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Comparison of Some Methods for Evaluation of Reaction of Different Winter Faba Bean Genotypes to Botrytis fabae

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Abstract

Reaction of nine genotypes of faba bean (*Vicia faba*) and one of narbon vetch (*Vicia narbonensis*) to *Botrytis fabae* was studied using the detached leaf technique and under artificial infection in pots and in the field. Although the genotypic differences could be identified with each method, there was little consistency in the ranking perhaps because each method tends to express a particular phenomenon related to the crop's defence mechanism against the disease.

Introduction

In France, the winter faba bean (*Vicia faba* L.) is subjected to attacks of several pathogens which can cause considerable losses in yield. *Ascochyta fabae* is the first to infect faba bean plants during winter, followed by *Botrytis fabae* in spring, and *Uromyces fabae* and *Peronospora* sp. in June. *B. fabae* is considered to be the most common and serious of these pathogens. Chemical control and modified cultural practices provide only partial control. Therefore breeding for disease resistance is of major importance in controlling this disease.

This work aimed to study the performance of seven lines and two varieties of *V. faba* and one related species (*Vicia narbonensis*) to *B. fabae* using three methods of artificial inoculation; detached leaves maintained at survival level (Mohamed *et al.* 1980; Khalil and Harrison 1981; Abou-Zeid 1985), whole plants grown in pots (Gondran 1978; Elliot and Whittington 1979), and field inoculation (Hanounik 1983).

Materials and Methods

The plants

Based on Gondran's (1978) findings the following lines and varieties were selected for this study. Seven lines (two English LCF and S45, five French 245.17, 48B, 3.33, 29E, and 29H), two synthetic varieties (Bourdon and Soravi), and one related species *V. narbonensis* which is slightly susceptible to *B. fabae*.

The experiments were conducted at Le Rheu Station. In the field, plants were grown in 5.4 m^2 plots with a population density of 25 plants/m^2 using Fisher blocks in triplicate. Sowing was on 27 October 1983 and 27 November 1984 for the cropping seasons 1983/84 and 1984/85, respectively. In pots, three seeds were sown per pot (14 cm in diameter), each of which contained equal proportions of arable soil, brown peat, and sand. After 5 weeks of vernalization at 4°C (8 h of photoperiod) the plants were kept in a greenhouse until inoculation.

Inoculum increase

The fungus was grown on two different media. Faba bean leaf extract medium, as described by Leach and More (1966), was used to prepare the spore suspension for inoculation of detached leaves and whole plants in pots. On this medium the fungus produced a large number of spores, which tend to lose their infectivity with time (Last 1960). After 10 days' incubation at 20°C under black light (435 nm), the spores were collected in 5 ml sterile water by gently passing an elbowed Pasteur pipette over the surface of the colonies, then filtered through sterile gauze. Barley grain medium was used to produce inoculum for field inoculation. The fungus was propagated on barley grains which were placed in Erlenmeyer flasks, moistened, and autoclaved twice at 120°C for 1 h, at 24 h intervals. The inoculum was added to the grains and the flasks were incubated at 20°C. After 1 month the grains were covered with large numbers of *B. fabae* sclerotia.

To preserve the aggressiveness of *B. fabae*, the fungus was reisolated from infected plants each year, kept in a malt extract agar medium, then plated on the above media to stimulate sporulation.

Methods of inoculation and data collection

Detached leaves maintained at survival level

Detached leaves were placed on slides in petri dishes, 14 cm in diameter, containing filter paper saturated with 10 ml of sterile water. The petioles of each leaflet were cut longitudinally to avoid possible interactions among leaflets of the same leaf. Each end was covered with moistened cotton to preserve leaf turgidity. A drop (20 µl) of spore suspension

containing 0.005 million spores/µl was applied to the center of each leaf. Inoculated leaves were then incubated at 20°C. Data were recorded on the number of spots/leaflet (recorded every 12 h after the appearance of disease symptoms), disease development (recorded daily after the first lesions started to coalesce using the scale established by Abou-Zeid in 1985), and sporulation (evaluated by collecting the spores of each leaflet in a fixed volume of sterile water).

Whole plants in pots

The plants were inoculated twice, at the 5-7 leaf stage and at flowering (28 February 1984 and 19 March 1985). Each plant was inoculated by spraying a spore suspension containing 0.05 million spores/ml. After inoculation the plants were kept in a phytotronic enclosure at 96% relative humidity, at a temperature of 20 and 15°C during the day and night, respectively, and with 16 h of photoperiod.

Whole plants in the field

To simulate natural infection, barley grains infected with *B. fabae* were distributed among the plants at the rate of 5 grains/plant.

In both seasons, plants were inoculated 4 months after sowing (26 March 1984 and 25 April 1985). To enhance the disease development inoculated plots were sprinklered as soon as the daytime average temperature reached 18 °C.

For both whole plants in pots and in the field the data were recorded according to Gondran's (1977) disease scoring scale.

Results

Detached leaves

Chocolate spot symptoms were evident on faba bean lines 7-8 h after inoculation and after 9 h on narbon vetch (*V. narbonensis*). The disease symptoms started as a few small spots which significantly increased in number with time (Fig. 1) The average number of spots/leaflet varied significantly among the different lines (Table 1): 15 h after inoculation it was 6.1 and 19.4 on faba bean lines S45 and 29 H, respectively, and 2.3 on narbon vetch. The average time needed for new spots to appear ranged from 0.5 h on narbon vetch to 4.1 h on faba bean lines 29 H and 3.33.

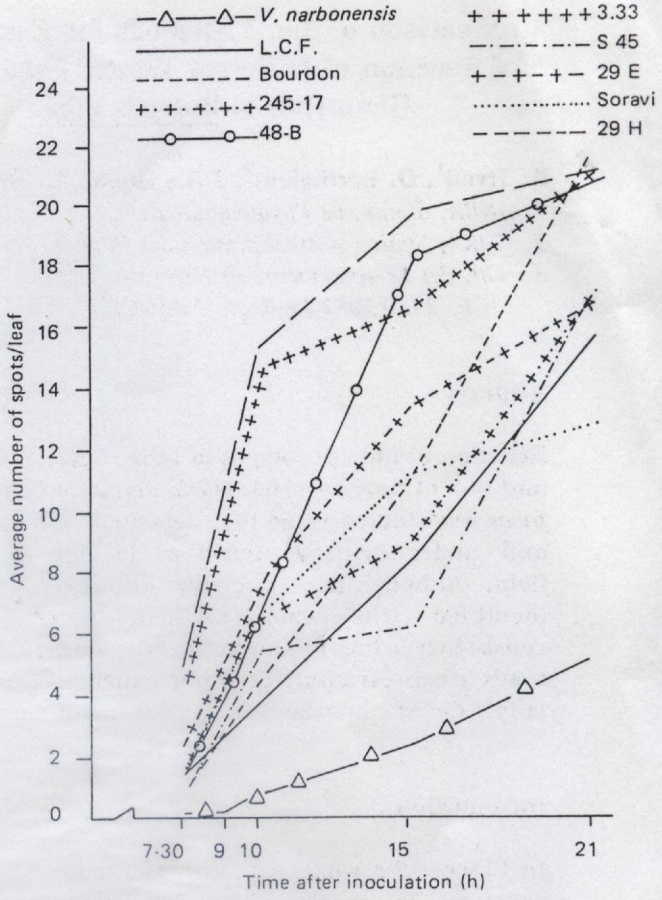

Fig. 1. Average number of spots appearing on detached leaves of nine genotypes of *V. faba* and one of *V. narbonensis* (v.n.) after inoculation with *B. fabae*.

Spot coalescence started 24-36 h after inoculation and the size of the spots significantly increased with time. Six days after inoculation the average disease scores were 4.4-7.9 for faba bean lines 29 H and 48B, respectively, and 5.2 for narbon vetch (Table 1).

Sporulation was estimated 11 days after inoculation. Considerable differences in sporulation were detected among the 10 lines tested, with the average number of spores produced by each line ranging from 0.02 to 1.0 million spores/leaflet on faba bean lines 29 E and Bourdon, respectively. Sporulation on vetch was intermediate with 0.6 million spores/leaflet (Table 1).

Whole plants in pots

Table 2 shows the average disease scores of plants inoculated at the 6-8 leaf stage and at flowering. At both stages there were significant differences in disease score, but the differences among lines

Table 1. Average reaction of detached leaves of nine genotypes of *V. faba* and one of *V. narbonensis* (*V.n.*) to *B. fabae*.

Appearance of symptoms				Disease development		Sporulation	
No. of spots 15 h after inoculation		Speed of new spot formation (spot/h)		Disease score 6 days after inoculation		No. of spores/leaflet 11 days after inoculation (million)	
V.n	2.3	*V.n.*	0.5	29H	4.4	29E	0.02
S45	6.1	LCF	1.1	Soravi	4.9	S45	0.04
LCF	8.7	Bourdon	1.6	29E	5.0	29H	0.09
245.17	9.1	29E	1.8	S45	5.1	245.17	0.10
Bourdon	11.0	S45	1.9	*V.n.*	5.2	3.33	0.30
Soravi	11.1	245.17	1.9	LCF	6.4	Soravi	0.30
29E	13.3	48B	2.2	Bourdon	6.6	*V.n.*	0.60
3.33	16.6	Soravi	2.5	245.17	6.8	LCF	0.70
48B	18.4	29H	4.1	3.33	6.8	48B	0.80
29H	19.4	3.33	4.1	48B	7.9	Bourdon	1.00
F calculated	6.62		9.08		5.55		2.35
F at P=0.05	2.45		2.45		2.45		3.07
LSD	6.28		1.12		1.4		NS

Table 2. Average disease scores of nine genotypes of *V. faba* and one of *V. narbonensis* (V.n.) grown in pots in response to *B. fabae* inoculum applied at two different stages of growth.

Young plant stage		Flowering stage			
		Inoculation 28/02/84		Inoculation 19/03/85	
LCF	3.3	V.n	2.4	Soravi	2.3
S45	3.4	29H	5.7	V.n.	3.0
Bourdon	3.5	Soravi	5.8	245.17	4.3
48B	3.8	29E	7.0	S45	4.4
3.33	4.5	LCF	7.3	29E	4.6
245.17	4.6	S45	8.1	29H	5.0
Soravi	4.9	Bourdon	8.2	Bourdon	5.8
29H	5.0	245.17	8.4	LCF	6.1
29E	5.6	48B	8.5	3.33	6.1
		3.33	8.5	48B	6.8
F calculated	92.7		14.4		18.3
F at 0.05	2.3		2.2		2.1
LSD.	0.5		0.6		0.9

inoculated at the flowering stage were more evident. Plants inoculated early in the season reacted differently to the disease compared to those inoculated later in the season. Moreover, in 1984 plants inoculated at flowering showed a different reaction to the disease compared to those grown during 1985.

Whole plants in the field

Table 3 shows the floral damage and disease reaction of the inoculated plants during 1983/84 and 1984/85. It is evident that the disease pressure was much higher in 1984/85. In 1984, the disease score ranged from 1.2

Table 3. Average disease scores and the relative values for undamaged floral parts of nine genotypes of *V. faba* and one of *V. narbonensis* (V.n.) to *B.fabae* under field conditions.

1983/84			1984/85		
Disease score		**Relative values for undamaged floral parts**	**Disease score**		**Relative values for undamaged floral parts***
V.n.	1.2	132	*V.n.*	6.2	Bourdon 86
Bourdon	1.4	113	29E	6.8	*V.n.* 83
Soravi	1.5	100	Bourdon	7.1	3.33 77
S45	1.6	92	S45	7.1	S45 74
48B	1.7	91	LCF	7.2	Soravi 71
245.17	1.8	89	48B	7.2	29E 68
LCF	1.8	88	Soravi	7.4	LCF 62
29E	1.9	83	29H	7.6	245.17 61
29H	2.0	83	3.33	7.8	29H 52
3.33	2.1	77	245.17	7.8	48B 50
F calculated	9.9	20.1		4.4	2.7
F at P= 0.05	2.5	2.5		2.5	2.5
LSD	0.3	10.0		0.7	17.0

* Numbers over 100 indicate that the disease did not reach the first floral stage.

for narbon vetch to 2.1 for line 3.33, while in 1985 it ranged from 6.2 for vetch to 7.8 for line 245.17. Differences among the 10 lines tested were significant in both years.

Discussion and Conclusion

These results show that although the methods for the evaluation of performance of faba bean in relation to *B. fabae* are relatively simple, the relations between these different methods are not always obvious, because each one shows a particular phenomenon.

On detached leaves infected by *B.fabae* two important aspects are observed: the rate of appearance of the symptoms and the rate of development of the disease. The first one varies considerably from one genotype to another, while the second is less discriminating. So, a line with good performance in the first phase of fungal attack could be totally affected by the disease once the infection has set in. The most spectacular example is provided by the narbon vetch which considerably delayed the initial establishment of infection but was unable to limit its spread later in the rest of the tissue. This situation shows without doubt the importance of two different mechanisms in the host resistance to the disease.

The performance studied after inoculation in pot culture in the greenhouse integrates at a certain moment of the plant life, the plant's intrinsic reaction to infection in a more drastic manner than may naturally occur in the field. The field technique, on the other hand, simulates a natural attack, progressive but slower than the previous method, and shows an overall performance of the plant including the interaction of the fungus with different plant organs in relation to different phases of the disease epidemiology. This overall performance in the field, which is the consequence of intrinsic or "phenotypic" reaction of plants, constitutes the reference performance.

Although the relationship between the different methods used in this study is not always evident, the delayed appearance of symptoms in narbon vetch probably explains the superior performance of this species as already noted by Gondran (1977). The faba bean genotypes varied in their response to *B. fabae* in each test used but this can be understood in view of the fact that the characters being used for evaluation differed from one test to another. However, these studies showed consistent good performance of narbon vetch, Bourdon variety, and line S 45 and consistent susceptibility of lines 3.33 and 48B.

The results obtained from this experiment revealed that the nine faba bean genotypes reacted differently in the three methods used for studying their reaction to *B. fabae*, because each method permits expression of some particular mechanisms of resistance more clearly than others. For example in the detached leaf test, line 29H, which was the most susceptible line during the early stages of infection showed limited disease development and later sporulation indicating a different mechanism of resistance. We therefore suggest that crosses should be made between different faba bean plants with different mechanisms of resistance to combine such resistance in one line.

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MAJOR FABA BEAN PRODUCING COUNTRIES

Area, yield, and production of faba bean in the major faba bean producing countries ranked on 1984 production.

Country	Area (1000 ha)					Yield (kg/ha)					Production (1000 MT)				
	1974/76	1977/81	1982	1983	1984	1974/76	1977/81	1982	1983	1984	1974/76	1977/81	1982	1983	1984
China	2300	4100	1900	1920	1800	1058	1069	1368	1198	1250	2433	4280	2600	2300	2250
Ethiopia	288	301	381	381	343	998	1152	1579	1577	1312	287	348	601	600	450
Egypt	106	107	115	128	120	2267	2132	2255	2305	2258	241	229	260	295	271
France	21	21	37	52	67	2039	2691	2946	3077	3149	44	57	109	160	211
Italy	209	165	150	148	148	1207	1290	1191	1092	1241	253	213	178	162	183
Morocco	213	181	111	171	200	1234	621	892	834	610	263	114	99	142	122
Turkey	31	30	36	43	42	1612	1729	1831	1812	1810	51	52	65	77	76
Spain	117	86	55	50	59	1005	1001	982	820	1085	118	87	54	41	64
Tunisia	80	66	66	58	65	563	567	593	858	846	45	39	39	50	55
Mexico	49	53	39	27	30	722	1034	1299	1425	1333	35	53	51	39	40
Brazil	192	176	135	141	142	379	348	312	234	246	73	63	42	33	35
Czechoslavakia	27	46	27	18	15	1626	1787	1553	1700	2037	44	84	41	31	30
Germany FR	17	5	6	6	8	2977	3226	3422	2987	3579	51	17	21	17	27
Algeria	35	41	43	50	50	864	717	458	423	500	30	29	20	21	25
Sudan	15	16	16	16	16	1203	1279	1375	1375	1375	18	21	22	22	22
Peru	23	23	23	24	24	915	924	927	936	936	21	21	22	22	22
Portugal	45	35	32	32	32	667	572	647	563	619	30	19	21	18	20
Canada			15	15	19			784	784	789			12	12	15
Syria	6	8	8	7	8	1608	1670	1843	1864	1750	10	13	14	13	14
German DR	6	6	7	6	6	1989	2123	2438	2117	2109	11	13	16	14	14

Source: FAO Production Yearbooks

Area, yield, and production of faba bean in different geographical regions.

Region	Area (1000 ha)					Yield (kg/ha)					Production (1000 MT)				
	1974/76	1977/81	1982	1983	1984	1974/76	1977/81	1982	1983	1984	1974/76	1977/81	1982	1983	1984
Africa	744	721	839	811	802	1199	1089	1418	1403	1189	891	783	1048	1139	954
N & C America	73	80	85	75	83	676	868	957	945	923	49	69	81	71	77
South America	252	237	186	194	195	506	501	508	466	474	127	119	94	90	93
Asia	2361	4153	1954	1978	1858	1066	1079	1379	1214	1265	2517	4368	2695	2401	2350
Europe	455	397	319	318	340	1254	1421	1409	1420	1638	570	567	449	452	556
World Total	3886	5596	3299	3393	3294	1070	1076	1327	1227	1226	4156	5915	4377	4164	4039

Source: FAO Production Yearbooks.