Comparative aggressiveness of *Mycosphaerella pinodes* on peas from different regions in western Algeria

**Benali Setti**¹, **Mohamed Benchekh**¹, **Jamel Henni**² and **Claire Neema**³

**Summary.** Mycosphaerella blight caused by *Mycosphaerella pinodes* (Berk. et Blox.) Vestergr. is now recognized as one of the major problems limiting yield of pea crops in Algeria. The present work was carried out to study the aggressiveness of 75 *M. pinodes* isolates collected from different pea-growing areas forming four population groups representing four geographic areas in western Algeria. The latent period, incubation period and disease severity were measured in the greenhouse for each isolate × cultivar combination. All three aggressiveness components differed significantly between isolates and between cultivars. No significant interaction however was noted between isolates and cultivars. Both principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to analyze the variation pattern within and between population groups. Cluster analysis, which summarizes the relationship between isolates according to their distance of similarity, sorted isolates into six distinct aggressiveness groups. Aggressiveness group 1 was the most represented, with 34% of all isolates. Both PCA and cluster analysis revealed that many isolates were closely related irrespective of the geographic area or the host cultivar from which they were collected. At the same time, and based on the same aggressiveness components, the cv. Onward, Lucy and DP were the most susceptible, whereas the cv. Rondo and MK were partially resistant.

**Key words:** Mycosphaerella blight, HCA, PCA, aggressiveness group.

**Introduction**

Ascochyta blight caused by *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr. is one of the most destructive pathogens of peas (Moussart et al., 1998). It is widespread throughout the major pea-growing areas worldwide (Wallen 1965; Lawyer, 1984; Bouznad, 1988; Bretag et al., 2006; Setti et al., 2008). The disease has caused yield losses of over 50% in Canada in some years (Wallen, 1965; Xue et al., 1997), and similar losses in Australia (Bretag, 1989). In France, Ascochyta blight causes yield losses of up to 30%. In view of its complexity and economic importance, the disease has been investigated in many studies on pea-growing regions around the world. In recent years, an increased incidence of Ascochyta blight has been seen in different production areas in Algeria and has led to increasing yield loss (Setti et al., 2008). This could be due to an increased pathogenicity
of the pathogen population or a greater inoculum pressure.

Damage is reduced by fungicides, but multiple sprays are needed during the growing season. Breeding resistant cultivars is a good alternative to chemical control. However, there are no cultivars with sufficient field resistance to counteract the disease. Breeding pea varieties with resistance to *M. pinodes* is difficult because many varieties have only partial resistance. Although a large number of accessions have been screened to identify sources of resistance to *M. pinodes* (Ali-Khan *et al.*, 1973; Ali *et al.*, 1978; Bretag, 1989, Xue *et al.*, 1996; Kraft *et al.*, 1998), high levels of resistance have never been found.

To manage and control *M. pinodes*, it is therefore important to know the amount of phenotypic variation within a given pathogen population. Variations in aggressiveness have been used to describe variations in the severity of the disease reaction produced by virulent biotypes. Hence, variations in quantitatively measured traits of *M. pinodes* populations such as latent period, incubation period and the disease severity index have been studied in several plant *M. pinodes* pathosystems (Wicker *et al.*, 2001; Bouhassan *et al.*, 2003; Suasuna *et al.*, 2004; Milus *et al.*, 2006). This aspect is of great importance for the management of a plant disease. If such variation is found in *M. pinodes* population, increased aggressiveness could conceivably be selected for over time (Latin *et al.*, 1981; James and Fry, 1983; Alexander *et al.*, 1985; Beasse *et al.*, 2000), perhaps reducing the durability of resistance (Latin *et al.*, 1981; Newton, 1989).

Differences in aggressiveness also affect *Mycosphaerella* blight epidemics, and consequently, blight management. It has been shown that epidemics caused by the more aggressive isolates are more severe, cause greater crop losses and require a greater number of fungicide sprays (Wroth, 1998). Studies of the pathogenicity of *M. pinodes* have found considerable variation in aggressiveness as well as differences between isolates in their relative ability to cause disease on a host (Buddenhagen, 1981; Ovichinnikova and Andryukhina, 1984; Nasir and Hope, 1991).

The objectives of the current study were (i) to evaluate the aggressiveness of a large collection of isolates of *M. pinodes* from different parts of western Algeria, (ii) to determine the relationship between aggressiveness, geographic area and host cultivar origin, and (iii) to evaluate the reaction of seven commercial cultivars grown in this area to *M. pinodes* isolates.

**Materials and methods**

**Plant material**

The pea cv. Onward, Merveille de Kelvedon (MK), Douce de Provence (DP), Akel, Rondo, Grillevert, and Lucy are cultivated in most parts of western Algeria. Seeds of these cultivars were sown in 20-cm diameter pots containing an unsterilized soil/compost mixture. Ten seeds were planted per pot and the seedlings were thinned to five. The plants were maintained in a growth chamber. Three replicates were used for each combination.

**Fungal material**

Seventy-five isolates of *M. pinodes*, obtained from different parts of western Algeria and collected during 2001–2005 were used in the study (Table 1).

Strains were raised on potato dextrose agar (PDA) for 10 days at 21°C. Conidia from 10-day-old cultures were collected by adding 10 mL of sterile distilled water to dislodge the spores. The spore suspension was filtered through two layers of cheesecloth to remove the mycelium and agar fragments. The concentration of spores was determined using a hemocytometer. The suspension was diluted with sterile distilled water to obtain a final concentration of 3.5×10⁶ conidia mL⁻¹.

**Inoculation**

Plants were inoculated by spraying to runoff with the spore suspension using a spray atomizer with an adjustable nozzle to form a high density of fine droplets on the aerial parts of the plants. Control plants were sprayed with sterile distilled water. The plants were covered for 48 h with transparent polyethylene bags immediately after inoculation and sprayed inside the bags with distilled water to facilitate infection. After incubation, the plants were uncovered and kept in an uncontrolled greenhouse at temperatures from 15 to 25°C.

**Disease assessment**

*Mycosphaerella pinodes* infection on the leaves
Table 1. Geographic origin of the *Mycosphaerella pinodes* isolates collected in western Algeria and used for the aggressiveness test.

<table>
<thead>
<tr>
<th>Population group</th>
<th>Isolate</th>
<th>Cultivar</th>
<th>Geographic origin</th>
</tr>
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<tbody>
<tr>
<td>pg1</td>
<td>HU1</td>
<td>Onward</td>
<td>Talassa, Chlef</td>
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<tr>
<td>&quot;</td>
<td>HU2</td>
<td>MK</td>
<td>Talassa, Chlef</td>
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<td>Mezhrene, Chlef</td>
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<td>Onward</td>
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<td>Onward</td>
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<td>Marsa, Mostaganem</td>
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<td>Onward</td>
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<td>Onward</td>
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<td>HU18</td>
<td>Onward</td>
<td>Sidi ali, Mostaganem</td>
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<td>HU19</td>
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<td>Sidi ali, Mostaganem</td>
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<td>SHU1</td>
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<td>MK</td>
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<td>MK</td>
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<td>SHU9</td>
<td>DP</td>
<td>Sidi khat, Mostaganem</td>
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<td>DP</td>
<td>Ain tedless, Mostaganem</td>
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<td>Onward</td>
<td>Ain tedless, Mostaganem</td>
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<td>MK</td>
<td>Attaf, Ain defla</td>
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<td>DP</td>
<td>Attaf, Ain defla</td>
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<tr>
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<td>AR3</td>
<td>DP</td>
<td>Attaf, Ain defla</td>
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<tr>
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<td>Onward</td>
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<td>Onward</td>
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<td>AR8</td>
<td>Onward</td>
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<td>&quot;</td>
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<tr>
<td>&quot;</td>
<td>AR13</td>
<td>Onward</td>
<td>Ouled fares, Chlef</td>
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<tr>
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</tr>
<tr>
<td>&quot;</td>
<td>AR16</td>
<td>MK</td>
<td>Ouled fares, Chlef</td>
</tr>
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</table>

(continued on the next page)
was recorded 21 days after inoculation using a 0–5 disease severity (DS) scale according to Tivoli et al. (1996), where 0, no lesion; 1, a few scattered flecks; 2, numerous flecks; 3, 10–15% leaf area necrotic and presence of flecks; 4, 50% of leaf area covered by lesions; 5, 75–100% of leaf area dehydrated or necrotic. To determine the incubation period (IP) and the latent period (LP), plants were inspected daily for up to 20 days.

The IP was defined as the period (in days) from host inoculation to the appearance of the first symptoms on the leaves, and the LP as the time (in days) from inoculation to the first formation of pycnidia in the lesions. Each lesion with pycnidia on the leaves was recorded with the aid of a hand lens (10×).

Data analysis

Analysis of variance was assessed for IP, LP and DS of both the isolates and the cultivars. Means of cultivars was performed using Tukey’s honestly significant difference (HSD) test. Principal component analysis (PCA) was performed to detect the main components that defined significant structures within the data set. PCA was followed by an ascending hierarchical classification (AHC). Isolates were then classified in groups by their aggressiveness on the seven cultivars. To ascertain how the components were associated with each other, Pearson’s linear correlation coefficients were calculated between components across cultivars. All statistics were determined using the R Statistics software (Version 2.5.0).

Results

Isolate aggressiveness

There were significant differences ($P<0.001$) in aggressiveness between isolates from different geographic areas. These differences occurred in all fitness parameters measured, including IP, LP and DS. The DS index, based on the 1–5 scale for individual isolates, ranged from 2.96 to 3.75, with a mean of 3.29 ($SD=1.12$). Variation in the distribution of the mean DS for the 75 isolates of $M. pinodes$ across the seven cultivars was essen-
Mycosphaerella pinodes on peas in western Algeria

tially continuous. Isolates from the same area were always different from each other and had different disease indices. PCA revealed that the first and second principal coordinates accounted for 41.97 and 21.72% of total variation respectively. The two principal axes thus explained 63.69% of total variation between isolates. These two coordinates separated isolates into groups (Fig. 1). These group separations were confirmed by hierarchical cluster analysis (Fig. 2). At a similarity distance of 0.769, the isolates of *M. pinodes* from the different geographic areas were classified into six aggressiveness groups (Table 2). The first group (AG1), was the largest, with 27 isolates (34%). It contained isolates from the four geographic areas. AG2 only included isolates from population group 4. AG3 had 13 isolates (17%) coming from all population groups. AG4 and AG5 had isolates from all four geographic areas; AG4 consisted of 14 (19%) and AG5 of 10 isolates (14%). AG6 had 4 isolates. The dendrogram also indicated that isolates collected from the same location were similar to those from widely dispersed sites, or from different cultivars. Moreover, mean comparison with the t test of DS

![Principal component analysis of 75 isolates of *Mycosphaerella pinodes* under axis 1 and 2.](image-url)

Fig. 1. Principal component analysis of 75 isolates of *Mycosphaerella pinodes* under axis 1 and 2.
revealed no significant differences between population groups ($P<0.05$).

**Cultivar susceptibility**

Cultivar reactions varied significantly between each other (Table 3) ($P<0.05$). The disease index of pea cultivars varied from 2.86 to 3.68, with a mean of 3.27 and a standard deviation of 1.12 on ‘MK’ and ‘Rondo’, partially resistant cultivars; the DS index was 2.90 and 2.86 respectively. The most susceptible cultivars were ‘Onward’, ‘Lucy’ and ‘DP’, with a disease index greater than 3.63. Mean comparison of the disease index of the seven cultivars revealed significant differences. Tukey’s honestly significant difference (HSD) test classified the cultivars into three groups based on their DS index (Table 3). The cv. Rondo and MK had a significantly longer LP (1 to 2 days)
Table 2. Percentage of *Mycosphaerella pinodes* isolates of each aggressiveness group in the four geographic areas determined by hierarchical classification (HCA) analysis.

<table>
<thead>
<tr>
<th>Geographic area</th>
<th>AG1</th>
<th>AG2</th>
<th>AG3</th>
<th>AG4</th>
<th>AG5</th>
<th>AG6</th>
</tr>
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<tbody>
<tr>
<td>pg1</td>
<td>38</td>
<td>0</td>
<td>5</td>
<td>28</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>pg2</td>
<td>18</td>
<td>0</td>
<td>39</td>
<td>6</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>pg3</td>
<td>37</td>
<td>0</td>
<td>25</td>
<td>31</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>pg4</td>
<td>37</td>
<td>8</td>
<td>8</td>
<td>17</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>All areas</td>
<td>34</td>
<td>3</td>
<td>17</td>
<td>19</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3. Mean disease severity (DS), latent period (LP), and incubation period (IP) of seven commercial cultivars exposed to isolates of *Mycosphaerella pinodes*.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Latent period</th>
<th>Incubation period</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rondo</td>
<td>2.863 a*</td>
<td>12.83 ab</td>
<td>4.12 b</td>
</tr>
<tr>
<td>MK</td>
<td>2.908 a</td>
<td>12.86 a</td>
<td>4.32 a</td>
</tr>
<tr>
<td>Grillevert</td>
<td>2.917 a</td>
<td>13.07 a</td>
<td>3.72 c</td>
</tr>
<tr>
<td>Ekel</td>
<td>3.384 b</td>
<td>12.50 b</td>
<td>3.60 cd</td>
</tr>
<tr>
<td>DP</td>
<td>3.632 c</td>
<td>11.88 c</td>
<td>3.22 f</td>
</tr>
<tr>
<td>Lucy</td>
<td>3.665 c</td>
<td>11.68 c</td>
<td>3.46 de</td>
</tr>
<tr>
<td>Onward</td>
<td>3.688 c</td>
<td>11.92 c</td>
<td>3.32 ef</td>
</tr>
</tbody>
</table>

*Overall means within the study were compared using the Tukey’s honestly significant difference (HSD) test. Means followed by the same letter are not significantly different at $P=0.05$.

than the cv. Onward, DP and Lucy. Differences in IP between cultivars were very small. However, Tukey’s test distinguished seven groups, with ‘MK’ having the highest IP (Table 3).

**Incubation and latent period**

There were significant differences in IP and LP between isolates and cultivars, but not in the interaction isolate–cultivar. The IP of 75 isolates across cultivars was from 3.09 to 4.16, with a mean of 3.68 and a standard deviation of 1.07. The mean comparison of IP with the t test revealed no significant differences between population groups. The LP of isolates followed a similar trend. The t test revealed no significant differences in LP between population groups.

The three aggressiveness components were strongly correlated with each other ($P<0.0001$) (Table 4). The DS was the least correlated with the others. The DS and the IP were well correlated with a coefficient of determination $R^2$ of 0.56.

Table 4. Pearson linear correlation coefficient between three aggressiveness components measured across seven cultivars.

<table>
<thead>
<tr>
<th></th>
<th>Latent period</th>
<th>Incubation period</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent period</td>
<td>1</td>
<td>0.704***</td>
<td>-0.611***</td>
</tr>
<tr>
<td>Incubation period</td>
<td>0.744***</td>
<td>1</td>
<td>-0.744***</td>
</tr>
<tr>
<td>Disease severity</td>
<td>-0.611***</td>
<td>-0.744***</td>
<td>1</td>
</tr>
</tbody>
</table>

***, $P<0.0001$
Discussion

*Mycosphaerella pinodes* isolates from various locations in four areas of western Algeria were compared for their aggressiveness against seven commercial cultivars with different levels of resistance ranging from susceptible to partial resistant. The aggressiveness of a biotype of a pathogen is denoted by the DS produced by the biotype on the host (Burnett, 1975). The aggressiveness of a parasitic genotype is its capacity to persist in a population (Nelson, 1972, Nelson, 1979). In the experiment described here, significant variations were found in the aggressiveness components, LP, IP and DS.

Both cluster analysis and PCA grouped isolates by their level of aggressiveness to all cultivars tested. These two type of analysis detected that many of the isolates were pathogenically very similar to each other despite coming from widely dispersed locations. The study found that 50% of the isolates were highly aggressive and 34% were moderately aggressive, and that these isolates occurred in almost all Algerian pea-growing areas. Furthermore, the mean comparison test did not identify any particular group as being significantly different from the others: only continuous variation being observed. These findings were consistent with previous studies (Ali et al., 1978; Clulow et al., 1991; Nasir and Hoppe, 1991, Bretag et al., 2006).

There was also no significant interaction between isolates and cultivars. According to Van der Plank (1984), the lack of a significant interaction between isolate and cultivar indicates that isolates differ in their aggressiveness and vary in other respects independently of the cultivar tested. This is consistent with numerous reports in the literature, where variation between isolates has been reported for various fungi (Allingham and Jackson, 1981; Nelson and Marshall, 1990; Krupinsky, 1997).

On the other hand, the study shows that the cultivars had different levels of quantitative resistance. The mean comparison test of the DS showed that the seven cultivars fell into three groups (P<0.0001) going from susceptible to partially resistant. This is consistent with previous reports indicating that small but heritable differences in susceptibility to Mycosphaerella blight exist among accessions and cultivars of *Pisum sativum* (Bretag, 1989; Xue et al., 1996; Wroth, 1998; Fondevilla et al., 2005; Bretag et al., 2006). As expected, some cultivars that were not completely resistant were identified. The study found that the use of cultivars with partial resistance to *M. pinodes* delayed the onset of epidemics due to a significant reduction in disease efficiency. Hence, partial resistance to *M. pinodes* could be expected to delay progress of the disease, due to a higher LP and IP.

The more aggressive isolates of *M. pinodes* had a shorter LP and IP and a greater DS. Pea-cultivars with quantitative resistance to Mycosphaerella blight tend to have a longer LP (by 1 to 2 days) than the susceptible cultivars. LP and IP are important components of quantitative resistance (Edden et al., 1996; Webb et al., 1997; Hartman et al., 1999; Lovell et al., 2004; Buloviene and Surviliene, 2006; Setti et al., 2008). At the seedling stage, differences in LP between susceptible and resistant cultivars are very small, but Zadoks (1971) and Teng et al. (1977) demonstrated that even a small change in the LP can have a strong impact on the development of Mycosphaerella blight epidemics. LP was also discussed by Leonard and Mundt (1984), who stated that for a pathogen like *M. pinodes*, with a high reproduction rate, increases in LP would decrease in the growth of the pathogen. In some plant diseases, the length of LP is reported to be affected by the density of infection (Leonard, 1969; Leonard and Mundt, 1984). Plants with higher lesion densities generally have shorter LPs.

Although pathotype groups were not identified in this study, differences in aggressiveness were found using seven commercial cultivars grown in western Algeria.

Fifty percent were highly aggressive and these isolates were distributed in all Algerian pea-growing areas. This represents a serious risk when susceptible cultivars are grown. The aggressiveness data failed, however, to structure the population according to their geographic or host cultivar origin. The molecular characterization of *M. pinodes* is required to ascertain whether variations in aggressiveness are associated with genotypic variations. It should then be possible to study the genetic structure of the population and compare it with other population groups. An insufficient understanding of the genetic structure of *M. pinodes* population is a serious obstacle to breeding pea resistant to Mycosphaerella blight (Onfroy et al., 1999; Bease et al., 2000; Bretag and Ramsy, 2001).
**Literature cited**


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