Phytopathol. Mediterr. (2002) 41, 99–108

New faba bean genotypes resistant to chocolate spot caused by *Botrytis fabae*

Azza Rhaïem[1], Mohamed Cherif[1], Mohamed Kharrat[3], Mejda Cherif[2] and Moncef Harrabi[2]

[1]Laboratoire de Phytopathologie, Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1082 Cité Mahrajène, Tunis, Tunisia
[2]Laboratoire de Génétique, Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1082 Cité Mahrajène, Tunis, Tunisia
[3]Laboratoire des Légumineuses à Graines, Institut National de la Recherche Agronomique de Tunisie, Rue Hédi Karray, Ariana, Tunisia

Summary. The response of sixty-seven genotypes of faba bean (*Vicia faba*) to the virulent isolate IBf24 of *Botrytis fabae* was studied under field conditions. Five of these genotypes (LPF39, LPF113, LPF44, LPF237 and LPF05) were moderately resistant to chocolate spot according to their MDI (mass disease index). Ten lines (LPF38, LPF41, LPF64, LPF95, LPF106, LPF132, LPF225, LPF228, LPF23, LPF233) were moderately susceptible, and fifteen lines (LPF54, LPF61, LPF66, LPF89, LPF124, LPF129, LPF131, LPF134, LPF138, LPF152, LPF173, LPF174, LPF190, LPF274, BPL710) susceptible as shown by their MDI values but with low AUDPC (area under disease progress curve) values and were believed to have an overall tolerance to the disease. Fourteen of the genotypes tested in the field, as well as the resistant BPL710 and the susceptible Rebaya 40 controls, were further screened under greenhouse conditions in order to confirm the field evaluation. In both the field and the greenhouse trials, the four lines LPF44, LPF237, LPF05 and LPF113 showed the highest level of resistance to the disease. These trials also revealed that genotypes with only overall tolerance may yet constitute interesting sources of resistance. Genotype BPL710, with known resistance to *B. fabae* races in the Mediterranean region, was found to be susceptible in the field but moderately susceptible in the greenhouse, suggesting the appearance of new races of this pathogen.

Key words: *Vicia faba*, resistance, *Botrytis fabae*.

Introduction

Faba bean is cultivated on more than 56% of the total area cultivated with leguminous crops in Tunisia (Anonymous, 1999), mainly in the northern parts of the country (Kharrat *et al*., 1996). However, in spite of the high potential of this crop and the fact that cultural practices are very well mastered, yields are unstable from one year to another. This instability is attributed mainly to pests and diseases affecting both yield and quality (Ibrahim and Nassib, 1979; Tivoli *et al*., 1990) and particularly to chocolate spot caused by *Botrytis fabae* and *B. cinerea*. Epidemics of this disease can cause severe yield losses (up to 100%) especially when favourable conditions prevail (Sardina, 1930; Yi, 1980). Chemical control combined with preventive measures have proved impractical, mainly because of widespread fungicide resistance to the two chocolate spot agents. Boris (1997) reported finding both *B. fabae* and *B. cinerea* with resistance to the benzimidazoles and the dicarboximides. At present, although genetic resistance to these pathogens generally provides

Corresponding author: M. Cherif
Fax: +216 71 799 391
E-mail: cherif.mohamed@inat.agrinet.tn

partial protection, the use of resistant cultivars remains the major means to reduce yield losses. The objective of the present work was to identify new sources of resistance to chocolate spot. The response of sixty-seven genotypes of faba bean from Tunisia were tested against the highly virulent *B. fabae* isolate IBf24 first under field conditions and then in the greenhouse to confirm the field assessment.

Materials and methods

The pathogen

The monoconidial isolate IBf24 of *B. fabae* used in the trials was obtained from naturally infected faba bean leaves in Beja (Tunisia) during the 1998–99 season. It was maintained on potato dextrose agar (PDA). The fungus was transferred to faba bean leaf dextrose agar medium (Tivoli *et al.*, 1986), incubated at 20±2°C in a cycle of 12 h darkness and 12 h near ultraviolet light to induce sporulation. After 14 days of growth, an inoculum suspension was prepared by adding sterile distilled water and stirring with a sterile loop. Spore suspensions were then adjusted to 500,000 spores ml^{-1}.

Field trial

Sixty-seven inbred lines were tested. They were developed from local germplasms by the 'Laboratoire de Légumineuses à Graines de l'INRAT' (Institut National de la Recherche Agronomique de Tunisie), except for line LPF237 which came from Spain, LPF284 from Morocco, BPL710 from Colombia and Rebaya 40 from Egypt. The field trial was conducted at INAT (Institut National Agronomique de Tunisie). Seeds were sown on 24 December 1999 in two blocks 3 m apart. Ten seeds per genotype were planted in a single row 1.5 m long and 0.5 m distant from the others, with cv. BPL710 repeated as a resistant control every two entries (rows). The randomised complete block statistical design was adopted. Plants were inoculated twice, before flowering and 20 days later, by spraying the fungal spore suspension on the foliage with a high-volume sprayer.

Greenhouse trial

Fourteen genotypes that had shown a promising reaction in the first experiment, as well as BPL710 and Rebaya 40, as resistant and susceptible controls respectively, were tested in order to confirm the results obtained in the field. Plastic pots with a capacity of 2800 cm^3, filled with sand, peat and garden soil in equal proportions (Khalil and Harrisson, 1981) were used. Four plants were grown in each pot and five pots were used for each genotype. The experiment was carried out with a simple randomised design in five replications. Inoculation was on four-week-old plants, before flowering. The plants were then covered with plastic sheets to insure a high level of humidity and kept in the greenhouse at 20±2°C.

Disease assessment and statistical analysis

Chocolate spot symptoms on the foliage were recorded at regular intervals using a 0–9 scale according to Ding *et al*. (1993). The following infection levels on the scale were used: 0, no visible infection on leaves; 1, a few dot-like accounting for less than 5% of total leaf area; 3, discrete spots less than 2 mm in diameter, accounting for 6–25% of leaf area; 5, numerous scattered spots with a few linkages, diameter 3–5 mm, on 26–50% of leaf area with a little defoliation; 6, confluent spot lesions accounting for 51–75% of leaf area, mild sporulation, half the leaves dead or defoliated; 7, complete destruction of the larger leaves, spot lesions covering more than 76% of leaf area, abundant sporulation, heavy defoliation and plants darkened and dead. In the field, both the percentage of infected leaves and the extent of defoliation were taken into account when scoring infection levels. All the plants of the row (or the pot) were scored individually. The mass disease index (MDI) (Ding *et al*., 1993) and the AUDPC (area under disease progress curve) (Steffenson and Webster, 1992) of genotypes were determined. For the greenhouse trial, disease severity was also recorded on the stems using a 0–3 scale where: 0, no visible infection; 1, a few scattered lesions; 2, numerous lesions; 3, very numerous and coalescent lesions (William, 1975), and disease indexes on the stems (DIS) were determined. MDI, AUDPC and DIS were calculated according to the following formula:

$$\text{MDI} = \{[(n1\times 0) + (n2\times 1) + (n3\times 3) + (n4\times 5) + (n5\times 7) + (n6\times 9)] / N \times 9\} \times 100,$$

where 0, 1, 3, 5, 7 and 9 are the infection severity levels on the leaves; *ni*, the number of plants having the same infection level; N, the total number of plants.

$$AUDPC = \sum^{n}_{i=1} [(Y_{i+1} + Y_i) \times 0.5] [T_{i+1} - T_i],$$

where Y_i is the infection index on the leaves (MDI) or on the stems (DIS) at the i^{th} observation, T_i the time (in days) at the i^{th} observation, and n the total number of observations;

$$DIS=[(n1\times0) + (n2\times1) +(n3\times2) + (n4\times3)] / N,$$

where 0, 1, 2 and 3 are the infection severity levels on the stems; *ni* the number of plants having the same infection level; and N the total number of plants.

The response of the genotypes was expressed as the MDI values according to Ding *et al*. (1993). Six resistance levels were used: HR (highly resistant), MDI ranging between 0 and 2.0; R (resistant), MDI=2.1–15.0; MR (moderately resistant), MDI=15.1–40.0; MS (moderately susceptible), MDI=40.1–60.0; S (susceptible), MDI=60.1–80.0; HS (highly susceptible), MDI=80.1–100.

The data collected from the two experiments were subjected to statistical analysis using the STATISTICA computer statistical package (Statsoft France 1997, Maisons-Alfort, France). ANOVA was applied to each of the disease indexes at different dates either on the foliage or on the stems and to the AUDPC. Means were separated using the Least Significant Difference (LSD) Test.

Results

Field experiment

ANOVA analysis of infection data 7, 14, 21, 30, 37 and 52 days after inoculation revealed significant effects of the tested genotypes at the first and last inspection dates after inoculation. A significant effect was also obtained with the AUDPC (Table 1), confirming differences in disease reaction among the genotypes. According to AUDPC (Fig. 1) and MDI (Table 2) values, the five genotypes LPF39, LPF237, LPF44, LPF05 and LPF113 showed the highest resistance to chocolate spot. They were classified as moderately resistant (MR) on the basis of their MDI scores. These lines slowed down disease development immediately after infection and later in the cycle, maintaining low MDI levels until 52 days after inoculation (Fig. 2A, 3). Lines LPF228, LPF41, LPF225, LPF64, LPF106, LPF38, LPF231, LPF233, LPF95, LPF132, were moderately susceptible (MS), and lines BPL710, LPF174, LPF152, LPF54, LPF131, LPF138, LPF134, LPF274, LPF173, LPF190, LPF129, LPF66, LPF61, LPF89, LPF124, were susceptible (S) 52 days after inoculation (Table 2) and also presented low AUDPC values according to the LSD test (Fig. 1). These particular genotypes were relatively resistant to the disease at the earlier stages but were unable to limit it or slow it down later (Fig. 2B, 2C, 3). Eight genotypes were highly susceptible (HS) with high AUDPC values: LPF160, LPF148, LPF58, LPF222, LPF59, LPF83, LPF102 and LPF80 (Table 2; Fig. 1). These genotypes showed no resistance to disease establishment and development (Fig. 2D, 3). Genotype BPL710, used as a resistant control, did not show the highest level of resistance to the disease. Twenty genotypes were moderately resistant or moderately susceptible according to MDI values, and thus showed greater

Table 1. Analysis of variance of the area under disease progress curve (AUDPC) and of the mass disease index (MDI) on leaves 7 to 52 days after inoculation with *Botrytis fabae* under field conditions.

Source of variation	df	Measurements/ (days after inoculation)[a]						AUDPC[a]
		MDI1 (7d)	MDI2 (14d)	MDI3 (21d)	MDI4 (30d)	MDI5 (37d)	MDI6 (52d)	
Genotypes	66	11.396***	40.814 ^{ns}	38.567 ^{ns}	71.582 ^{ns}	175.293^{ns}	380.719*	137265**
Blocks	1	12.333 ^{ns}	77.937 ^{ns}	61.683 ^{ns}	1485.6***	496.511 ^{ns}	0.042 ^{ns}	409175.7*
Residue	130	4.813	33.172	28.538	55.273	134.160	250.413	83048.69

[a] Values are mean squares.
* Significant at $0.01<P<0.05$; ** significant at $0.001<P<0.01$; *** significant at $P<0.001$; ns, not significant at 5%.

resistance than BPL710. But only 15 of these lines showed low AUDPC and hence they were more tolerant to chocolate spot disease than BPL710, which had relatively low AUDPC values and was susceptible (Table 2; Fig. 1 and 2C).

Greenhouse trial

In the greenhouse, disease symptoms on the leaves and stems of the 14 genotypes selected because of their promising reactions to chocolate spot under field conditions, as well as symptoms on the susceptible control Rebaya 40 and the resistant control BPL710, showed significant differences among the tested genotypes both on the foliage (Table 3) and on the stems (Table 4). According to AUDPC values for foliage infection (Fig. 4), genotype LPF05 presented the highest resistance. This line resisted infection particularly at the earlier stages and showed relatively low levels of disease with MDI values of about 25 three days after inoculation, and lower than 55 on day 11. On the basis of the MDI 7 days after inoculation (Table 5), when the susceptible Rebaya 40 was already fully infected, LPF05 was classified as moderately susceptible, and so were LPF44, LPF113, LPF38, LPF95, LPF237, LPF233 and BPL710, the resistant control. These lines limited disease progression only by the end of the trial and thus had higher AUDPC values than LPF05. It is important to note that the genotypes most tolerant to disease progression on foliage were not necessarily those most tolerant to stem infection (Fig. 4 and 5). Six genotypes were susceptible and showed relatively high levels of AUDPC for foliage infection: LPF41, LPF39, LPF64, LPF89, LPF152 and LPF131. Genotype LPF174, like Rebaya 40, was highly susceptible. These last two genotypes showed no resistance to infection and did not slow down disease progression. MDI values for these two genotypes were higher than 80 seven days after inoculation.

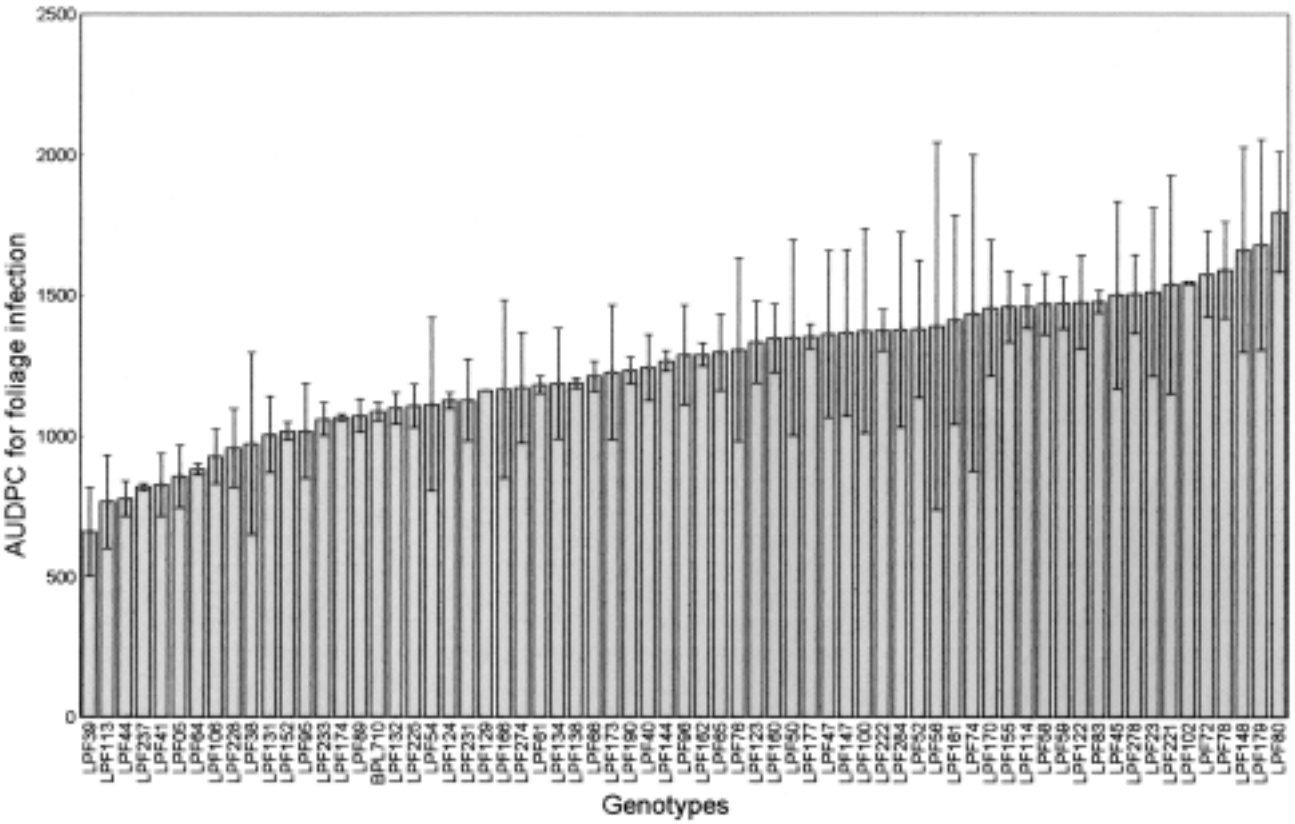

Fig. 1. Classification of 67 genotypes of *Vicia faba* according to area under disease progress curve (AUDPC) values determined under field conditions. Bars represent standard deviation.

Table 2. *Vicia faba* reaction to chocolate spot according to mass disease index (MDI) values under field conditions.

Genotype	MDI6[a]	Classification[b]	Genotype	MDI6[a]	Classification[b]
LPF39	33.33	MR	LPF138	68.88	S
LPF237	34.39	MR	LPF284	70	S
LPF44	38.14	MR	LPF134	70.16	S
LPF05	38.33	MR	LPF177	70.37	S
LPF113	39.68	MR	LPF144	70.37	S
LPF228	42.69	MS	LPF274	70.37	S
LPF41	42.85	MS	LPF173	70.63	S
LPF225	48.57	MS	LPF221	70.83	S
LPF40	48.88	MS	LPF278	71.42	S
LPF64	49.2	MS	LPF179	71.69	S
LPF106	51.38	MS	LPF162	71.74	S
LPF38	55.55	MS	LPF170	71.85	S
LPF231	55.95	MS	LPF190	72.22	S
LPF233	57.14	MS	LPF129	73.41	S
LPF52	57.4	MS	LPF66	74.07	S
LPF95	57.4	MS	LPF72	74.07	S
LPF147	57.4	MS	LPF61	74.16	S
LPF74	57.4	MS	LPF23	75.13	S
LPF114	58.73	MS	LPF123	75.92	S
LPF132	60	MS	LPF89	77.4	S
BPL710	60.84	S	LPF100	77.46	S
LPF174	61.11	S	LPF124	77.77	S
LPF65	61.9	S	LPF122	79.36	S
LPF152	63.33	S	LPF78	79.76	S
LPF54	64.44	S	LPF166	81.48	HS
LPF76	64.81	S	LPF160	81.58	HS
LPF161	65.08	S	LPF148	81.94	HS
LPF155	65.92	S	LPF58	83.33	HS
LPF96	66.66	S	LPF222	84.12	HS
LPF47	67.59	S	LPF59	84.72	HS
LPF50	67.59	S	LPF83	86.5	HS
LPF131	68.25	S	LPF102	87.96	HS
LPF45	68.51	S	LPF80	90.79	HS
LPF56	68.51	S			
			$LSD_{0.05}$	1.56	

[a] MDI6 was recorded 52 days after inoculation.
[b] MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

Table 3. ANOVA of mass disease indexes 3 to 23 days after inoculation (MDI1–MDI5) and of area under disease progress curves (AUDPC) (for foliage infection) under greenhouse conditions.

Source of variation	df	Notations/ (days after inoculation)[a]					AUDPC[a] foliage
		MDI1 (3d)	MDI2 (7d)	MDI3 (11d)	MDI4 (15d)	MDI5 (23d)	
Genotypes	15	408.31***	724.05***	469.91***	505.57***	360.03***	204710.3***
Residue	64	122.37	197.711	151.67	117.77	57.82	26927.19

[a] See Table 1.
*** Significant at $P<0.001$.

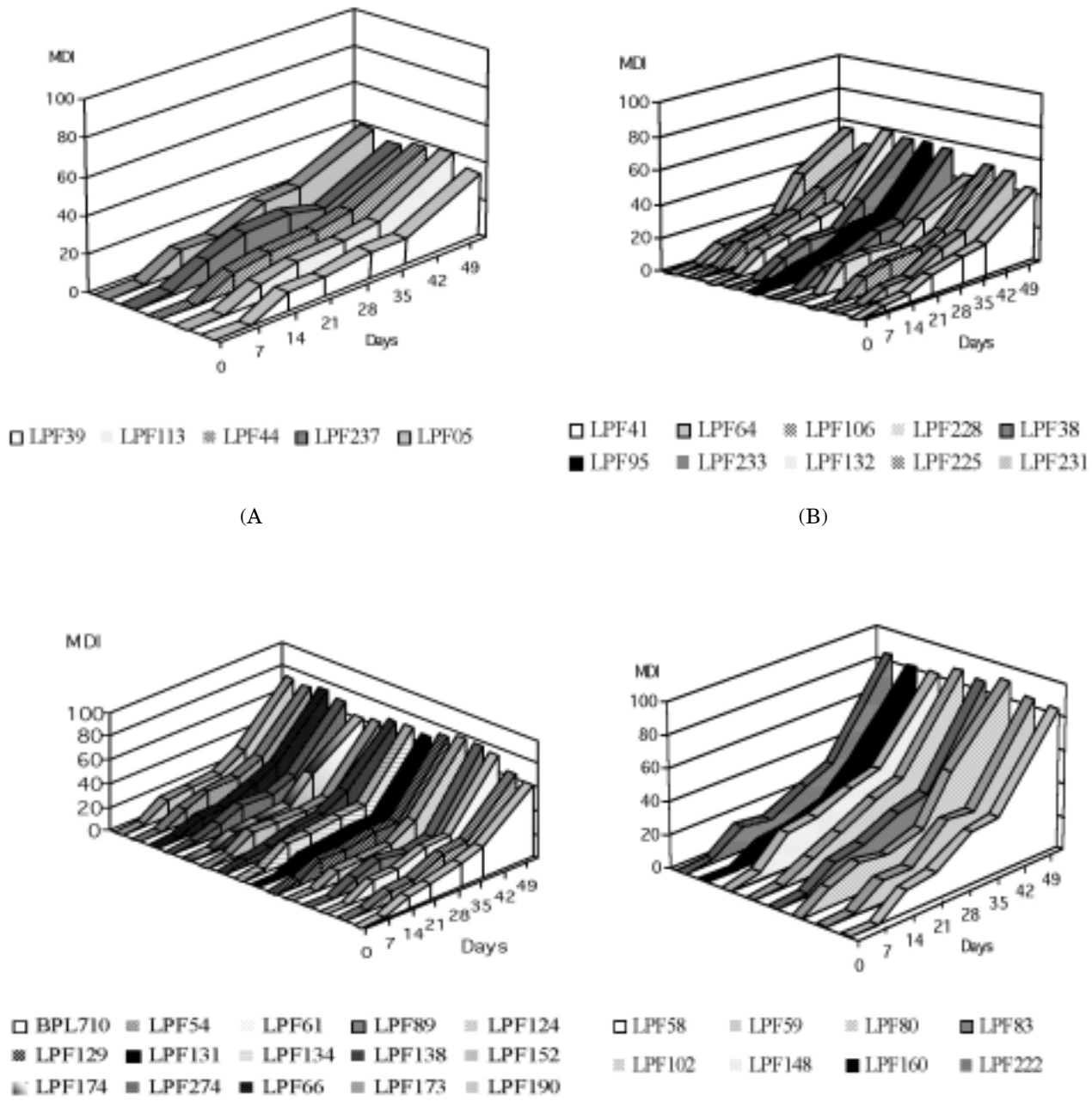

Fig. 2. Mass disease index (MDI) of chocolate spot infection under field conditions for moderately resistant lines (A); moderately susceptible lines with low area under disease progress curve (AUDPC) values (B); susceptible lines with low AUDPC values (C); highly susceptible lines (D).

Discussion

Under field conditions, the 67 tested genotypes differed significantly in their relation to chocolate spot at the first and the last measurements 7 and 52 days after inoculation. These differences were not however observed between the two dates, since, after infection, disease progression slowed down for all lines. Newly formed tissue showed fewer symptoms than older tissue, probably because high temperatures during this period lowered disease pressure. Nevertheless, although temperatures were very high, reaching sometimes 30°C, particularly at the end of the experiment (from 13 April to 5 May 2000, that is, between the fourth and the last measurements), the infection resumed its progression later on with greater severity. This may

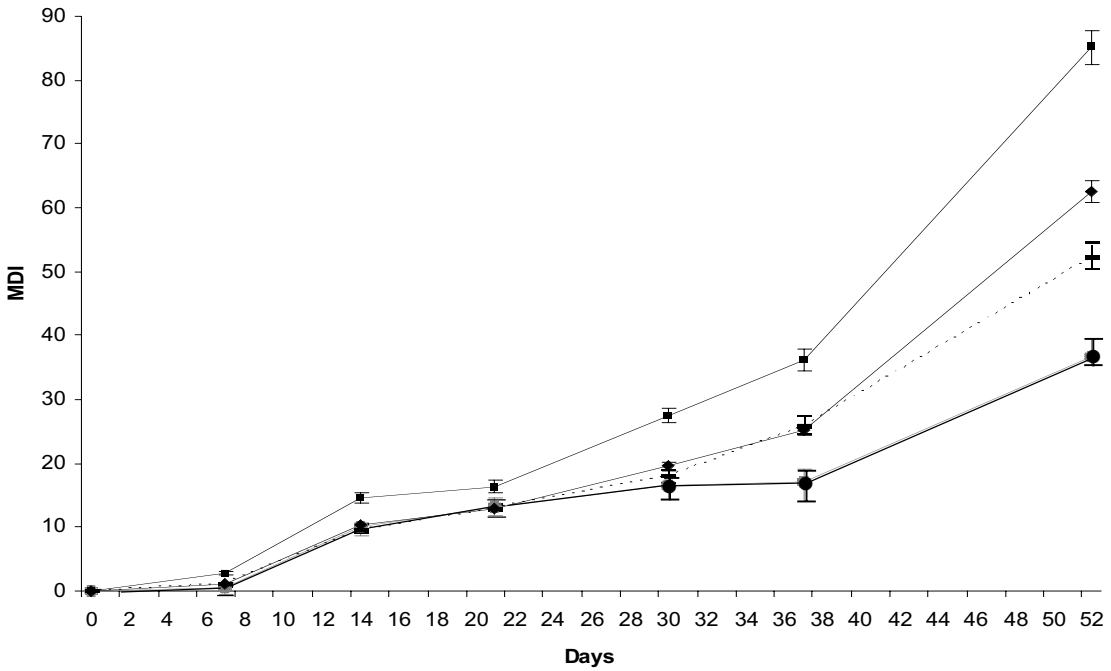

Fig. 3. Mass disease index (MDI) of chocolate spot on leaves under field conditions for: moderately resistant lines with low area under disease progress curves (—●—); highly susceptible lines with high AUDPC (—■—); moderately susceptible lines with low AUDPC (····▬····); and susceptible lines with low AUDPC (—♦—). Bars represent standard deviation.

Table 4. ANOVA of stem infection 3 to 23 days after inoculation (DIS1–IS5) and of area under disease progress curves (AUDPC) (for stem infection) under greenhouse conditions.

Source of variation	df	Mesaurements/ (days after inoculation)[a]					AUDPC[a] stems
		DIS1 (3d)	DIS2 (7d)	DIS3 (11d)	DIS4 (15d)	DIS5 (23d)	
Genotypes	15	0.52**	0.57*	0.69*	0.54	0.14	155.88**
Residue	64	0.21	0.31	0.32	0.35	0.11	61.92

[a] See Table 1.
* See Table 1.

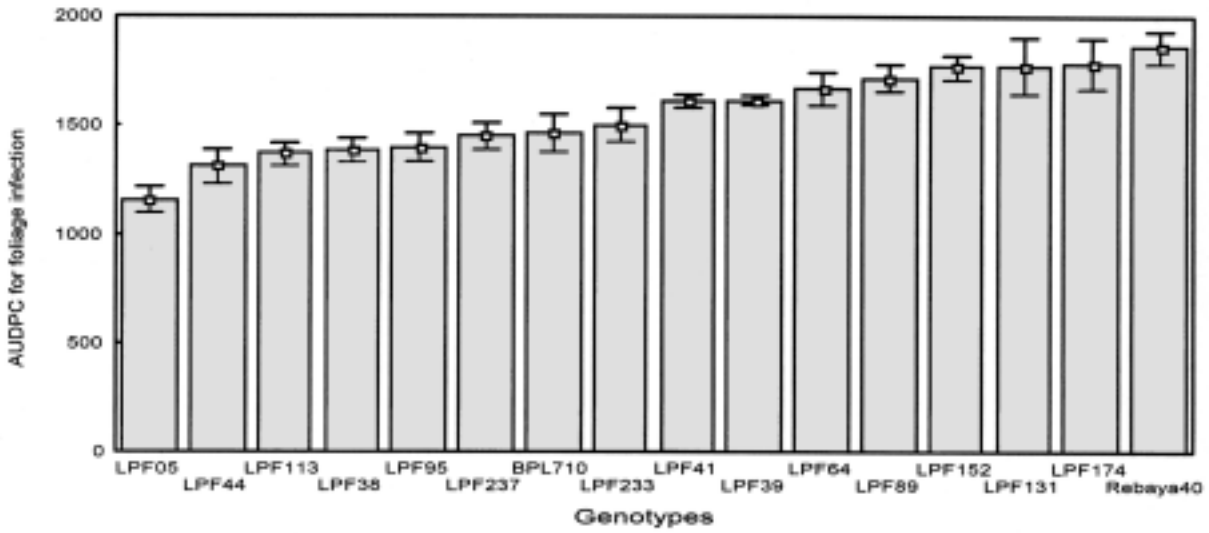

Fig. 4. Sixteen genotypes of *Vicia faba* classified according to area under disease progress curves (AUDPC) for foliage infection under greenhouse conditions. AUDPC values were calculated from the mass disease indexes determined 0, 7, 14, 21, 30, 37 and 52 days after inoculation, and ranged from 0 to 100. Bars represent standard deviation.

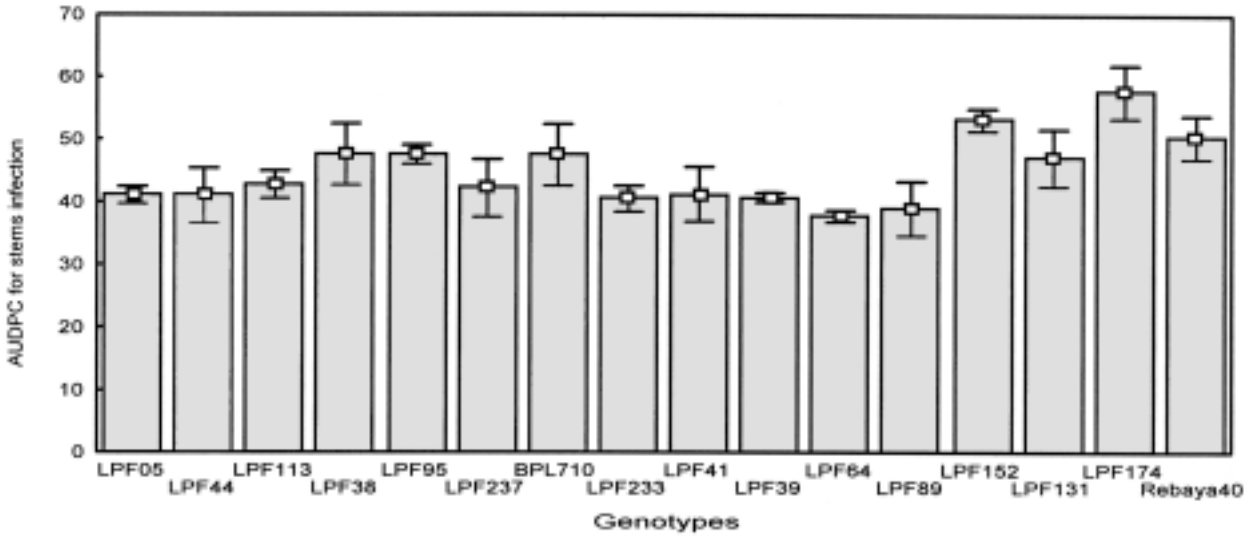

Fig. 5. AUDPC for stem infection of 16 genotypes of *Vicia faba* tested under greenhouse conditions and classified according to foliage infection. AUDPC values were calculated from the disease indexes on the stems (DIS), determined 0, 7, 14, 21, 30, 37 and 52 days after inoculation, and ranged from 0 to 3. Bars represent standard deviation.

Table 5. Fourteen genotypes of *Vicia faba* tested under greenhouse conditions according to their mass disease index (MDI).

Genotypes	MDI2 [a]	Resistance [b]
LPF44	41.1 a	MS
LPF237	53.3 ab	MS
LPF95	54.4 ab	MS
LPF05	54.4 ab	MS
BPL710	57.0 ab	MS
LPF38	57.4 abc	MS
LPF113	57.8 abc	MS
LPF233	58.0 abc	MS
LPF39	66.7 bcd	S
LPF89	66.7 bcd	S
LPF41	67.0 bcd	S
LPF64	70.2 bcde	S
LPF131	74.8 cde	S
LPF152	76.7 de	S
LPF174	80.7 de	HS
Rebaya 40	87.8 e	HS

[a] MDI2, mass disease index on foliage 7 days after inoculation; $LSD_{0.05}$: values followed by the same letter are not significantly different at 5%.
[b] See Table 2.

be explained by the fact that older tissues are generally more susceptible to disease than younger ones (Deverall and Wood, 1961; Abou Zaïd, 1974). The increase in symptom severity varied significantly with the genotype, which could explain the significant effect obtained at the last measurement. This observation led to the conclusion that while some genotypes slowed disease progression immediately after its initiation, others did not do so until later in the experiment. Genotypes thus differed in quantitative resistance to the virulent IBf24, quantitative resistance being the amount of tissues (leaves, stems) infected by the pathogen (Steffenson and Webster, 1992). These variations are represented by significant differences in the AUDPC values.

Of the 67 genotypes tested under field conditions, only 5 were moderately resistant to the disease and were potential sources of resistance. However, their resistance must be confirmed by further screening, particularly under controlled conditions and using other isolates of *B. fabae* from different parts of Tunisia. Plants in pots, growing under greenhouse conditions, may at certain points in their life cycle react more strongly to infection than they would naturally in the field (Tivoli *et al.*, 1986). By contrast, a field trial mimics a natural infection and takes place more gradually and more slowly than under the controlled conditions of the greenhouse, revealing more clearly the overall resistance of the plant, and the interaction of the pathogen with different plant organs at different stages of disease progression (Tivoli *et al.*, 1986). In a field trial, phenotypic and metabolic characters express themselves in a quantitative manner, reflecting the numerous infection and defence mechanisms involved in successive phases of the disease (Rapilly, 1991). The field performance of some of the genotypes suggested they might well be new sources of resistance (Tivoli *et al.*, 1986).

Although some genotypes were moderately susceptible or susceptible 52 days after inoculation, they had low AUDPC values. This may reflect the ability of these lines to slow down the disease at different phases of its progression. For example, the acceleration of disease at the end of the experiment could not be attributed to favourable conditions. At that period temperatures were very high and not conductive to the disease. Moreover, chocolate spot severity was significantly greater in susceptible and highly susceptible genotypes that in moderately resistant and moderately susceptible genotypes. The significant difference in severity 52 days after inoculation could be due to the ability of certain genotypes to slow down fungal development and multiplication at an advanced stage of the disease; this may be what happens with moderately resistant genotypes. Moderately susceptible or susceptible lines with low AUDPC values did not limit disease spread as effectively as did resistant lines. Nevertheless, although these moderately susceptible or susceptible genotypes slowed disease development only at the earlier stages (immediately after infection), they may yet represent possible sources of resistance and should not be eliminated. At the epidemiological level, host resistance to parasite infection is measured by three components: infection by primary inoculum (Xo), host colonisation and production of infective propagules (Xn). Each of these components involves many subsequences. The effect of all these components is perceptible in the course of an epidemic and is expressed by the AUDPC (Rapilly, 1991).

According to the two screening tests, genotypes

LPF44, LPF237, LPF05 and LPF113 were moderately resistant. Lines LPF95, LPF38 and LPF233 were moderately susceptible but, with low AUDPC values under field conditions, they were also tolerant to disease development in the greenhouse. Therefore, based on the hypothesis that genotypes with overall resistance may represent interesting sources of resistance, these genotypes merit inclusion in breeding programs. Line LPF39 showed the highest level of resistance in the field but was not as tolerant under greenhouse conditions. The susceptibility to chocolate spot shown in the greenhouse by this line in particular, and the higher levels of infection with all the fourteen lines in the field than in the greenhouse may be due to conditions in the greenhouse being more favourable to the disease. The tolerance shown by some genotypes in the field could be broken under certain conditions of temperature and light, which may make these genotypes susceptible (Tivoli *et al*., 1992).

Genotype BPL710 was susceptible to *B. fabae* isolate IBf24 in the field and moderately susceptible to that isolate in the greenhouse. This contrasts with previous findings that this line is resistant to the four prevalent races in the Mediterranean region, particularly in Egypt, Italy, Morocco and Tunisia (Halila *et al*., 1990). Such conflicting results of this and the other lines could be due to varying levels of virulence of isolate IBf24, and this may indicate the appearance of new races of *B. fabae* in Tunisia.

Acknowledgements

This research was supported by funds from FRYMED: "Yield stability and resistance of faba bean to major pathogens in the western Mediterranean basin" (Projet INCO/DC ERBIC18-CT98-0300) and IRESA, Tunisia (Projet Fédérateur Légumineuses à Graines).

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Accepted for publication: May 31, 2002