningen, the Netherlands. E-mail: m.vlami@nvwa.nl

The diversity of Xylella fastidiosa in imported ornamental Coffea arabica plants was assessed through a MLST analysis, and compared with X. fastidiosa infecting different host plants worldwide. Different sequence types (STs) of X. fastidiosa were found, such as ST 53 and ST 73 (X. f subsp. pauca) and ST 72 and ST 76 related to X. f subsp. fastidiosa. Additionally, a novel ST, ST 77 has been assessed, that is related to X. f subsp. fastidiosa, but shares alleles from at least two different subspecies of X. fastidiosa. Isolation of X. fastidiosa from infected C. arabica plants was successfully performed only after the application of a brief ultrasonication step during extraction. The acquired X. f subsp. pauca isolates belonged to either ST 53 or ST 73. Data acquired from PACBIO/Illumina next generation sequencing (NGS) on X. f subsp. pauca isolate PD 7202 (ST 53) demonstrated that, at the chromosomal level, PD 7202 is identical...
to CoDiRo-ST53 found in Italy on olive. However, at plasmid level, clear differences have been assessed in individual genes. Virulence studies are currently ongoing after inoculation of *X. f* subsp. *pauca* isolates PD 7202 (ST 53) and PD 7211 (ST 73) on several plant species including *Coffea arabica*. Preliminary results on virulence will be presented.

This study was supported by research grant OS 2015330 project for *X. fastidiosa* of the Ministry of Economic Affairs in the Netherlands and partly by H2020 programme – SFS-09-2016, XF-ACTORS, grant agreement 727987.

**Fast and sensitive detection for Xylella fastidiosa through recombinase polymerase amplification.** R. LI¹, P. RUSSELL¹, S. ZHANG¹, B. DAVENPORT¹, A. EADS², K. SCHUETZ¹, S. BERKANI², M. AMATO².

¹Agdia Inc., Elkhart, IN 46514, U.S.A. ²Agdia-EMEA, 91350 Grigny, France. E-mail: Rugang.li@agdia.com

*Xylella fastidiosa* (Xf), living and multiplying in host xylem, is regulated in many countries. Xf originated in the American continent, but in recent years has appeared in Mediterranean countries including Italy, France, and Spain, and is causing grave concern from damage in olive trees of southern Italy and rapid spread to other crops and areas. The genetic diversity indicates that these new introductions are independent of one another. A fast and sensitive detection method is critical to reduce the likelihood of Xf introduction into new areas. Agdia has developed a rapid and sensitive DNA test for specific detection of Xf using the advanced recombinase-polymerase amplification technology (AmplifyRP). The assay performs both as a real-time and an endpoint test, from a single reaction tube at 39°C for 20 min. Reaction template is simply prepared by soaking 50 mg of petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 min, or by suspending one culture colony in 100 μL AMP1 buffer. The assay reacts to 28 Xf isolates from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry, while consistently detecting 22 and even less copies of spiked Xf genome in soaking extract (1:10, w:v). No reaction background was observed in host tissues, and no cross-reaction was observed to *Xanthomonas, Erwinia, Pseudomonas,* and *E. coli*. This DNA test provides a reliable tool to fight against Xf spread, as it can be performed directly on site.

This research is supported by Agdia, Inc.

**Current situation in France regarding Xylella fastidiosa: methods of detection and subspecies characterization, strains and host plant diversity.** F. POLIAKOFF¹, B. LEGENDRE¹, V. OLIVIER¹, C. DOUSSET¹, S. PAILLARD¹, D. MOLUSSON¹, A. SAINTE-LUCE¹, V. JUTEAU¹, N. DENANCE¹,², M.A. JACQUES. ¹Bacteriology, Virology and GMO Unit - ANSES / Plant Health Laboratory – 49044 Angers, France. ²IRHS, INRA, AGROCAMPUS-Ouest, Université d’Angers, SFR4207 QUASAV, 42, rue Georges Morel, 49071 Beaucouzé, France. E-mail: francoise.poliakoff@anses.fr

In conjunction with the emergence of *Xylella fastidiosa* (Xf) in Europe, several interceptions of coffee plants contaminated with Xf occurred in France. Different Xf subspecies and sequence-types (ST) were identified: *fastidiosa* (ST75), *sandyi* (ST72 and ST76) and *pauca* (ST53 and ST74). Since the discovery of a focus of *Polygala myrtifolia* (Pm) in natural settings in 2015 in Corsica and the French Riviera, this pathogen has been detected on thirty plant species with a validated method based on real time PCR (Harper et al, 2010) associated with a DNA extraction kit (BioNobile). Characterization of isolates directly on plants, or for strains isolated on modified PWG medium is performed according to multilocus sequence typing (MLST) (http://pubmlst.org/xfastidiosa/). Following EPPO protocol PM 7/24, isolates were mostly allocated to sequence types ST6 and ST7 (subspecies *multiplex*). Mixed infections by ST6 and ST7 was demonstrated by isolation of both strains from one Pm. Modifications to a proposed amplification protocol revealed infections linked to the subspecies *pauca, sandyi,* one recombinant and some mixed infections. The EPPO protocol MLST confirmed identification of four Pm contaminated with subsp *pauca* but not the identification of other contaminants. These contaminations were not observed again in the immediate environment after plant eradication. Subspecies assignation directly from plant material is not always successfully linked to PCR inhibitors depending on host plants. This study confirms the diversity of subspecies of Xf in France, but the subspecies *multiplex* was found to greatly predominate.

This research is partly supported by the Project H2020 PONTE.

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Anova-Plus is developing Flashdiag®XF, a diagnostic kit for field use that will detect *Xylella fastidiosa* from a wide spectrum of plant hosts using DNA isothermal amplification. Samples from public collections were obtained for sub-species *X. f. fastidiosa*, *X. f. pauca* and *X. f. multiplex*, isolated from different plant hosts (grapevine, coffee, almond, olive or plum). The isothermal amplification method was conceived to test in one reaction all these *X. fastidiosa* sub-species. DNA from healthy plants was used as negative control and absence of cross-reaction was ensured with closely related species (i.e. several *Xanthomonas* species). Flashdiag®XF was designed to be used directly in the field, and is adapted for users without laboratory experience. From a symptomatic plant leaf/petiole, and in less than one hour, the test clearly indicates the presence or absence of *X. fastidiosa* for a given plant sample. In 2017, DNA samples from infected plants of olive, almond, oleander, cherry, *Polygala mirtifolia*, laurel, lavender and rosemary, collected by the University of Bari Aldo Moro, will be tested. In 2018, the kit will be tested with other host species. A field validation will be conducted by the end of 2017 on olive trees in Apulia (Italy), to test the kit in field conditions with a high number of samples. Flashdiag®XF aims to provide a rapid diagnostic leading to quick monitoring of *X. fastidiosa* the pathogen in a field-based, user-friendly format.

This project was supported by Bpifrance (the French Public Investment Bank).

**The Android application XylApp for the survey of *Xylella fastidiosa* infections.** F. SANTORO, S. GUALANO, G. FAVIA, A.M. D’ONGHIA. CIHEAM - Istituto Agronomico Mediterraneo di Bari, Via Ceglie 23, Valenzano (BA) 70010, Italy.

*Xylella fastidiosa* is an important quarantine bacterium, vector-transmitted, which infects more than 380 plant species worldwide. Following the EU implementation of Decision 789/2015, surveys for *X. fastidiosa* are mandatory in member states, but these surveys time- and human resources- consuming, and require accuracy in field data acquisition and rapid data transmission. Support for inspectors could be provided by handheld devices, such smartphones and tablets. A dedicated application for Android smart devices, named ‘XylApp’, has been designed and developed for the accuracy in the monitoring activity of *X. fastidiosa* in Apulia, Italy. Improved versions of XylApp have been made to enhance the accuracy and rapid use of survey data, and to support statistical analyses for the epidemiological studies. The version dedicated to inspectors is composed of five independent modules: ‘Sample’, for data acquisition and geolocalization without map support; ‘Browse and Sample’, for data acquisition and geolocalization using the regional cartographic grid; ‘Find’, for finding one or more targets through geographic coordinates; ‘Archives’, for field data storage and transmission to a remote database; and ‘Vademecum’, for providing inspectors with a valuable information as a practical guide. An additional module is ‘Improve localization’, for manual improvement of geolocalization. A light version of the application, composed of three modules (Check, Mail, Learn), was also developed for rapid reporting of suspected symptomatic host plants by stakeholders (XylApp<sub>all</sub>). XylApp facilitates, optimizes and rationalizes data acquisition, geolocalization, storage and realtime transmission to the a central server of the Plant Protection Service.

**Flashdiag®XF Kit, a rapid field diagnostic tool for detection of *Xylella fastidiosa*.** T. VANDEWALLE<sup>1</sup>, K. OULDELKABLA<sup>1</sup>, C. FABRE<sup>1</sup>, G. LOCONSOLE<sup>2</sup>, M. MASSON<sup>1</sup>.<sup>1</sup> Anova-Plus, 4 rue Pierre Fontaine, Genopole Campus 3, Evry 91030, France. <sup>2</sup> Department of “Scienze del Suolo, della Pianta e degli Alimenti”, University of Bari Aldo Moro, Via Amendola, 165/A 70126 Bari, Italy. E-mail: thomas.vandewalle@anova-plus.com

Anova-Plus is developing Flashdiag®XF, a diagnostic kit for field use, that will detect *Xylella fastidiosa* from a wide spectrum of plant hosts using DNA isothermal amplification. *Xylella fastidiosa* samples from public collections were obtained for sub-species *X. f. fastidiosa*, *X. f. pauca* and *X. f. multiplex*, isolated from several plant species (grapevine, coffee, almond, ol-
The emergence of Xylella fastidiosa in the Balearic Islands, Spain, is associated with several subspecies and sequence types of the bacterium. D. OLMO1, M. MONTES-BORREGO2, A. NIETO1, F. ADROVER1, A. URBANO1, O. BEIDAS1, A. JUAN4, E. MARCO1, M.M. LÓPEZ3, I. NAVARRO3, A. MONTERDE3, J.A. NAVAS-CORTÉS3, B.B. LANDA2. 1Serveis de Millora Agrària. Govern Balear. Eusebi Estada 145. 07009, Palma de Mallorca, Spain. 2Department of Crop Protection, Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain. 3TRAGSA, Empresa de transformación Agraria, Delegación de Baleares. Pasaje Cala Figuera, 6. 07009, Palma. 4Servicio de Agricultura. Conselleria de Medi Ambient, Agricultura i Pesca. C/Reina Constança, 4. 07006 Palma. 5Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera km 4,5, Moncada 46113, Valencia, Spain

Xylella fastidiosa is a quarantine organism in the European Union (EU), that was first detected in Europe in Italy in 2013 where it is associated to a severe epidemic on olive trees. The bacterium has also been detected in France (2015) and Germany (2016). Due to the recent outbreaks and to different interceptions, mainly on ornamental coffee plants, the EU has implemented annual surveys in its member states to prevent new introductions or the spread of this harmful organism. During official surveys in late autumn 2016 in Mallorca Island, Spain, the bacterium was first detected in a garden centre near the locality of Manacor. Since then a total of 189 positive samples in 11 different host species have been found in different disease foci in the islands of Mallorca (124), Menorca (16) and Ibiza (49). Sequence analysis of the RNA polymerase sigma 70 factor sequence and multilocus sequence analysis (MLST)/typing revealed the presence of X. fastidiosa subsp. fastidiosa ST1 and X. fastidiosa subsp. multiplex ST6* (a new ST closest to ST6) and ST7 in Mallorca island, X. fastidiosa subsp. multiplex ST6* in Menorca island, and X. fastidiosa subsp. pauca ST80 (a new ST) in Ibiza island. Polygala myrtifolia was found to be infected by all subspecies and ST types. These results suggest that the emergence of X. fastidiosa in the Balearic Islands is likely due to several introduction events of diverse strains and different subspecies. Eradication measures were taken in the garden centre according to the Spanish contingency plan and EU legislation. Following the Commission Decision 2015/789/EU of establishing a 10 km radius delimiting buffer zone for each infection focus, 80% of the territory of Mallorca 50% of Menorca, and 90% of Ibiza are considered as demarcated areas. The best strategies to control the different outbreaks are under study.

This study was supported by funding from the European Union’s Horizon 2020 research and innovation programme, under grant agreements No. 635646 POnTE (Pest Organisms Threatening Europe) and No. 727987 XF-AC-TORS (Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy).

Fast and sensitive detection for Xylella fastidiosa through recombinase polymerase amplification. R. LI1, P. RUSSELL1, S. ZHANG1, B. DAVENPORT1, A. EADS1, K. SCHUETZ2, S. BERKANI1, M. AMATO2. 1Agdia Inc., Elkhart, IN 46514, U.S.A. 2Agdia-EMEA, 91350 Grigny, France. E-mail: Rugang.li@agdia.com

Xylella fastidiosa (Xf), living and multiplying in host xylem systems, is regulated in many countries. Xf originates from the American continent. In recent years the pathogen has appeared in Mediterranean...
countries, including Italy, France, and Spain, and is causing grave concern through damage in olive trees of southern Italy and rapid spread to other crops and areas. The genetic diversity of Xf indicates that these new introductions are independent of one another. Therefore, a fast and sensitive detection method is required to reduce the likelihood of Xf introduction into new areas. Agdia has developed a rapid and sensitive DNA test for specific detection of Xf, using advanced recombinase-polymerase amplification technology (AmplifyRP). The assay performs both as a real-time and an endpoint test, from a single reaction tube held at 39°C for 20 min. Reaction template is simply prepared by soaking 50 mg of plant petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 min, by suspending one culture colony in 100 μL AMP1 buffer. The assay reacts to 28 Xf isolates, from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry, while consistently detecting 22 (and even less) copies of spiked Xf genome in soaking extract (1:10, W/V). No reaction background was observed in host tissues. No cross-reaction was observed to Xanthomonas, Erwinia, Pseudomonas, and E. coli. This test provides users with a reliable tool to assist against Xf spread as it can be performed directly on site.

This research is supported by Agdia, Inc.

**Isolation, genetic characterization and phenotypic profiling of Xylella fastidiosa strains from Costa Rica.** N. RODRÍGUEZ-MURILLO, I. ABDALLAH-QUIROS, A. BADILLA-LOBO, G. GONZÁLEZ-ES-PINOZA, C. CHACÓN-DÍAZ. Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San Pedro 2060, Costa Rica. E-mail: carlos.chacondiaz@ucr.ac.cr.

*Xylella fastidiosa* is endemic in Costa Rica. In the last decade this pathogen has been detected and isolated from more than 20 different economically important crops and ornamentals, extending the geographic range of detection of the bacterium. However, although *X. fastidiosa* has great potential to cause disease, and is widespread throughout Costa Rica, the symptoms related to infected plants are usually mild or infections are asymptomatic. In recent years, the presence of *X. fastidiosa* in Europe has had important social and economic consequences, and also in plant exporting countries such as Costa Rica. From previous reports it is known that *X. fastidiosa* strains isolated from Costa Rica have broader genetic variability than strains in other countries. There is genetic similarity among ST53 isolates from Costa Rica and the CoDiRo strains from affected olives in Italy. The parallel study of *X. fastidiosa* circulating strains from Costa Rica can contribute to outline of specific traits of the European *X. fastidiosa* strains. We isolated and characterized *X. fastidiosa* strains from different hosts to broaden genetic and phenotypic information on our circulating strains. We have isolated *X. fastidiosa* ST33, ST21 and ST61 strains from coffee and ST33 from guava, and these sequence types are related to *X. fastidiosa* subspecies *fastidiosa*. Complementary to genetic profiling, we are phenotypically characterizing our strains through biochemical and fatty acid profiling and through biofilm formation assays. Our goal is to standardize a series of *In vitro* assays that could eventually be used in reference and research units for *X. fastidiosa* profiling.

This research was supported by the European Union’s Horizon 2020 research and innovation programme, under grant agreements No. 635646: POnTE (Pest Organisms Threatening Europe) and No. 727987: XF-ACTORS (*Xylella fastidiosa* active containment through a multidisciplinary oriented research strategy).

**A new molecular LAMP tool for *Xylella fastidiosa* early detection.** C. AGLIETTI1, L. GHELARDINI1,2, P. CAPRETTI1, A. SANTINI1, N. LUCHI1. 1Department of Agrifood production and Environmental Sciences (DISPAA), University of Florence, Piazzale delle Cascine 18, 50144, Firenze, Italy. 2Institute for Sustainable Plant Protection- National Research Council (IPSP-CNR), Via Madonna del piano 10, 50019, Sesto fiorentino (Firenze), Italy. E-mail: chiara.aglietti@unifi.it

*Xylella fastidiosa* is a Gram-negative bacterium that causes considerable economic damage by xylem occlusion in over 200 different plant hosts. This pathogen was confined to America until 2013, when it was found in Italy (Apulia) and thought to be responsible of olive quick decline syndrome. The pathogen was also reported in Europe on oleander (*Nerium oleander*) and on Polygala myrtifolia. As an invasive pathogen, its spread might cause severe environmental and economic damage. An effective control
plan is necessary to contain *X. fastidiosa* impacts, and this requires specific and sensitive diagnostic tools. PCR-based methods are favoured for their sensitivity and specificity, but these require laboratory facilities. Advantages might be gained from moving testing closer to sampling sites. A diagnostic assay based on loop mediated isothermal amplification (LAMP) was developed to detect *X. fastidiosa*. This assay, optimized on the portable instrument Genie II (Optigene, UK) and based on RimM target region, can recognize the pathogen DNA with high levels of specificity, identifying only *X. fastidiosa*, and sensitivity, detecting DNA as little as 0.128 pg/μL, equaling results obtained with the compared *X. fastidiosa* qPCR assay. The LAMP method used for detecting *X. fastidiosa* on symptomatic and asymptomatic samples could assist checking of imported and exported live plants, limiting the uncontrolled spread of this pathogen. Simplicity, sensitivity and specificity, high speed (only 30 min) and minimum required equipment make the assay ideal for field applications, helping routine plant testing in cities and forests.

**Wood, root and foliar diseases in fruit and forest crops in the Mediterranean region**

Pine wilt disease: insights into the biology of *Bursaphelenchus xylophilus*-associated *Serratia*. C.S.L. VICENTE¹, K. HASEGAWA², M. MOTA³. ¹ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal. ²Department of Environmental Biology, Chubu University, Kasugai, Japan. E-mail: cvicente@uevora.pt

Pine wilt disease (PWD) is caused by the parasitic nematode *Bursaphelenchus xylophilus* (pinewood nematode; PWN), which infects mainly *Pinus* species with the aid of an insect-vector, *Monochamus* sp.. Bacteria isolated from *B. xylophilus* are being considered as a fourth element in this disease complex. Their precise roles of these organisms in this interaction are unclear, as both beneficial and pathogenic bacteria have been found associated with PWD. Previously, we have shown the high oxidative stress tolerance of the PWN-associated bacteria *Serratia* sp. LCN16 and *Serratia marcescens* PWN146, and their beneficial effects towards the nematode under harsh oxidative stress conditions. Here, we present a detailed analysis of the genome sequences of these two PWN-associated bacteria and provide new insights into their biology and contributions to PWD and the PWN. *Serratia* sp. LCN16 is phylogenetically most closely related to the phytosphere group of *Serratia*, and shares many features with endophytes (plant-associated bacteria). These include genes coding for plant polymer degrading enzymes, iron uptake/transport, siderophore and phytohormone synthesis, aromatic compound degradation and detoxification enzymes. *Serratia marcescens* PWN146 can also withstand and colonize the plant environment, without having any deleterious effects towards *B. xylophilus* nor to the nematode model *C. elegans*. PWN146 has the potential to interfere with plant metabolism via hormonal pathways or nutritional acquisition (i.e. iron), and to be competitive against other bacteria and fungi, through resource acquisition or production of antimicrobial compounds.

This research was supported by the JSPS KAKENHI Grant numbers P14394 (to CSLV) and 26450204 (to KH); and by National Funds through FCT—Foundation for Science and Technology under the Project UID/AGR/00115/2013.

**Comparative study of Pseudomonas syringae pv. syringae strains isolated from mango trees distributed worldwide with over 25 years apart.** F. APRILE, J.A. GUTIERREZ-BARRANQUERO, F.M. CAZORLA, A. DE VICENTE. Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (IHSM-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: aprile@uma.es

Mango (*Mangifera indica* L.) is one of the most important world fruit crops. In 1992, the disease bacterial apical necrosis (BAN) of mango was described for the first time in southern Spain. BAN is caused by *Pseudomonas syringae* pv. syringae (Pss), and is mainly associated with Mediterranean climate. The disease has been described in other mango-producing areas with similar weather (Portugal, Italy, Israel, Egypt, Florida and northeast Australia). Different Pss isolates from mango have been studied for years, to decipher their virulence and epiphytic fitness mechanisms. Genes associated with these biological characteristics have been described: *mbo* operon involved in the mangotoxin production, *copABCD* or *cusCBA*...
operons involved in copper resistance, and the production of cellulose by rrs genes. Phylogenetic studies have revealed a differentiated phylotype of the Pss strains isolated from mango characterized by mangotoxin production. This study analysed epidemiology and evolution of different Pss isolates from mango from different Spain, Portugal, Italy, Israel and Australia, isolated in 2000 (UMA lab collection), and recently obtained isolates (2016 and 2017). A comparative genomic analysis representative strains of each collection will be carried out to unravel the evolutionary processes which have occurred during the last 25 years.

This research is supported by Incentivos a Proyectos de Excelencia de la Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía (P12-AGR-1473), cofinanced by FEDER (EU).

Survey of Cylindrocarpon-like anamorphs in Spanish forest nurseries. B. MORA-SALA, A. CABRAL, M. LEÓN, C. AGUSTÍ-BRISACH, J. ARMENGOL, P. ABAD-CAMPOS. 1Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022-Valencia, Spain. 2Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomía, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal. 3Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain. E-mail: beamosa@upvnet.upv.es.

Cylindrocarpon-like anamorphs infect herbaceous and woody plants, mainly in agricultural situations, but also in forests. This study characterized, by DNA analysis, a wide collection of Cylindrocarpon-like isolates recovered from roots of a broad range of forest hosts showing decline symptoms in nurseries. From 2009 to 2012, 18 Spanish forest nurseries were surveyed and a total of 103 Cylindrocarpon-like isolates were obtained. The isolates were identified based on sequencing a fragment of the histone H3 gene (HIS), which was amplified by PCR with the primer pair CylH3F and CylH3R. Some isolates were additionally sequenced for the Internal Transcribed Spacer (ITS) region, and partial β-tubulin (TUB) and translation elongation factor 1-α (TEF) genes, to better resolve their phylogenetic positions. Thirteen species of Cylindrocarpon, Dactylonectria, Ilyonectria and Neonectria were identified from damaged roots of 15 different hosts. The species C. alicantinum, D. macrodidyma, D. noovelandica, D. pauciseptata, D. piniola, D. torresensis, I. capensis, I. cyclaminicola, I. liriodendri, I. pseudodestructans, I. robusta, I. rufa and Neonectria sp. were identified. In addition, two new Dactylonectria and one new Ilyonectria species were described. This study is the first characterization of a wide collection of Cylindrocarpon-like anamorphs obtained from forests plants, and demonstrates the prevalence of this fungal group associated with seedlings of diverse hosts showing decline symptoms in Spanish forest nurseries.

This research was supported by the project AGL2011-30438-C02-01 (Ministerio de Economía y Competitividad, Spain).

Simulation of the potential infectivity range of Phytophthora cinnamomi under climate change. M.C. CABALLERO, I.M. PÉREZ-RAMOS, L. MATÍAS, M. SERRANO. Instituto de Recursos Naturales y Agrobiología de Sevilla, Avenida Reina Mercedes, 10, 41012, Sevilla, Spain. E-mail: maria.serrano@irnas.csic.es

Quercus open-woodlands, dehesas in Spain, are one of the most important ecosystems of the Mediterranean Basin, but their sustainability and persistence could be seriously affected by global change and exotic pathogen introductions. This study examines the interactive effects of climate change and land-use (over-grazing) changes on production of sporangia by Phytophthora cinnamomi in high risk area of trees suffering from root rot disease. An experiment of reduced rainfall and increased temperature (simulating the future climate conditions predicted by climate change models) was set up in three dehesas systems facing different grazing intensities (low, medium or high) in open areas and below Q. ilex trees, during September 2016. A total of 48 replicates were established, with six replicates per site, location and climate treatment. Five months later (January 2017), soils obtained under trees were more stimulatory to sporangia producee with the high risk area of trees suffering from root rot disease.
among the climate change treatments have not yet observed, but differences were recorded for plant composition related to treatments and sites. These differences increased during spring. Climate change and grazing effects on plant communities and their relationships with sporangium production with time will be presented.

This research was supported by the Project DECAFUN: CGL2015-70123-R (Ministry of Economy, Industry and Competitiveness) and Marie Sklodowska-Curie Actions-H2020 (European Union).

Increasing diversity of vegetative compatibility types in *Cryphonectria parasitica* in the Eastern Black Sea region of Turkey and its relation to sexual reproduction. E. MANGİL, O. ERİNCİK. Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection, 09100, Aydın, Turkey. E-mail: oerincik@adu.edu.tr.

This study aimed to determine the vegetative compatibility (vc) type diversity of *Cryphonectria parasitica* in the Eastern Black Sea Region of Turkey, and the role of sexual reproduction in this diversity. Vc types of 344 *C. parasitica* isolates collected from Artvin, Trabzon and Rize provinces in 2016 were identified by growing pairs of isolates on media. Mating types were detected using a PCR with specific primers. Single ascospore isolates were obtained from perithecia of 21 field cankers and at least 25 isolates per peritheciun were subjected to vc type assay. There is large vc type diversity in the region. Among 344 isolates, 293 were compatible with the European vc testers, EU-1 (68%), EU-17 (6.7%), EU-12 (6%), and EU-3 (4%), whereas 51 isolates were not compatible with any of the European testers. The unidentified vc types were in six groups, designated TU-1 (6.4%), TU-2 (2.6%), TU-3 (1.2%), TU-4 (1.7%) and TU-5 (0.9%). MAT-1 and MAT-2 comprised, respectively, 37.4% and 55.3%, respectively. Thirteen isolates were heterokaryotic carrying both mating alleles. Perithecia were found in 130 bark samples, which indicates widespread occurrence of sexual reproduction. Number of vc types within the group of single ascospore isolates from single perithecia ranged from 1 to 13. Diversity of vc types increases in Turkey, and this increase can be partially related to the recombination of vegetative incompatibility genes through sexual reproduction.

This research was supported by the Scientific Research Fund of Adnan Menderes University through the grant no: ZRF-15077.

Phenotypic, molecular and pathogenic characterization of *Phlyctema vagabunda*, the cause of olive leprosy. J. ROMERO, M.C. RAYA, L.F. ROCA, C. AGUSTÍ-BRISACH, J. MORAL, A. TRAPERO. 1Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain. 2Department of Plant Pathology, University of California, Davis, Kearney Agricultural Research and Extension Center, 9240 South Riverbend Ave., Parlier 93648, CA, USA. E-mail: ag1trcaaduco.es

Olive leprosy, caused by the fungus *Phlyctema vagabunda*, is a classic fruit ro disease widespread in the Mediterranean basin. From 2009-2013, new disease symptoms consisting of small circular necrotic leaf lesions, coin branch canker and shoot dieback were observed in Spanish and Portuguese olive orchards showing intense defoliation. *Neo_fabraea*-like fungal colonies were consistently isolated from symptomatic leaves and shoots. Representative isolates from affected leaves, shoots and fruits were characterized by morphology of colonies and conidia, optimal growth temperatures and comparison of DNA sequence data from four regions: ITS, tub-2, MIT and rpb2. Pathogenicity tests were performed on apple and olive fruits, and on branches and leaves of olive trees. Maximum mycelial growth rate ranged between 0.54 and 0.73 mm d⁻¹. Morphology of conidia produced on apple fruit and phylogenetic analysis showed homogeneity among fungal isolates, which were identified as *Phlyctema vagabunda*. On fruits, influence of wounding, ripening and cultivar resistance were studied, with cv. Blanquet being the most susceptible cultivar. On branches, mycelial plug inoculation reproduced olive leprosy symptoms and caused shoot dieback. On leaves, Koch’s postulates were fulfilled and the pathogen caused characteristic necrotic spots and plant defoliation. Wounds had a key role on olive leprosy development. This is the first time that *Ph. vagabunda* is described as a causal agent of leaf spot and defoliation in olive trees. The integration of mechanized practices in olive crop management could be the cause of re-emergence of this disease.
This research was funded by Bayer Crop Science and ELAIA companies. C. Agustí-Brisach is holder of a ‘Juan de la Cierva-Formación’ fellowship from MINECO. J. Moral holds a Marie Skłodowska-Curie fellowship launched by the European Union’s H2020 (contract number 658579).

The importance of identifying the vegetative compatibility types of chestnut blight (Cryphonectria parasitica) at local level; case study in a chestnut stand in El Bierzo (León). A. LORENZANA1, D. RODRÍGUEZ2, M.F. CAMPELO1, F. CASTEDO-DORADO1. 1Departamento de Ingeniería y Ciencias Agrarias, Escuela Superior y Técnica de Ingeniería Agraria, Universidad de León, Campus de Ponferrada, Avda. de Portugal s/n, 24401 Ponferrada, León, Spain. 2Grupo de Investigación de Ingeniería y Agricultura Sostenible, Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad, Universidad de León, Avda. de Portugal, 41, 24071 León, Spain. E-mail: alorc@unileon.es

The most used treatment for control of chestnut blight (caused by Cryphonectria parasitica) is based on the use of hypovirulent fungal strains. The success of this method depends on the knowledge of the vegetative compatibility (vc) types present in the stand of chestnut trees. Our research highlights the importance of establishing the vc types at local levels, through the case study of a stand of 250 ha located in Oencia (El Bierzo, León province). The aims were: (i) to determine the prevalence of chestnut blight canker in the stand; (ii) to identify vc types existing; and (iii) to compare these vc types with the most common European vc types in El Bierzo, according to the literature. A systematic sampling was carried out in 60 plots in which samples of bark with symptoms of the disease were collected. The results indicated that more than 70% of the trees and 100% of the sampled plots were affected. Furthermore, four vc types were identified in the stand, a large numbers considering that recent studies found five vc types throughout the province of León and nine vc types in Galicia. Of the fourvc types found, only two were compatible with the European vc testers EUI and EUIII. The high diversity found could be due to genetic recombinations caused by previous infections by hypovirulent strains.

Diversity of subtypes of Cryphonectria hypovirus 1 in the chestnut areas of Turkey where hypovirulence is present. O. ERİNÇİK, S. AÇIKGÖZ, S. HOSSEİNALİZADEH, M.T. DÖKEN, Adnan Menderes University, Faculty of Agriculture, The Department of Plant Protection 09100, Aydın, Turkey. E-mail: oerincik@adu.edu.tr

Biological control, based on the use of hypovirulent strains of Cryphonectria parasitica, is one the most effective methods for management of chestnut blight. Success in biological control on the subtype of Cryphonectria hypovirus 1 (CHV1) infecting hypovirulent strains. This study aimed to determine the diversity of the subtypes of CHV1 in Turkey. In 2014 and 2015, C. parasitica isolates were obtained from hypovirulent-type cankers from 14 provinces of the Marmara and Black Sea Regions, where hypovirulence is present. Among the 215 double-stranded RNA (dsRNA) positive isolates, 92 CHV1-infected C. parasitica isolates were sampled to use in subtype determination. The dsRNA of the virus was extracted and reverse transcription (RT) PCR product was obtained, using the primer pair hvep-1 and EP721-4. This amplifies a polymorphic DNA fragment from the open reading frame A region of the viral genome. Nucleotide sequence and phylogenetic analyses of the PCR products showed evidence of low diversity of subtype in CHV1 throughout the sampling area. The two subtypes of CHV1, Subtype I and Subtype F2, were found. Subtype I comprised of 78% of the isolates (76) and was dominant and found in 11 provinces. Subtype F2 accounted for 12% of the isolates (16) and was found in six provinces and restricted mostly to the Eastern Black Sea Region.

This research was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) through the grant no: 114O403.

Investigation of mycovirus double-stranded RNA in Phomopsis viticola isolates from grapevine in the Aegean Region of Turkey. S. ACIKGOZ, S. HOSSEINALIZADEH, O. ERINCİK. Adnan Menderes University, Faculty Of Agriculture, Dep. of Plant Protection. E-mail: oerincik@adu.edu.tr

Certain dsRNA viruses found in fungi have been associated with hypovirulence, and they are recommended as biological control agents in the management of several plant diseases caused by fungi. The
most successful example this strategy is the use of mycoviruses for management of chestnut blight. One of the important fungal diseases of grapevine that leads to economic damage in the Aegean Region, is dead arm (Phomopsis cane and leaf spot), caused by *Phomopsis viticola*. This study determined the presence of dsRNA in *P. viticola* isolates from grapevines in Turkey. Eighty samples were collected in 2016 from grapevine dark fissure-like lesions on canes and leaf spot symptoms, in the Manisa-Salihli and İzmir provinces of Aegean Region. A total of 75 *P. viticola* isolates were obtained. Nucleic acids were extracted from freeze-dried fungal mycelia and dsRNA was separated by cellulose CF-11 chromatography. The dsRNA electrophoretic pattern of 18-20 kb was detected in eight *P. viticola* isolates, on agarose gel. The diagnosis of this new mycoviral dsRNA in *P. viticola* has not yet been made, and it is not known whether this mycovirus is associated with hypovirulence. In future studies, the dsRNAs found be diagnosed by full genome sequence analysis. Virulence tests will be conducted on potted grapevine plants to determine the relationship between the mycoviral dsRNA and *P. viticola* hypovirulence.

This research was supported by the Project BAP2016-ZRF16009 (Adnan Menderes University, Turkey).

**Characterization of Fusarium oxysporum isolated from a young vineyard affected by grapevine decline.**

T. CINELLI¹, P. REVEGLIA², C. COMPARINI¹, M. NOCENTINI¹, A. EVIDENTE², L. MUGNAI¹. ¹Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. ²Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy. E-mail: tamara.cinelli@unifi.it

A young vineyard (2 years old) of cv. Pinot Gris, located in Veneto, North-Eastern Italy, showed two large areas of declining grapevines. The affected vines were stunted or dead. Sixteen plants were sampled from the two areas following a defined sampling scheme. The explanted vines showed large numbers of aerial roots, while the root systems of 87% of the vines were severely damaged. The majority of the few roots of the affected plants were fully necrotic or showed internal necrotic tissues. The necroses extended into the rootstocks and, in some, also into the scions. Fourteen of the 16 plants were mostly colonized by a single species, which was isolated from 70 to 90% of the woody tissues of the roots, of the rootstock and of the cultivar, and occasional *Cylindrocarpon*-like isolates were also obtained. For identification, multigene phylogenetic analyses were carried out. The internal transcribed spacer (ITS1-5.8S-ITS2) region and parts of the translation elongation factor 1-α (TEF1) and β-tubulin (TUB) genes of four isolates were sequenced. Nucleotide sequences were compared with those in the NCBI databases, showing a 100% identity with those belonging to *Fusarium oxysporum*. Since this is a species known for the production of phytotoxic metabolites that could have roles in symptom induction, EtOAc-pH6 and EtOAc-pH2 extracts from culture filtrates were tested on tobacco leaves. Both the extracts showed toxicity on tobacco leaves, inducing necrosis of the tissues. The phytotoxic compounds produced are currently being purified, and chemically and biologically characterized.

**Grapevine trunk diseases: the relevance of disinfection of propagation material.**

L. MUGNAI¹, T. CINELLI¹, C. COMPARINI¹, M. NOCENTINI¹, E. BATTISTON¹, M. BENANCHI², F. OSTI², T. NEM-CIK³, S. DI MARCO². ¹Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente, University of Florence, Piazzale delle Cascine 28, 50144 Firenze, Italy. ²Istituto di Biometeorologia (IBIMET), CNR, Via Gobetti 101, 40129 Bologna, Italy. ³510 Las Lomas Road, Sonoma, CA 95476, USA. E-mail: laura.mugnai@unifi.it

Grapevine trunk diseases (GTDs) are a major threat for viticulture, in all grape-growing countries. The main diseases affecting vineyards in Europe are the Esca complex: grapevine leaf stripe disease, black wood streaking, Petri disease and white rot. Cankers caused by Botryosphaeriaceae are also found with increasing frequency, associated with the death of grapevine cordons and spurs. Nursery production has a major role in producing plants strong enough to withstand aggressive wood pathogens, once they are planted in the field. At the same time, they must be as free as possible from pathogen infections at early life stages. Many years of trials have been carried out to evaluate strategies for reducing early nursery infections, comparing different new with established methods. Plant material infections by *Phaeomoniella*...
chlamydospora, Phaeoacremonium minimum, and species of Botryosphaeriaceae were assessed in either non-inoculated or artificially inoculated graftings, treated with different products. Promising results were obtained in the control/limitation of GTDs pathogen infections by treatment with innovative, low impact products (e.g. electrolysed water, ozone) and biological control methods. The benefits and relevance of superior quality planting stock are only realized when subsequent agricultural activities follow well-planned and balanced vineyard management practices.

**Occurence of Hop stunt viroid (HSVd) in Turkish pistachio trees.** S.C. BALSAK, N. BUZKAN, M.Z. AY and M. GÜRBUZ. 1Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü Imam, 46060 Kahramanmaraş, Turkey. E-mail: nbuzkan@gmail.com

Turkey is one of the greatest world producers of pistachio (Pistacia vera) after Iran and the USA. Plantations are generally in semi-arid areas of the southern part of Turkey. Recently, Hop stunt viroid (HSVd) (Hostuviroid, Pospiviroidae) infection was reported from Tunisia, although information of diseases associated with viruses and viroids is scarce. HSVd has a wide host range including trees, shrubs and herbaceous plants. In Turkey, HpSVd has been detected in grapevine, plum, peach, apricot, sweet cherry and almond, by RT-PCR, but without molecular characterization. We investigated HpSVd in a pistachio tree collection in Turkey. In July 2016, leaf and shoot samples were collected from 50 pistachio trees with virus-like symptoms, from the research and experimental orchard of the University of Kahramanmaraş Sütçü Imam in the Kahramanmaraş province of eastern Mediterranean. RT-PCR detection of HSVd was performed with dsRNAs using VP-19 and VP-20 primers. One sample of positive PCR was directly sequenced in two directions and was aligned with isolates from GenBank using CLUSTALX 1.8. Blast analysis of the Turkish HSVd pistachio isolate showed 99% nucleotide similarity with an HSVd isolate from Japan (Accesion number: X00009). A phylogenetic tree was constructed with 17 HSVd isolates from various hosts, using the neighbour-joining method. The Turkish HSVd isolate from pistachio trees aligned with an HSVd-grapevine isolate from Turkey. To our knowledge, this is the first report of HSVd in pistachio trees in Turkey. No symptom association was made with HSVd in pistachio trees.

This research was supported by the Research fund of Kahramanmaraş Sütçü Imam University (2016/5-35YLS).

**Comparative study of Pseudomonas syringae pv. syringae strains isolated from mango trees distributed worldwide and separated by 25 years.** F. APRILE, J.A. GUTIERREZ-BARRANQUERO, F.M. CAZORLA, A. DE VICENTE. Instituto de Horticicultura Subtropical y Mediterránea “La Mayora” (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Spain. E-mail: aprile@uma.es

Mango (Mangifera indica) is one of the most important fruit crops in the world. In 1992, the disease bacterial apical necrosis (BAN) was described for the first time on mango in southern Spain. BAN is caused by *Pseudomonas syringae* pv. syringae (Pss), and is mainly associated with Mediterranean climate. BAN has also been described in other mango producing areas with similar weather (Portugal, Italy, Israel, Egypt, Florida and northeast Australia). Different Pss isolates from mango have been studied, to decipher their virulence and epiphytic fitness mechanisms. Different genes associated with these biological characteristics have been described: *mbo* operon involved in the mangotoxin production, *copABCD* or *cusCBA* operons involved in copper resistance, and *wss* genes involved with cellulose production. Phylogenetic studies have revealed the presence of a differentiated phylotype of Pss strains isolated from mango, and characterized by mangotoxin production. This study included epidemiological and evolutionary analyses of different Pss isolates from mango from different growing areas (Spain, Portugal, Italy, Israel, Australia), isolated by 2000 (UMA lab collection), and new isolates obtained in 2016 and 2017. We will perform a selection of the most representative strains of each collection, to carry out a comparative genomic analysis to unravel the evolutionary processes which have taken place through more 25 years.

This research is supported by Incentivos a Proyectos de Excelencia de la Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía (P12-AGR-1473), cofinanced by FEDER (EU).
Almond anthracnose, caused by Colletotrichum spp., is a serious and emerging disease in the major almond-growing areas worldwide. All isolates causing almond anthracnose have been assigned to the C. acutatum s.l. complex, in which only C. fioriniae and C. godetiae have been associated with the disease. This study characterized Colletotrichum isolates recovered from almond fruits affected by anthracnose from ten commercial orchards located in Andalusia, between 2014 and 2016. Additionally, two Colletotrichum isolates causing olive anthracnose were also included for comparison. Morphological characters, mainly colony colour and conidial shape, were useful to separate the isolates within fungal groups or species. Pathogenicity tests were conducted on detached fruits from almond, olive and apple. Results showed differences in virulence and some degree of pathogenic specialization among isolates. Molecular characterization using six genomic regions was essential to clarify the identification of Colletotrichum isolates tested. Olive isolates were identified as C. godetiae and C. nymphaeae, which had been identified before in Andalusian olive orchards. For isolates from almond, two phylogenetic species were identified: C. godetiae (grey colony subpopulation), which is well known in other countries; and C. acutatum sensu stricto, (pink colony subpopulation), which was more virulent and did not match with C. fioriniae, the pink colony subpopulation described in other countries. This is the first report of a new Colletotrichum species causing almond anthracnose within the C. acutatum s.l. complex.

This research was supported by the Junta de Andalucía (project ‘Transforma de Fruticultura Mediterránea’ from Andalusian Institute for Research and Formation in Agriculture and Fishery, IFAPA) with the collaboration of ‘Crisol/Arboreto’ and ‘Mañán’ OPFHs, and the private company ‘Almendras Francisco Morales’. C.A.B. is the holder of a ‘Juan de la Cierva-Formación’ fellowship from MINECO.

Phenotypic, molecular and pathogenic characterization of Phlyctema vagabunda, causal agent of olive leprosy. J. ROMERO1, M.C. RAYA1, L.F. ROCA1, C. AGUSTI-BRISACH1, J. MORAL1,2, A. TRAPERO1. 1Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain. 2Department of Plant Pathology, University of California, Davis, Kearney Agricultural Research and Extension Center, 9240 South Riverbend Ave., Parlier 93648, CA, USA. E-mail: ag11rcca@uco.es

Olive leprosy, caused by Phlyctema vagabunda, is a classic fruit rot disease widespread in the Mediterranean region. From 2009-2013, new disease symptoms consisting of small circular necrotic leaf lesions, coin branch canker and shoot dieback were observed in Spanish and Portuguese olive orchards showing intense defoliation. Neofabraea-like fungal colonies were consistently isolated from symptomatic leaves and shoots. Representative isolates from affected leaves, shoots and fruits were characterized using morphology of colonies and conidia, optimal growth temperature and comparison of DNA sequence data from four regions: ITS, tub-2, MIT and rpb2. Pathogenicity tests were also performed on apple and olive fruits, and on branches and leaves of olive trees. Maximum mean mycelium growth rate ranged from 0.54 to 0.73 mm d-1. Morphology of conidia produced on apple fruit and phylogenetic analysis showed homogeneity among fungal isolates, which were identified as Phlyctema vagabunda. On fruits, influence of wounding, ripening and cultivar resistance was studied, with cv. Blanqueta being the most susceptible cultivar. On branches, mycelial-plug inoculation reproduced olive leprosy symptoms and caused shoot dieback. On leaves, Koch’s postulates were fulfilled, and the pathogen caused characteristic necrotic spots and plant defoliation. Wounds had a key role on olive leprosy development. This is the first description of Ph. vagabunda as a causal agent of leaf spot and defoliation in olive trees. The integration of mechanized practices in olive crop management could be the cause of the disease re-emergence.

This research was supported by Bayer Crop Science and ELAIA companies. C. Agustí-Brisach is holder of a ‘Juan de la Cierva-Formación’ fellowship from MINECO. J. Moral holds a Marie Skłodowska Curie fellowship launched by the European Union’s H2020 (contract number 658579).

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This research was supported by the Project AGL-2012-37521 from ‘Ministerio de Economía y Competitividad’ of Spain, Project P12-AGR-1486 from ‘Consejería de Economía, Innovación y Ciencia’ of Junta de Andalucía, and FEDER financial support from the European Union.

Olive trees host many plant-parasitic nematodes (PPNs). Understanding the factors that maintain biodiversity in communities depends on identification of diversity. We investigated the effects of environmental conditions, soil properties, agronomic management practices and spatial structure, on the variation of species community composition (β-diversity) and species richness of PPNs infesting rhizosphere soil from 376 commercial olive orchards widely distributed in Andalusia, southern Spain. We identified 128 species of PPNs with different feeding behaviours. Constrained ordination analysis showed that all explanatory variables together accounted for approx. 13% of the variation of community composition and 30% of species richness. These low values showed that spatial variability in the distribution of plant-parasitic nematodes is generally very stochastic. Also, with redundancy analysis and variation partitioning, we determined the relative importance of environmental conditions, soil properties, agronomic management and spatial structure, as well as different tendencies among species composition and richness. Environment (6% of community composition variance), soil (35%), agronomic management (7%) and spatial structure (18%) explained variance from the total explained variance of community composition. For species richness, environment explained 0% of variance, soil 5%, agronomic management 14%, and spatial structure 34%. Overall, the diversity of PPNs species infesting soils from cultivated olive is mainly influenced by land properties and spatial habitat, and to a lesser extent by environmental conditions and agronomic management.


Phaeoaclenomium aleophilum is one of the first colonizers in grapevine trunk disease, and is the main pathogen isolated in Castile-Leon vineyards. The general growth media PDA and MEA have been used for growing this pathogen, but its growth rate is very slow compared to other grapevine trunk pathogens. A new growth medium, containing vine sawdust, dextrose and agar (VSDA), has been assayed, and compared with PDA (potato, dextrose and agar). Using compounds from vines could improve pathogen growth rates, and provide information on which compounds have roles in development of disease. Phaeoaclenomium aleophilum strain Y-38-05-03-a from ITACyL was taken from 14-d-old mycelium. This strain was put onto VSDA and PDA in Petri plates, and the plates were each marked with two perpendicular crosses. The experiment was repeated, using four replicates of each time. The plates were incubated at 28°C in a phytotron for 15 d, and colony growth (mean colony diameters) was then measured. In VSDA, mean P. aleophilum colony diameter was 2.49 (±0.25) (typical error 0.09), and on PDA was significantly less (P < 0.05) (1.25 ±0.17) (typical error 0.06), as indicated by Tukey LSD tests. After this positive first assay, other parameters fungal will be evaluated on VSDA, including spore production, growth of other Phaeoaclenomium aleophilum strains, and other growth media.
The Mediterranean Phytopathological Union (MPU) is a non-profit society open to organizations and individuals involved in plant pathology with a specific interest in the aspects related to the Mediterranean area considered as an ecological region. The MPU was created with the aim of stimulating contacts among plant pathologists and facilitating the spread of information, news and scientific material on plant diseases occurring in the area. MPU also intends to facilitate and promote studies and research on diseases of Mediterranean crops and their control.

The MPU is affiliated to the International Society for Plant Pathology.

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- **Arab Society for Plant Protection (ASPP), Beirut, Lebanon** - [http://www.asplantprotection.org/](http://www.asplantprotection.org/)
- **French Society for Phytopathology (FSP), Bordeaux, France** - [http://www.sfp-asso.org/](http://www.sfp-asso.org/)
- **Italian Phytopathological Society (SIPAV), Reggio Calabria, Italy** - [http://www.sipav.org/](http://www.sipav.org/)
- **Spanish Society for Plant Pathology (SEF), Valencia, Spain** - [http://www.sef.es/SEF/](http://www.sef.es/SEF/)
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