Influence of growth stage and leaf age on expression of the components of partial resistance of faba bean to *Botrytis fabae* Sard.

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Summary. In detached leaf tests on faba bean (*Vicia faba* L.), genotypes partially resistant and susceptible to *Botrytis fabae* were examined. Expression of four components of partial resistance to a virulent isolate of *B. fabae* differed depending on the plant age and the leaf age of the genotypes. The incubation period of resistant genotypes at the podding stage was longer than that of susceptible genotypes at the same stage. The area under disease progress curve (AUDPC) of the lesion size increased from the seedling to the flowering stage but declined at the podding stage in all genotypes. Differences between resistant and susceptible genotypes for lesion size were significant except on old leaves from plants at the podding stage. The latent period decreased, and spore production increased with increasing growth and leaf age but there was significant interaction with the genotype. These last two components of partial resistance were more clearly expressed at all growth stages on FRY167 (highly resistant) but were expressed only at the seedling and podding stages on FRY7 (resistant). The resistant line BPL710 was not significantly different from the susceptible genotypes for the latent period at any growth stage, and for spore production at the seedling and flowering stages. Leaf age affected all genotypes, but with a significant interaction between leaf age and growth stage. Components of partial resistance were more strongly expressed on young leaves from plants at the seedling or flowering stage.

Key words: chocolate spot, plant age, disease severity, *Vicia faba* L.

Introduction

Chocolate spot caused by *Botrytis fabae* Sard. is a devastating disease of faba bean (*Vicia faba* L.) in many regions of the world (Gourley and Delbridge, 1973; Liang, 1986; Tivoli *et al*., 1988), including the Mediterranean basin (Hanounik and Robertson, 1988; Santorelli *et al*., 1992). In Morocco, chocolate spot occurs frequently in epidemic proportions (Mabsoute and Saadaoui, 1996; Sadiki *et al*., 2000). The pathogen attacks the leaves causing chocolate-colored lesions that may spread very quickly around the infection site, killing the tissue above the lesion. Conidia develop extensively in the lesions and constitute the inoculum for secondary infections. The disease can spread rapidly within a crop and from one field to another (Fitt *et al*., 1985). Chocolate spot causes substantial yield losses when suitable weather conditions prevail. Since the disease cannot be efficiently controlled chemi-
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Increasing resistance is a major objective of field breeding programs (Hanounik and Robertson, 1988; Tivoli et al., 1988; Porta-Puglia et al., 1994). Nevertheless, little work has been done to distinguish between total resistance, which is qualitative and can be broken, and partial resistance, which is quantitative, stable and durable (Parlevliet, 1993). Similarly, little information is available on the effects that different physiological factors have on the epidemiology of the disease. Previous works reported on the impact of plant age on chocolate spot by recording the spread of lesions and the sporulation of the pathogen, but on a susceptible faba bean cultivar and without previous study of partial resistance to this disease (Griffith and Amin, 1977; Creighton et al., 1986). In earlier studies we selected genotypes that had partial resistance to *B. fabae* under Moroccan field conditions (Bouhassan et al., 2000; 2004). Although tests on detached leaves did not always lead to the same ranking of faba bean genotypes to chocolate spot as did the field test, it was deemed suitable for separating highly resistant from highly susceptible genotypes (Tivoli et al., 1986; Bouhassan et al., 2004). The laboratory test also seemed to be useful for studies of the components of partial resistance (Bouhassan et al., 2003; 2004). Nevertheless, it is not clear whether leaf age and/or the growth stage of the plant affect the components of partial resistance.

The aim of this study was to examine the impact of leaf age and of the plant growth stage on the expression of and variations in four components of partial resistance in resistant genotypes as compared with susceptible genotypes, and the reliability with which these resistance components could be determined. Such information would also be useful in order to develop an integrated approach to chocolate spot control.

**Materials and methods**

**Plant material**

Five faba bean genotypes were used in the experiments. The genotypes FRY167, FRY7 and FRY30 were selected as highly resistant, resistant and susceptible to chocolate spot respectively, in preliminary field tests conducted in Rabat, Morocco in 1998 and 1999 (Bouhassan et al., 2000). The resistant line BPL710 from ICARDA (Jellis et al., 1982) and the cultivar Aguadulce, which is known to be susceptible to chocolate spot, were used as controls. Seeds were planted in March 2001 in 13-cm-diameter pots filled with arable soil, peat and sand (3:1:1; v:v:v), and germinated seedlings were thinned to five plants per pot. The seedlings were exposed to a temperature of about 20/16°C (day/night) and a 14-h photoperiod.

**Inoculum production**

A single virulent *B. fabae* strain (BFIAV99-1), obtained from infected leaves collected in the Rabat region, was cultured in 10-cm diameter Petri dishes on potato dextrose agar at 20–22°C and transferred to faba bean leaf extract medium to stimulate sporulation (Leach and Moore, 1966). After 10 days, cultures were flooded with sterile distilled water and conidia were dislodged by passing a curved Pasteur pipette gently on the surface of the colonies. The conidial suspension was filtered through two layers of sterile cheese cloth and diluted with tap water. The spore concentration was determined and adjusted to $3 \times 10^6$ spores ml$^{-1}$ using a Malassez haemacytometer slide (OSI, LAB’ 85, Paris, France).

**Detached leaf test**

For each genotype, six leaflets, each from a randomly selected leaf, were taken at the upper ($E_1$), middle ($E_2$) and lower nodes ($E_3$) of four-week-old plants (seedling stage), seven-week-old plants (flowering stage) and fourteen-week-old plants (podding stage). In the laboratory, leaflets were immediately placed in Petri dishes on two layers of filter paper previously sterilised and soaked in sterile water. A small humid cotton fragment was put on the cut end of the leaflet petioles to preserve leaf turgidity. In total, two Petri dishes per genotype were prepared each containing three leaflets of the same age and at the same growth stage. Leaflets were inoculated with a spore suspension containing $3 \times 10^6$ spores ml$^{-1}$ using a micropipette that deposited a 20 µl drop on each leaflet. At each condition and for each genotype six noninoculated leaflets were included as controls. Petri dishes were incubated in a growth chamber for 11 days at 15°C with a 14-h photoperiod.

**Measurements and data analysis**

Leaflets were examined for a short time (2 to 3
min) under a stereomicroscope every 2 h to determine the time from inoculation to the first appearance of symptoms (incubation period).

Disease progress was assessed daily starting 48 h after inoculation by measuring the lesion diameter (in mm). The AUDPC was computed for lesion size (Shaner and Finney, 1977).

The latent period, defined as the time required for the formation of the first spores (Parlevliet, 1979), was determined by daily examination of the lesions under sterile conditions. Eleven days after inoculation, leaflets were washed separately in a fixed volume of distilled water to collect the spores. The spore concentration of each suspension was determined by haemacytometer counts. Average spore production, expressed as the number of spores per leaflet, was then determined for each node position and for each plant growth stage.

The effects of genotype, leaflet age and plant age on each of the components of partial resistance were submitted to analysis of variance. Comparison of the means was done with the Duncan multiple range test (Dagnelie, 1984).

Results

Effect of leaf age

Leaf age did not affect the incubation period at any growth stage ($P=0.3$) but significantly affected the AUDPC of lesion diameters ($P=0.01$), the latent period ($P=0.001$) and spore production ($P=0.001$). Noninoculated leaflets were not infected at any plant or leaf age. In general, lesions were larger on old leaves than on young leaves (Table 1). Results averaged over genotypes showed that the latent period declined with increasing leaf age (Table 2). The mean number of conidia irrespective of genotype and the plant growth stage was significantly higher on old leaves than on young leaves (Table 3). Conidia were in general abundant on the oldest leaves ($3.6\times10^5$ conidia per leaflet) and less abundant on leaves from the middle of the stem and on the youngest leaves ($2.5\times10^5$ and $2.1\times10^5$ conidia per leaflet respectively).

Effect of growth stage

Plant age significantly influenced the incubation period of all genotypes ($P<0.001$). In general, this component of partial resistance was longer at the podding stage than at the seedling and the flowering stage. The AUDPC of the lesion diameters was also affected by the growth stage ($P<0.001$). Results averaged over all genotypes showed that AUDPC values increased from the seedling to the flowering stage without any significant differences between them (Table 1). These values then declined significantly from the flowering to the podding stage and tended to be similar to the means at the seedling stage. Differences between plant growth stages were not significant in young leaves (Table 1).

Table 1. Effect of growth stage and leaf age on chocolate spot severity expressed as the area under disease progress curve (AUDPC) values of lesion diameter in leaflets of five faba bean genotypes inoculated with Botrytis fabae.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seedling</th>
<th>Flowering</th>
<th>Podding</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_1^a$</td>
<td>$E_2$</td>
<td>$E_3$</td>
<td>$E_1$</td>
</tr>
<tr>
<td>FRY167</td>
<td>98 c</td>
<td>109 b</td>
<td>119 c</td>
<td>109 c</td>
</tr>
<tr>
<td>FRY7</td>
<td>118 bc</td>
<td>131 b</td>
<td>136 bc</td>
<td>132 bc</td>
</tr>
<tr>
<td>BPL710</td>
<td>143 ab</td>
<td>145 ab</td>
<td>152 b</td>
<td>144 abc</td>
</tr>
<tr>
<td>Aguadulce</td>
<td>159 a</td>
<td>177 a</td>
<td>211 a</td>
<td>165 ab</td>
</tr>
<tr>
<td>FRY30</td>
<td>158 a</td>
<td>175 a</td>
<td>203 a</td>
<td>179 a</td>
</tr>
<tr>
<td>Mean</td>
<td>135 B</td>
<td>148AB</td>
<td>164 A</td>
<td>146 B</td>
</tr>
</tbody>
</table>

a Leaflets from leaves at upper ($E_1$), middle ($E_2$) and lower ($E_3$) portions of the stems.
b Values followed by the same lowercase letter in columns or uppercase letter in rows are not significantly different at $P=0.05$ according to Duncan's multiple range test.
The growth stage affected the latent period only on the youngest leaves \((P<0.05)\) (Table 2). On these leaves, the latent period was significantly longer at the seedling and flowering stages than at the podding stage.

The number of conidia per leaflet was also affected by the plant growth stage \((P<0.001)\). In general, conidia production per leaflet increased as the plants matured (Table 3).

**Effect of genotype**

The mean incubation period, lesion size, latent period and spore production were all significantly affected by the genotype effect \((P<0.001)\). However, the genotype response to the incubation period, the latent period and spore production showed an irregular distribution and depended more often than not on the leaf age and the plant growth stage.

**Combined effect of leaf age, growth stage and genotype**

The interaction between genotype and leaf age was not a significant effect for the incubation period, but there was a highly significant interaction between genotype and growth stage for this component \((P<0.01)\). The results are presented as the

### Table 2. Effect of growth stage and leaf age on the latent period in the leaflets of five faba bean genotypes inoculated with *Botrytis fabae*.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seedling</th>
<th>Flowering</th>
<th>Podding</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E₁ᵇ</td>
<td>E₂</td>
<td>E₃</td>
<td></td>
</tr>
<tr>
<td>FRY167</td>
<td>10 aᵇ</td>
<td>6.7 a</td>
<td>6.0 a</td>
<td>6.7 a</td>
</tr>
<tr>
<td>FRY7</td>
<td>6.7 b</td>
<td>6.3 aᵇ</td>
<td>6.0 a</td>
<td>6.3 a</td>
</tr>
<tr>
<td>BPL710</td>
<td>6.3 b</td>
<td>6.3 aᵇ</td>
<td>5.0 b</td>
<td>6.0 b</td>
</tr>
<tr>
<td>Aguadulce</td>
<td>6.0 b</td>
<td>5.3 bᶜ</td>
<td>5.0 b</td>
<td>5.0 b</td>
</tr>
<tr>
<td></td>
<td>6.0 b</td>
<td>5.0 c</td>
<td>4.3 c</td>
<td>4.3 c</td>
</tr>
<tr>
<td>Mean</td>
<td>7.0 A</td>
<td>5.9 B</td>
<td>5.3 B</td>
<td>5.6 B</td>
</tr>
<tr>
<td>Overall mean</td>
<td>6.0 A</td>
<td>5.8 A</td>
<td></td>
<td>5.5 A</td>
</tr>
</tbody>
</table>

a See Table 1.
b See Table 1.

### Table 3. Effect of growth stage and leaf age on number of spores per leaflet in leaflets of five faba bean genotypes inoculated with *Botrytis fabae*.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Seedling</th>
<th>Flowering</th>
<th>Podding</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E₁ᵇ</td>
<td>E₂</td>
<td>E₃</td>
<td></td>
</tr>
<tr>
<td>FRY167</td>
<td>0.7 cdᵇ</td>
<td>0.9 bc</td>
<td>1.3 b</td>
<td>0.7 b</td>
</tr>
<tr>
<td>FRY7</td>
<td>0.3 d</td>
<td>0.2 c</td>
<td>1.8 ab</td>
<td>2.4 a</td>
</tr>
<tr>
<td>BPL710</td>
<td>1.5 bc</td>
<td>1.8 ab</td>
<td>2.0 ab</td>
<td>1.9 ab</td>
</tr>
<tr>
<td>Aguadulce</td>
<td>2.5 ab</td>
<td>1.7 ab</td>
<td>2.5 a</td>
<td>2.2 a</td>
</tr>
<tr>
<td>FRY30</td>
<td>3.0 a</td>
<td>2.4 a</td>
<td>2.1 a</td>
<td>3.1 a</td>
</tr>
<tr>
<td>Mean</td>
<td>1.6 A</td>
<td>1.4 A</td>
<td>1.9 A</td>
<td>2.0 B</td>
</tr>
<tr>
<td>Overall mean</td>
<td>1.7 C</td>
<td>2.7 B</td>
<td></td>
<td>3.9 A</td>
</tr>
</tbody>
</table>

a See Table 1.
b See Table 1.
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The incubation period varied significantly among genotypes only at the podding stage ($P=0.001$). The shortest incubation period was 10 h in the Aguadulce and FRY30 lines, and was not or only slightly affected by plant age. The longest incubation periods were 15.7, 14.6 and 14.3 h on BPL710, FRY7 and FRY167 lines respectively and were seriously affected by plant age ($P<0.05$, $P<0.01$ and $P<0.001$ respectively).

The interactions between the three studied factors and the AUDPC of diameter lesions were not significant. Nevertheless, results averaged over the genotypes showed that differences between the three leaf ages were not significant on plants at the podding stage and that all genotypes except Aguadulce had a significantly smaller lesion size at the podding stage than at the flowering stage (Table 1). Thus, differences between resistant and susceptible genotypes were large and statistically significant at the seedling and flowering stages, but not on old leaves at the podding stage (Table 1). However, at this last stage and in young leaves, FRY167 had a significantly lower AUDPC value than the more susceptible FRY30.

A significant effect of all interactions was found for the latent period component. In young leaves the latent period was, in general, significantly different from that in old leaves and intermediary insertion leaves except at the podding stage. This different response meant that there was a significant interaction between growth stage and leaf age ($P<0.001$). At the podding stage, leaves from the middle nodes had a significantly longer latent period than old leaves (Table 2). The shortening of the latent period with plant aging was highly marked only on the young leaves of genotype FRY167 and susceptible genotypes (Table 2). The genotype effect was significant on all leaves from the seedling to the podding stages. The FRY167 and FRY7 lines generally had a significantly longer latent period than the susceptible lines Aguadulce and FRY30. Nevertheless, the latent period of BPL710 was not significantly different from that of the susceptible Aguadulce.

The interaction between leaf age and genotype was the only non-significant effect on the number of spores per leaflet. There was no difference in spore production between plant growth stages on young leaves (Table 3). However, growth stage influenced production of spores, especially on old leaves of all genotypes. The number of spores also differed greatly between leaf ages at the flowering and the podding stages on all genotypes except FRY7 and BPL710. FRY7 was affected by leaf aging at the seedling and flowering stages, but BPL710 only at the podding stage. At the seedling and flowering stages, differences between the genotypes were significant, but on younger leaves only line FRY167 had a significantly lower number of conidia. In these young leaves, there was no statistically significant difference in mean conidia production per leaflet between line BPL710 and the susceptible Aguadulce. At the podding stage and on all leaves, FRY167, FRY7 and BPL710 lines had significantly lower sporulation than the susceptible genotypes Aguadulce and FRY30 (Table 3).

Discussion

The expression of the components of partial resistance to chocolate spot in faba bean was strongly affected by the genotype, growth stage and leaf age of the host plant. The significant effect of these interactions supports the conclusion that some genotypes often become more resistant and others more susceptible with leaf and plant age.

Generally, the incubation period was longer and the lesion size (AUDPC) was smaller at the podding stage, and more so on young than on old leaves. In contrast, the latent period was longer and production of spores was lower at the seedling and flowering stages, especially on old leaves. Although the incubation period and latent period have not been studied previously for chocolate spot, in other pathosystems it was reported that plant age did not affect the incubation period (Roumen, 1992; Pedersen and Morrall, 1994).

A shortening of the latent period with plant age and leaf age was also reported in pea infected with powdery mildew (Viljanen-Rollinson et al., 1997). In our study, the latent period was sometimes abnormally long on leaflets from middle-stem nodes as compared with that of the lower nodes. A possible reason for this may be genotypic differences in stem elongation.

Lesion sizes at the flowering stage were larger on old leaves. The increase of lesion size with plant age in the Vicia faba-Botrytis fabae pathosystem
was also reported by other authors (Griffith and Amin, 1977; Creighton et al., 1986) but these studies were based on detached leaf tests of only one susceptible faba bean line at the seedling stage. The reduction in lesion size at the podding stage is probably associated with the slow growth of *B. fabae* in leaf tissue due to a scarcity of nutrients because of lower photosynthesis in plants at this growth stage. The absence of a leaf age effect at this growth stage supports this idea because vegetative growth slows during pod filling and at that point the proportion of young tissue in the plant is reduced. This finding is consistent with studies in the wheat-leaf rust (Broers, 1989) and the rice-*Pyricularia oryzae* pathosystem (Roumen, 1992). In contrast, Chongo and Gossen (2001) reported that in the chickpea-*Ascochyta rabiei* pathosystem lesions increased as plants matured.

The greater resistance to infection of young leaves than old leaves at the seedling and flowering stages in the wheat-leaf rust pathosystem is probably due to differences in the morphology of the leaf tissue (Broers, 1989). The cells are smaller and more tightly packed in young than in older leaves, and this hinders the penetration and spread of the fungus within the young leaves (Broers, 1989). In our pathosystem, *B. fabae* kills and destroys cells as it progresses through the host and so smaller and more compact cells would not be expected to hinder growth. The increase of resistance in young leaves differs among genotypes and may be due to a strong expression of the partial resistance genes in these leaves, especially in FRY167.

The number of spores per leaflet increased considerably with increasing leaf age and plant age. This finding was similar to that in one faba bean line susceptible to chocolate spot at the seedling stage (Creighton *et al.*, 1986) and in barley susceptible to powdery mildew (Masterbroek and Balkema-Boomstra, 1991). Conversely, Roumen (1992) and Roumen *et al.* (1992) reported that sporulating *Pyricularia oryzae* in rice lesions declined with leaf age. In our pathosystem, senescent leaf tissue thus appears to be favourable for sporulation of *B. fabae*, but not for its penetration and colonisation.

Nevertheless, the incubation period contributed to partial resistance to chocolate spot in the resistant genotypes FRY167, FRY7 and BPL710, only at the podding stage. The resistance that reduced the size of lesions in these genotypes was lower at the flowering stage and on old leaves but was unchanged at the other growth stages. Partially resistant genotypes also showed longer latent periods and produced fewer spores on the leaflets. This resistance declined at the podding stage but remained unchanged at the seedling and flowering stages. The latent period component was however not expressed on genotype BPL710. The level of resistance in this genotype may be lower, or is regulated by different genes.

We conclude that in our genotypes, adapted to the Mediterranean environment, some components of partial resistance were more strongly expressed at the podding stage, while others were more strongly expressed at the seedling or flowering stage. A combination of high adult plant resistance and high resistance at the seedling or flowering stage as in genotype FRY167, may contribute to high partial resistance. The lower infection levels in young leaves of this genotype suggest that it may be suitable in programmes to breed for partial resistance to chocolate spot. The results of the study can help to manage disease severity and reduce the risk of epidemics if growers adopt an integrated approach combining resistant genotypes with the application of foliar fungicides, especially before the flowering stage when lesions become severe.

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