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Pathogenicity of *Botrytis* sp. isolates on *Vicia faba* based on two different methods

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Abstract - Pathogenicity of 20 isolates of *Botrytis* sp., 15 of which were isolated from different locations in Tunisia, was studied based on the reaction of five *Vicia faba* genotypes with known differential reaction to the disease. ANOVA of the chocolate spot disease infection over time revealed highly significant (p=0.001) differences among isolates and isolates×genotypes interaction. These differences were observed in greenhouse experiment using whole potted plants as well as *in vitro* assay using detached leaves. The twenty tested isolates were classified into 6 virulence groups based on genotypes reaction under greenhouse conditions. While the tested genotypes of *Vicia faba* reacted differently to *B. fabae* isolates, with results ranging from high susceptibility to moderate resistance, they have demonstrated a high resistance to all isolates of *B. cinerea* confirming the idea stating that this species is not important in disease development. Significant correlations were obtained between disease assessment under greenhouse conditions and *vitro* testing when a discriminating scale was used for detached leaves. However, the relationship between the two evaluation methods is likely to be not obvious and a combination of different methods should probably be adopted in order to have accurate estimation of the pathogenicity.

Keywords: Chocolate spot, Faba bean, *Botrytis fabae*, *B. cinerea*, pathogenicity

1. Introduction

Faba bean is among the most important food legumes in the world due to its richness in proteins and high nutritive value for humans and animals. Chocolate spot disease caused by *Botrytis fabae* is a devastating disease of faba bean inducing high yield losses (Terefe *et al.* 2015; Torres *et al.* 2006).
Treatment with fungicides and cultural management practices only provide partial control against the chocolate spot disease (Wilson 1937; Sundheim 1973; Tivoli et Gondran 1984). Efficient control strategies, including disease resistance as a main component should be considered. However, many attempts to identify sources of resistance allowed the identification of limited number of resistance genes which were not sufficient enough to develop *Vicia faba* cultivars with efficient resistance (Elliott et Whittington 1968; Russel 1978). In addition, many studies revealed the occurrence of high pathogenic variability among *B. fabae* isolates (Hanounik et Maliha 1984; Dereje 1996). Thus, each time new sources of resistance are identified, there is no guarantee that new races of the pathogen may not appear and be able to overcome this resistance (Russel 1978). Therefore, it is necessary, while screening for resistance among interesting cultivars, to take into account the wide range of pathogenicity of *B. fabae* isolates. In this context, many isolates with different origins and variable levels of pathogenicity should be used in screening programs for the identification of resistant cultivars (Dereje 1996). The main objective of this work was to study the pathogenicity of *Botrytis fabae* based on the reaction of 5 reference genotypes of *Vicia faba* generally used in many countries due to their differential reaction to the chocolate spot disease. Among the genotypes which were used in this study, two (BPL710 and BPL1179) were previously considered to be resistant to *B. fabae*, two (BPL1763 and BPL1821) were reported to show specific resistance to some isolates and one (LPF120) is known for its high level of susceptibility to the disease (Hanounik et Maliha 1986; Hanounik et Maliha 1984; Maliha 1983). The current study aimed to have an idea about the virulence of some Tunisian isolates of *B. fabae*. Two isolates of *B. fabae* from Morocco were also included in our study for comparison. In addition, in order to compare the pathogenicity of the two species *B. fabae* and *B.*

cinerea and their ability to induce chocolate spot typical symptoms, 4 isolates of *B. cinerea* were also included. In fact, the pathogenicity of the two species and their ability to induce chocolate spot symptoms have been subject to many controversies. Generally, *B. fabae* is considered to be the main causal agent of the disease, whereas *B. cinerea* is often considered to be secondary (Harrisson 1984). Some reports stated that *B. fabae* is more pathogenic than *B. cinerea*. More frequently, we agree to admit that both *B. fabae* and *B. cinerea* may induce the same typical chocolate spot symptoms but only *B. fabae* can lead to the aggressive stage under natural conditions (Hashim et al. 1997). Different evaluation methods, including the assessment of infected leaves, the extent of necrosis, the diseased leaf area or the number of lesions may be adopted (Tivoli *et al.* 1986).

2. Material and methods

Sampling

Leaves of *Vicia faba* showing typical chocolate spot symptoms were collected from different locations in Tunisia. Small pieces of 1-2 mm² in surface were cut from the lesions and deposited onto Water-Agar medium after surface disinfection with 3% Sodium Hypochlorite and incubated at 20±2°C. Purified cultures were transferred to Potato-Dextrose-Agar medium (PDA). Additional reference isolates from Tunisia, France and Morocco were also included in this study. All isolates were single-spored (Onfroy 1997) and maintained on PDA and Malt extract media after developing typical sclerotia. Inoculum was prepared by cultivating single-spored isolates on Faba bean-Leaf-Dextrose-Agar (FLDA) (Tivoli *et al.* 1986), incubating them under a cycle of 12h darkness and 12h near ultraviolet light UV light and a temperature of 20±2°C. Spore suspensions were prepared from 14-days old cultures and concentrations were adjusted to 5×10^5 spores/ml for each isolate. Only small lesions corresponding to the aggressive stage of infection were selected for isolating the pathogen. Widely spread lesions were avoided to minimize the risk of isolating saprobes, particularly *B. cinerea*. In fact, the latter may develop on primary lesions caused by *B. fabae* and can often be isolated and mistaken for being the causal agent (Harrisson 1984). *B. fabae* isolates were checked based on their growth and morphological characters as described by Onfroy (1997).

Pathogenicity testing

Greenhouse assay

Five genotypes, BPL710 and BPL1179 from Ecuador (Hanounik et Robertson 1996), BPL1821 and BPL1763 from Ethiopia (Dereje 1996) and LPF120 with unknown origin, were used. Seeds were sown in plastic pots under greenhouse conditions at 5 seeds/pot and 3 pots were used as replicates for each genotype. Thus, 15 plants per genotype and 75 plants per isolate were considered. The experiment, consisting of a total of 1500 plants, was organized according to a completely randomized factorial plot with 3 replicates. Inoculation was performed on 3-weeks-old seedlings using spore suspensions of each isolate of either *B. fabae* or *B. cinerea*. Pots were kept in the greenhouse under a temperature of 20±2°C, and individually covered with plastic bags to ensure high humid conditions more favorable for initiation and development of infection. Disease was scored 5, 12 and 19 days after inoculation using a 0-9 scale for disease assessment on leaves (Ding *et al.* 1993). The classification of the different genotypes regarding their resistance, from highly resistant (HR) to highly susceptible (HS) was based on mass disease index (MDI) (Ding et *al.* 1993).

Detached leaves assay

Following disease initiation in pots under greenhouse conditions, 6 leaflets per genotype and per isolate were detached from the 9^{nth} node of the plants 14 days after inoculation. All selected leaves were at the first stage of infection (1 with the scale) corresponding to the presence of only few chocolate spot dots covering less than 5% of the leaf surface. Detached leaves were surface cleaned with sterile distilled water, dried between two layers of sterile filter paper then placed in Petri dishes containing sterile filter paper humidified with 10 ml of sterile distilled water. Petiols uniting the two leaflets were longitudinally cut to avoid interactions between leaflets of the same leaf. A piece of sterile cotton humidified with sterile distilled water was placed onto petiols end in order to maintain their turgidity state and prevent an eventual dryness (Hashim *et al.* 1997; Khalil et Harrisson 1981). Three leaflets were deposited per plate and two replicates (2 plates) were adopted according to a completely randomized factorial plot with two factors. Plates were incubated at 20±2°C and disease assessment on detached leaves was performed 24, 48 and 96h later according to a 1-4 scale adopted for detached leaves (Hanounik et Maliha 1986). Mean disease indexes on detached leaves (IMF) were calculated. The infected leaf surface was assessed 120h after incubation, by determining the leaf

surface covered by the disease spots, using an area-measurement system (At-Delta-T. Devides; Durevell, Cambridge, England).

Statistical analysis

Data collected from the two performed experiments were subject 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 whole plants in the grennhouse or on detached leaves. Means were separated using the Least Significant Difference (LSD) Test. Correlations between disease assessment from the two assays were also determined.

3. Results and discussion

Sampling and identification

Sixteen single-spored isolates of *B. fabae* and 4 of *B. cinerea* were selected based on their growth and morphological features which were consistent with those stated by Onfroy (1997) (Table 1).

Table 1. Isolates of *Botrytis* spp. obtained and used in this study

Isolates	Species	location
IBf2	*Botrytis fabae*	Tunisia (Korba)
IBf6	*B. fabae*	Tunisia (Haouaria)
IBf7	*B. fabae*	Tunisia (Haouaria)
IBf12	*B. fabae*	Tunisia (Utique, Bizerte)
IBf23	*B. fabae*	Tunisia (Sidi Hothman, Beja)
IBf24	*B. fabae*	Tunisia (Beja)
IBf26	*B. fabae*	Tunisia (Beja)
IBf27	*B. fabae*	Tunisia (Beja)
IBf33	*B. fabae*	Tunisia (El Fahs)
IBf34	*B. fabae*	Tunisia (El Araybia)
Bf1	*B. fabae*	Tunisia (Tamazrat)
Bf2	*B. fabae*	Tunisia (Ariana)
Bf3	*B. fabae*	Tunisia (Mateur)
Bf6	*B. fabae*	Tunisia (Oued Beja)
Rabat I	*B. fabae*	Morocco
Rabat II	*B. fabae*	Morocco
Bc Kelibia	*Botrytis cinerea*	Tunisia (Cap Bon)
Bc II	*B. cinerea*	France
Bc air fr	*B. cinerea*	France
Bc Angl	*B. cinerea*	UK

On PDA and Malt extract media, *B. fabae* isolates presented thin, flush, slow-growing mycelia starting in locks, while *B. cinerea* isolates have rather aerial and loose rapid-growing mycelia with regular start. On FLDA medium, conidiophores of *B. fabae* were tight with dense sporulation while those of *B. cinerea* were loose with diffuse sporulation. ², developing on Malt extract and PDA media were smaller (1-1.7 mm) and more abundant for *B. fabae* isolates while they were quiet absent or very few but larger in size (2-5 mm) for *B. cinerea*. Conidia of *B. fabae* were clearly less abundant and larger in size (16-25 × 13-16µ) than those of *B. cinerea* (8-14 × 6-9µ). In addition, Conidiophores of *B. cinerea* presented more ramifications than those of *B. fabae* (Figure 1A-B).

Figure 1. Microscopic and pathogenic variation observed among *B. fabae* vs. *B. cinerea* isolates: **A.** Coniophores and conidia of *B. fabae*; **B.** Conidiophores and conidia of *B. cinerea*; **C.** Necrosis induced by *B. fabae* on *Vicia faba* plants under greenhouse conditions; **D.** limited faint spots induced by *B. cinerea* on *V. faba* plants in the greenhouse; **E.** Symptoms induced by *B. fabae* on detached leaves of resistant (left) and susceptible (right) *V. faba* genotypes *in vitro***; F.** Symptoms induced by *B. cinerea* on detached leaves of resistant (left) and susceptible (right) *V. faba* genotypes *in vitro*.

Pathogenicity of *Botrytis* sp. isolates under greenhouse conditions

ANOVA analysis revealed a highly significant effect of the isolates as well as the interaction (isolate ×genotype) on the disease development over time for Mass Disease Indexes MDI1, MDI2 and MDI3 determined respectively 5, 12 and 19 days after inoculation. These results demonstrated a highly significant difference between *Botrytis* spp. isolates regarding their virulence and aggressively and their specificity on infecting the different *V. faba* genotypes as well (Table 2).

Table 2. ANOVA of Mass Disease Indexex (MDI1, MD2 and MDI3) determined 5, 12 and 19 days after inoculation of 5 *Vicia faba* genotypes by 20 isolates of *Botrytis* spp.

Source	**Mass disease index/ (days after inoculation)[a]**		
of variation	MDI1 (5d)	MDI2 (12d)	MDI3 (19d)
Isolates	2740,797***	6238,495***	5817,267***
Genotypes	1634,129***	293,369ns	231,094ns
Isolates×Genotypes	160,959***	351,287***	407,971***
Residue	54,448	129,806	104,831
R^2	0,86	0,85	0,87
CV (%)	31,28	20,06	15,15

[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%.

Based on the values of MDI2 and on LSD significance test, the 20 tested isolates were classified into 12 virulence groups according to the reaction of the inoculated genotypes (Table 3).

Table 3. Classification of *Botrytis* sp. isolates into virulence groups based on mean values of mass disease index MDI2 determined 12 days after inoculation and determined accordingly to the 5 *Vicia faba* genotypes reaction[a] and LSD test[b].

Virulence groups	Isolates	Genotypes									
		BPL1821		BPL1763		BPL1179		BPL710		LPF120	
1	IBf24	**HS**	94,07	**HS**	85,92	**HS**	86,66	**HS**	92,59	**HS**	100
2	IBf26	**HS**	85,18	**HS**	81,85	**MS**	58,51	**HS**	88,14	**HS**	92,59
3	IBf7	**HS**	85,18	**S**	77,77	**S**	74,81	**HS**	82,22	**S**	77,77
	IBf27	**HS**	92,59	**S**	77,77	**MS**	60,00	**S**	73,33	**S**	73,33
4	IBf23	**S**	68,88	**S**	62,22	**S**	70,37	**MS**	52,59	**HS**	85,18
5	IBf6	**MS**	55,55	**S**	76,29	**MS**	60,00	**HS**	85,18	**S**	61,48
	IBf33	**S**	65,92	**S**	68,88	**MS**	51,11	**S**	71,85	**S**	64,44
6	IBf34	**S**	74,81	**S**	70,37	**MS**	57,03	**S**	68,88	**MS**	46,66
	Bf6	**S**	67,40	**S**	67,40	**S**	64,44	**MS**	54,07	**MS**	51,11
7	IBf12	**MR**	38,51	**HS**	85,18	**HS**	92,59	**S**	64,44	**S**	74,81
8	Rabat II	**MS**	51,11	**S**	61,48	**MS**	60,00	**MR**	39,25	**HS**	80,74
	Rabat I	**MS**	48,14	**S**	64,44	**MS**	46,66	**MR**	37,77	**MS**	51,11
9	IBf2	**MS**	48,14	**MS**	48,14	**S**	62,96	**MR**	39,25	**MS**	45,18
	Bf2	**MS**	46,66	**MS**	49,62	**MS**	51,11	**MR**	39,25	**MS**	60,00
10	Bf1	**MS**	43,70	**MS**	42,22	**MS**	54,07	**MS**	49,62	**MR**	34,81
11	Bf3	**MR**	36,29	**MS**	43,70	**MS**	49,62	**MR**	22,96	**S**	61,48
12	Bc Kel	**MR**	36,29	**MR**	25,92	**MR**	21,48	**MR**	34,81	**MR**	24,44
	Bc.air fr.	**MR**	21,48	**MR**	33,33	**MR**	16,29	**MR**	37,77	**MR**	39,25
	Bc. Angl	**MR**	25,92	**MR**	33,33	**MR**	15,55	**MR**	17,03	**R**	12,59
	Bc II	**MR**	36,29	**MR**	22,96	**MR**	18,51	**R**	14,07	**R**	14,07

[a] HR=highly resistant; R=resistant; MR=moderately resistant; MS=moderately susceptible; S=susceptible; HS=highly susceptible; [b] $LSD_{0.05}$ = 15,08

The highest level of infection was induced by isolate IBf24, representing the first group of virulence, with MDI values superior to 80% on the 5 tested genotypes that had all highly susceptible reaction, whereas the 4 isolates of *B. cinera*, classified in group 12, were the least pathogenic and induced moderate resistant to resistant reactions on the different genotypes. Differential levels of infection were observed for the other isolates. Based on the mean values of MDI2 corresponding to the global reaction on the 5 genotypes, isolate IBf24 of *B. fabae* from Beja was found to be the most virulent while Bf3, Bf1, Rabat I, IBf2 et Bf2 were the least virulent among *B. fabae* isolates. The four *B. cinerea* isolates were significantly less virulent than those of *B. fabae* (Table 4).

Table 4. Mean values of mass disease index MDI2 determined 12 days after inoculation for the 20 isolates of Botrytis sp.

Isolates	MDI2 (Means)*
Bc Angleterre	20,888 a
Bc II (France)	21,185 a
Bc Kelibia	28,592 ab
Bc air Fr.	29,629 b
Bf3	42,814 c
Bf1	44,888 c
Rabat I	46,074 c
IBf2	48,740 c
Bf2	49,333 c
Rabat II	58,518 d
Bf6	60,888 de
IBf34	63,555 def
IBf33	64,444 def
IBf6	67,703 efg
IBf23	67,851 efg
IBf12	71,111 fg
IBf27	75,407 gh
IBf7	79,555 h
IBf26	81,259 h
IBf24	91,851 i

* Values followed by the same letter are not significantly different at 5% ; LSD0.05 =6,744

Pathogenicity of *Botrytis* sp. isolates based on detached leaves *in vitro*

ANOVA results revealed significant effects of the isolates and the interaction isolates×genotypes on disease development *in vitro* on detached leaves (Table 5) demonstrating similarly to the greenhouse pathogenicity testing, the existence of pathogenic variability among *Botrytis* spp. isolates and a specific interaction between isolates and genotypes.

Table 5. ANOVA of mean disease indexes on detached leaves IMF1, IMF2, IMF3 and the infested leaf area determined respectively 24, 48 and 96 and 120 hours after incubation of detached leaves of 5 *Vicia faba* genotypes inoculated with 20 isolates of *Botrytis* spp.

Source of variation	Mean disease index/ (hours after incubation) [a]			Infested leaf area [a] (120 h)
	IMF1 (24h)	IMF2 (48h)	IMF3 (96h)	
Isolates	0,399***	0,685***	2,198***	3610,660***
Genotypes	0,297***	0,503***	1,217***	1797,550***
Isolates×Genotypes	0,154***	0,222***	0,291***	331,716***
Residue	0,049	0,061	0,122	148,620
R^2	0,80	0,83	0,85	0,87
CV (%)	18,68	19,19	20,88	43,52

[a] Values are mean squares.

*** Significant at $P<0.001$

However, a more pronounced effect of the genotypes was observed *in vitro*. Based on the extent of necrosis on leaf area 120 h after incubation, and accordingly to a resistance scale for detached leaves (Hanounik et Maliha 1986), *Botrytis* spp. isolates were classified into 7 virulence groups (Table 6).

Table 6. Classification of 20 *Botrytis* sp. isolates into virulence groups based on the reaction[a] of 5 inoculated *Vicia faba* genotypes and determined accordingly to the infected leaf area assessed 120h after incubation of detached leaves and the 1-4 scale for detached leaves of Hanounik et Maliha (1986)

Virulence groups	Isolates	Genotypes				
		BPL1821	BPL1763	BPL1179	BPL710	LPF120
1	Bf6	S	HS	HS	S	HS
2	IBf24	S	HS	R	R	HS
3	IBf26	S	HR	R	R	S
4	IBf7	HR	S	R	HR	HS
	Bf1	R	S	HR	R	S
5	IBf12	HR	R	S	HR	R
6	Bf3	HR	HR	R	R	S
	IBf34	R	R	HR	R	S
7	Bf2	R	R	R	HR	HR
	IBf27	R	R	HR	HR	R
	RII	R	HR	R	HR	R
	IBf6	R	HR	HR	HR	R
	IBf23	R	HR	HR	HR	HR
	RI	HR	R	HR	HR	HR
	IBf33	HR	HR	HR	HR	R
	IBf2					
	Bc. Kelibia					
	Bc. air Fr.	HR	HR	HR	HR	HR
	Bc. Angleterre					
	Bc. II					

[a] HR=highly resistant (0-25% necrosis); R=resistant (26-50% necrosis, few sporulation); S=susceptible (51-75% necrosis, medium sporulation); HS=highly susceptible (76-100% necrosis, abundant sporulation).

This classification presented some similarities with that obtained with the greenhouse pathogenicity assay, mainly regarding the fact that *B. cinerea* isolates were the least pathogenic, and the highest level of pathogenicity was in both cases observed for *B. fabae* isolates IBf24, IBf7 and IBf26. Symptoms induced by *B. cinerea* isolates were in both assays limited to some spots while *B. fabae* isolates induced very obvious and spread necrosis (Figure 1C-D). Some *B. fabae* isolates, namely IBf23, IBf6 and Bf27, were however less pathogenic *in vitro* in relation de some or all *V. faba* genotypes than under greenhouse conditions. In contrast, other isolates, essentially Bf6 but also Bf1 and Bf3, induced more necrosis on detached leaves than on whole plants. It is also important to notice that different virulence groups were obtained using the two evaluation methods. In general, the classification based on the 1-4 scale used for detached leaves method seems to surestimate the resistance of the genotypes as many isolates, namely IBf2, IBf23, IBf33, Rabat I, Rabat II, IBf6, IBf27 and Bf2 were judged to be with the same level of pathogenicity as *B. cinerea* isolates. This can be attributed to the fact that, based on this scale, genotypes are classified as resistant even for a percentage of coverage by necrosis that may reach 50%. In this context, and to prevent this eventual surestimation a new 0-9 scale (Ding *et al*. 1993- modified) for detached leaves was proposed and new disease indexes for detached leaves were calculated. In order to evaluate the reliability of using one method or another, correlations between disease assessment on whole plants under greenhouse conditions and on detached leaves *in vitro*, using both 1-4 and new 0-9 proposed scales, were determined. Correlations between diseaese assessments using the two evaluation methods were significant for most isolates. Correlations indexes were higher when the new 1-9 developed scale for

detached leaves was used instead of the 1-4 scale (Table 7). In particular, contrarily to the 0-4 scale which did not allow the establishment of any statistical correlations for *B. cinerea* isolates, significant correlations were obtained with the 0-9 proposed scale. In fact, since a level of infection of 1 is attributed to necrosis covering between 0 to 25 % of the leaf surface, the 1-4 scale is not likely to be able to allow any discrimination between *B. cinerea* isolates which were found to be in general less virulent with less pathogenic variability than *B. fabae* isolates. Previous studies showed that even the evaluation methods used to evaluate the pathogenicity of *B. fabae* or either to screen for resistance against this pathogen seem to be simple, the relationship between the evaluation methods is not often evident (Tivoli *et al*. 1986). Il is important to notice in this context, that according to the system plant pathogen-host, the initiation of infection may be slow at the beginning but the plant is unable to limit its spread later. This is is probably due to different defense mechanisms of the plant (Tivoli *et al.* 1986) which may not be taken into account when using the detached leaves evaluation method.

Tableau 7. Correlations between disease assessment under greenhouse conditions (MDI) and on detached leaves (IMF); where IMF-0-4 and IMF-0-9 are respectively mean disease indexes determined based on the 0-4 and the proposed 0-9 scales for detached leaves.

Isolates		**Correlations**	
		IMF_0_4	IMF_0_9
Bf1	MDI	0,472** p=0,008	0,487** p=0,006
Bf2	MDI	0,413* p=0,023	0,369* p=0,045
Bf3	MDI	0,737*** p=0,000	0,804*** p=0,000
Bf6	MDI	0,583** p=0,001	0,585** p=0,001
Rabat I	MDI	0,308 ns p=0,098	0,450* p=0,013
Rabat II	MDI	0,342 ns p=0,065	0,401* p=0,028
IBf 2	MDI	0,472** p=0,008	0,311 ns p=0,095
IBf 6	MDI	0,391* p=0,032	0,369* p=0,045
IBf 7	MDI	0,269 ns p=0,151	0,247 ns p=0,187
IBf 12	MDI	0,658*** p=0,000	0,611*** p=0,000
IBf 23	MDI	0,325 ns p=0,080	0,408* p=0,025
IBf 24	MDI	0,442* p=0,015	0,457* p=0,011
IBf 26	MDI	0,567** p=0,001	0,536** p=0,002
IBf 27	MDI	0,448* p=0,013	0,466* p=0,010
IBf 33	MDI	0,633*** p=0,000	0,702*** p=0,000
IBf 34	MDI	0,526** p=0,003	0,459* p=0,011
Correlation for *Botrytis fabae*	MDI	0,524*** p=0,000	0,522*** p=0,000
Bc Kelibia	MDI	-- p= ---	0,819*** p=0,000
Bc Air France	MDI	0,069 ns p=0,717	0,145 ns p=0,445
Bc Angleterre	MDI	-- p= ---	0,145 ns p=0,445
Bc II (France)	MDI	-- p= ---	0,528** p=0,003
Correlation for *Botrytis cinerea*	MDI	0,036 ns p=0,699	0,432*** p=0,000
Correlation for *Botrytis* sp.	MDI	0,578*** p=0,000	0,625*** p=0,000

* Significant at $0.01<P<0.05$; ** significant at $0.001<P<0.01$; *** significant at $P<0.001$; ns, not significant at 5%.

4. Conclusion

A high pathogenic variability was revealed among *Botrytis sp.* and particularly among *B. fabae* isolates using both greenhouse and detached leaves evaluation methods, which is in perfect agreement with many previous studies (Dereje 1996; Hanounik et Maliha 1984; Hanounik et Maliha 1986). Isolates were classified. Isolate IBf24 of *B. fabae*, from Beja region was found to be the most virulent among the 20 considered isolates of *Botrytis sp.* Isolates classified in the same virulence groups, determined either under greenhouse conditions or *in vitro* on detached leaves, do not necessary belong to the same geographic location. This is similar to what was previously shown in other studies (Dereje 1996). Very low levels of infection were observed on the 5 differential *Vicia faba* genotypes when inoculated with *B. cinerea* isolates. The latter induced generally limited spots, which evolved very slowly over time, contrarily to *B. fabae* isolates, which produced obvious necrosis that became darker, spread very fast and led even to the breakage of whole stems and plants collapsing in many cases. This confirms previous conclusions stating that *B. cinerea* is much less virulent than *Botrytis fabae* (Hashim *et al*. 1997) and that *B. cinerea* may enter the agressive stage only under particularly very favorable conditions (Harrisson 1983).

Adopting a 1-4 scale for disease assessment on detached leaves was shown to overestimate resistance among *V. faba* genotypes and to be unable to discriminate accurately between pathogenic variations. The 1-9 scale for detached leaves proposed in this study allowed the establishment of more significant correlations with the greenhouse evaluation method. However, based on these results, the *in vitro* evaluation method is likely to be not really reliable when used alone and should probably be associated with other confirmation methods.

5. References

Dereje g (1996) Morphological, cultural and pathogenic variability among nine isolates of *Botrytis fabae* from Ethiopia. Fabis News 38/39: 37-41

Ding G, Xung L, Oifang G, Pingxi L, Dazhao Y, Ronghal H (1993) Evaluation and screening of faba bean germaplasm in China, Fabis News 32: 8-10.

Elliot JE, Wettington WJ (1979) An assessment of varietal resistance to chocolate spot (*Botrytis fabae*) infection of field beans (*Vicia faba*) with some indications of its heritability and its mode of inheritance. J Agric Sci Camb 93: 411-418

Hanounik SB, Maliha N (1986) Horizontal and vertical resistance in *Vicia faba* to chocolate spot caused by *B. fabae*. Plant Dis 70: 770-773

Hanounik S, Maliha N (1984) pathogenic and cultural variability in *Botrytis fabae*. Fabis News 10: 21-24

Hanounik SB, Robertson LD (1988) New sources of resistance in *Vicia faba* to chocolate spot caused by *Botrytis fabae*. Plant Dis 72: 696-698.

Harrisson JG (1983) Dinstinguishing between lesions caused by *Botrytis fabae* and *Botrytis cinerea* on field bean leaves. Trans Br Mycol Soc 81: 663-664

Harrisson JG (1984) *Botrytis cinerea* as an important cause of chocolate spot in field beans. Trans Br Mycol Soc 83: 631-637

Hashim M, Roberts JA, Rossall S, Dickinson MJ (1997) Leaflet abscission and phytoalexin production during the response of two faba bean breeding lines to *Botrytis* infection. Plant Pathol 46: 989-996

Huston RA, Mansfield JWB (1980) A genetical approach to the analysis of mechanisms of pathogenicity in *Botrytis* / *Vicia faba* interactions. Physiol Plant Pathol 17: 309-317

Jacobs TH, Parlevliet JE (1993) Durability of disease resistance - Kluwer Academic Publishers, Boston/London, 374 p

Khalil SA, Harrisson JG (1981) Methods of evaluating faba bean materials for chocolate spot. Fabis News 3: 51-52

Maliha NF (1983) Resistance of faba bean selections to different isolates of *Botrytis fabae*. American Univ. of Beirut (Lebanon), Thesis (Master of science); 58 pp.

Onfroy C (1997) Maladies fongiques aériennes des légumineuses alimentaires - INRA- Station de Pathologie Végétale, Le Rheu, 11 pp.

Purkayastha RP, Deverall BJ (1964) Physiology of virulence and avirulence of *Botrytis* spp. on leaves of broad bean (*Vicia faba*). Transactions of Brit Mycol Soc 47: 461

Rapilly F (1991) L'épidémiologie en pathologie végétale: mycoses aériennes- INRA Editions, 317 p

Russel GE (1978) Plant breeding for pest and disease resistance. Betterworths, London. 485 p.

Sundheim L (1973) *Botrytis fabae*, *Botrytis cinerea* and *Ascochta fabae* on broad bean (*Vicia faba*) in Norway. Acta. Agriculturae Scandidavia 23: 43-51.

Terefe H, Fininsa C, Sahile S, Tesfaye K (2015) Effect of temperature on growth and sporulation of Botrytis fabae, and resistance reactions of faba bean against the pathogen. J Plant Pathol Microb 6: 285. doi: 10.4172/2157-7471.1000285

Tivoli B, Berthelem D, Le Guen J, Onfroy C (1986) Comparison of some methods for evaluation of reaction of different genotypes to *B. fabae*. Fabis News 16: 46-50

Tivoli B, **Gondran J (1984)** Les tâches chocolat de la féverole causées par *Botrytis fabae* Sard. Persp Agric 77: 22-25.

Torres AM, Roman B, Aviva CM, Satovic Z, Rubiales D *et al.* (2006) Faba bean breeding for resistance against biotic stresses: Towards application of marker technology. Euphytica 147: 67-80.

Wilson AR (1937) The chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis cinerea* Pers. Ann App Biol 24: 258-288.