

Resistance in *Vicia faba* Germ Plasm to Blight Caused by *Ascochyta fabae*

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ABSTRACT

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Of the 672 germ plasm lines evaluated in 1980 and 1981 for resistance to *Ascochyta fabae* in Syria, 19 were resistant and 653 were susceptible. Resistant lines were tested again in Syria, England, Canada, Poland, France, and Tunisia in 1983, 1984, and 1985. Lines BPL 471, 460, 646, 74, and 2485 were resistant at all locations, whereas lines BPL 472, 818, and ILB 1814 were resistant at some, but not all, locations. BPL 471 and 2485 revealed no differential host-pathogen interactions, whereas BPL 818 and ILB 1814 consistently showed significant differential interactions when inoculated with eight isolates of the pathogen from Syria. Therefore, BPL 471 and 2485 seemed to carry genes for a broad-based general resistance compared with those for a narrow-based specific resistance of BPL 818 and ILB 1814. Based on their interactions on BPL 818 and ILB 1814, the eight isolates of *A. fabae* were classified into four groups representing races 1, 2, 3, and 4.

Blight, caused by *Ascochyta fabae* Speg., is one of the most widely distributed diseases of faba beans (*Vicia faba* L.) throughout Northern Europe, England, Canada, the Middle East, North Africa, China, and the USSR (1,6,8,20). The disease could inflict significant losses in faba bean production, particularly under wet and cool weather conditions (5,6,13,17).

Although the use of chemicals (10,12) and certified seeds (9) may provide partial crop protection, effective disease control can be achieved only if host resistance is used as an essential component in the disease management strategy. However, attempts in the past to identify useful sources of resistance to *A. fabae* resulted in the detection of few genes, which were not effective enough to develop acceptable disease-resistant cultivars (2,16,19).

Large germ plasm collections could vary considerably for both specific and general disease resistance (7,14,18). Therefore, the differentiation between these types of resistance requires specialized techniques to suppress specific resistance while favoring the expression of general resistance (7,14). Inoculum consisting of wide mixtures of isolates (15) or virulent races (3) favors the detection of general resistance. However, Parlevliet (14) suggested using a single race with the broadest possible range in virulence to avoid confounding specific with general resistance. Vanderplank (18) reported that the presence or absence of specific resistance can be determined by differential interactions between genotypes of the host and genotypes of the pathogen.

Different isolates of *A. fabae* differ appreciably in their virulence and cultural characteristics (4,11). No reports have been published on physiological specialization in the pathogen.

The objectives of this study were to: 1) identify useful sources of resistance to *A. fabae*, 2) examine if races exist in the pathogen, and 3) differentiate faba bean lines with specific resistance from those with general resistance to *A. fabae*. The work was done in Syria at the International Center for Agricultural Research in the Dry Areas (ICARDA), and also in England, Canada, Poland, France, and Tunisia with the help of ICARDA's collaborators.

MATERIALS AND METHODS

Local evaluations. Germ plasm lines were evaluated in 1980 and 1981 in the field at ICARDA's substation near Lattakia in northwestern Syria. In October of each year, 20 seeds of each faba bean line were planted in rows 2 m long and 50 cm apart, with the *A. fabae*-susceptible faba bean cultivar Giza 4 grown as a check every five test entries.

A mixture of 25 isolates of *A. fabae* was prepared from a wide range of naturally infected faba bean seeds of susceptible local cultivars from major production regions in Syria. Seeds were surface-disinfected with a 10% sodium hypochlorite solution for 3 min, plated in petri dishes on FDA medium (extracts of 200 g of faba bean seeds, 20 g of dextrose, and 18 g of agar), incubated at 20°C for 6 days, and subcultured until pure colonies of *A. fabae* were obtained. These colonies were exposed to seven cycles of 12 hr darkness and 12 hr light from a 40W fluorescent tube to induce sporulation (17) and were incubated at 20 ± 2°C. After 14 days, the contents of 100 petri dishes per isolate were submerged separately in 10 L of tap water inside plastic containers

for 2 hr. The containers were shaken vigorously for 30 min, the contents filtered through two layers of cheesecloth, and leachates diluted with tap water until 4×10^5 pycnidiospores of *A. fabae* per milliliter were obtained. Equal amounts of spores of each isolate of *A. fabae* were mixed. The mixed inoculum (25 ml/plant) was applied at 1800 hr to the foliage of 10-wk-old plants with a knapsack sprayer. After inoculation, plants were sprinkled with a fine mist of water three times daily (800, 1200, and 1600 hr) until susceptible lines developed severe blight symptoms (3 wk after inoculation).

Sources of resistance were identified using a two-cycle screening technique (7). In the first cycle (1980), 672 germ plasm lines were inoculated with the original broad mixture of 25 isolates of *A. fabae*. Resistant lines developed a few scattered sporulating lesions that may have been induced by highly virulent forms of *A. fabae* (4,11). To evaluate resistant materials more rigorously, 25 isolates of *A. fabae* obtained precisely from these lesions were combined and used in the second screening cycle in 1981. Progenies of the resistant lines in 1980 were inoculated in the second cycle (1981) employing the same procedures.

The 25 isolates used for inoculum of the first screening cycle (IA) were designated IA₁ to IA₂₅ and the 25 isolates used for inoculum of the second screening cycle (IB) were designated IB₁ to IB₂₅.

International evaluation. Based on evaluation in Syria, nine faba bean lines (Table 1) were included in the Faba Bean International Ascochyta Blight Nursery (FBIABN) for multilocation testing in different geographical regions where blight caused by *A. fabae* has long been known to be endemic. Line numbers 1-7 were resistant to inocula IA and IB, line 8 was resistant to IA and susceptible to IB, and line 9 was susceptible to both IA and IB (Table 1). These lines were evaluated with the help of ICARDA's collaborators at the Plant Breeding Institute, Cambridge, England; The University of Manitoba, Manitoba, Canada; The Agricultural Research Center, Strzelce, Poland; the National Agricultural Research Institute, Le Rheu, France; and the National Agricultural Research Institute, Tunis, Tunisia. In each country, seeds were planted in rows 1 m long and 30 cm apart with two replicates. Local faba bean standard checks were repeated every four test entries.

Disease reactions of different faba

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bean lines, in local and international evaluations, were recorded 3 wk after inoculation using ICARDA's 1-9 disease scoring scale (Table 1).

Host-pathogen interactions. Isolation chambers were designed to conduct two tests on host-pathogen interactions in the field. Each chamber had a cylindrical metal frame (40 cm diameter × 90 cm high), a polyethylene jacket, and was covered at the top with an inverted aluminum pan.

The first test compared the disease reaction of the faba bean lines BPL 471 and ILB 1814 inoculated separately with isolates of inocula IA or IB. Seeds of both lines were sown in 22-cm plastic pots and were placed in the field. After 10 wk, pots were placed in isolation chambers in the shade and plants were inoculated separately with different isolates of *A. fabae* using the same procedures described for field inoculation. Each chamber contained one pot with five plants. After 14 hr of incubation, plants were sprinkled with water three times a day until severe blight symptoms developed on ILB 1814 (15 days after inoculation).

The second test was carried out to check the presence or absence of differential interactions between isolates of *A. fabae* and lines of faba beans. Eight isolates (IA₅, IA₉, IA₁₃, IA₂, IB₃, IB₂, IB₂₅, and IB₄) representing the widest possible range of virulence in the mixed inocula IA and IB in the first test were used to inoculate five faba bean lines, BPL 818 and ILB 1814 (resistant at some

but susceptible at other locations), BPL 471 and 2485 (resistant at all locations), and Giza 4 (susceptible at all locations). Plants of these lines were grown in plastic pots placed in isolation chambers and were inoculated separately with the eight isolates mentioned earlier, using the same procedures described in the first test.

This second study was done twice with treatments replicated four times in a split-plot design. Faba bean lines were placed in the main plot and isolates of *A. fabae* in the subplot. Disease readings were made 15 days after inoculation in both tests.

RESULTS

Local evaluations. Most of the faba bean germ plasm lines evaluated for resistance to *A. fabae* were susceptible. Of the 672 lines evaluated in the first cycle, 19 were resistant and 653 were susceptible. Of the 19 lines rated as resistant in the first cycle, seven lines (BPL 471, 460, 646, 74, 2485, 472, and 818) remained resistant in the second cycle (Table 1). Many large lesions with abundant sporulation were produced in both cycles on plants of the highly susceptible local check Giza 4, indicating a consistent disease reaction throughout these tests.

International evaluations. Results from evaluations in Syria were in general agreement with those obtained from the FBIABN (Table 1). Of the nine lines tested 13 times in six distant countries between 1980 and 1985, lines BPL 471, 460, 646, 74, and 2485, with resistance to

both inocula IA and IB in Syria, were consistently rated as resistant at all locations, whereas ILB 1814, with resistance only to inoculum IA in Syria, was rated as resistant at some, but not all, locations in the FBIABN. Although BPL 472 and 818 exhibited resistant reactions to inocula IA and IB in Syria, and were also rated as resistant in England, Canada, Poland, and Tunisia, they were rated as susceptible in France. However, Giza 4, which was rated as susceptible to inocula IA and IB in Syria, was consistently rated as susceptible at all locations.

Host-pathogen interactions. The first test on host-pathogen interactions indicated the presence of a greater percentage of pathogenic isolates (Fig. 1) and a narrower range of virulence (Table 2) in inoculum IB compared with inoculum IA. These differences varied from one line to another. On BPL 471, only 4% of the isolates of inoculum IA induced susceptible reactions compared with 8% of those of inoculum IB. However, on ILB 1814, 20% of the isolates of inoculum IA induced susceptible reactions compared with 84% of those of inoculum IB.

The decrease in the percentage of pathogenic isolates of inoculum IB from 84% on ILB 1814 to only 8% on BPL 471 suggested that BPL 471 has a broader range of resistance to isolates of *A. fabae* compared with ILB 1814 (Fig. 1). Similar, but less dramatic, results were obtained with isolates of inoculum IA.

Results from the second test on host-

Table 1. Faba bean (*Vicia faba*) reactions to *Ascochyta fabae* at different locations

Line no.	Accession	Pedigree	Origin	Disease reaction ^y													
				Syria ^w					England			Canada ^x		Poland	France	Tunisia	
				1980	1981	1983	1984	1985	1983	1984	1985	1983	1984	1985	1985	1985	1985
1	BPL 471	Sel. 80 Lat. 14434-2	Lebanon	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2	BPL 460	Sel. 80 Lat. 14422-2	Lebanon	R	R	R	R	R	R	R	R	R	R	R	R	R	R
3	BPL 646	Sel. 80 Lat. 14998-2	England	R	R	R	R	R	R	R	R	R	R	R	R	R	R
4	BPL 74	Sel. 80 Lat. 70015	Iraq	R	R	R	R	R	R	R	NT	R	R	NT	NT	NT	NT
5	BPL 2485	Sel. 81 Lat. 10026	Spain	NT ^y	R	R	R	R	R	R	NT	R	R	NT	NT	NT	NT
6	BPL 472	Sel. 80 Lat. 14435-3	Lebanon	R	R	R	R	R	R	R	R	R	R	R	S	R	R
7	BPL 818	Sel. 80 Lat. 15035	Ethiopia	R	R	R	R	R	R	R	R	R	R	R	R	S	R
8	ILB 1814	Syrian local ^z	Syria	R	S	S	S	S	R	R	R	R	R	R	S	S	S
9	Giza 4	Egyptian local ^z	Egypt	S	S	S	S	S	S	S	S	S	S	S	NT	NT	NT
10	Hylon	England local ^z	England	NT	NT	NT	NT	NT	S	S	S	NT	NT	NT	NT	NT	NT
11	Erfordia	Canadian local ^z	Canada	NT	NT	NT	NT	NT	NT	NT	NT	S	S	NT	NT	NT	NT
12	Jasny-II	Polish local ^z	Poland	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	S	NT	NT	NT
13	48-B	French local ^z	France	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	S	NT	NT
14	TL-S	Tunisian local ^z	Tunisia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	S

^v Resistance (R) denotes 1, 3, or 5 and susceptibility (S) denotes 7 or 9 on a disease rating scale of 1-9, where 1 = no lesions or very small nonsporulating flecks covering up to 1% of leaf surface, with little or no stem bronzing; 3 = few small localized nonsporulating lesions covering 1.1-5% of leaf surface, with moderate stem bronzing; 5 = lesions common covering 5.1-25% of leaf surface, with poor sporulation and high level of stem bronzing; 7 = lesions coalesced covering 25.1-50% of leaf surface, sunken on stems and pods with intermediate sporulation and some defoliation; and 9 = lesions large and coalesced covering more than 50% of leaf surface, deeply sunken on stems and pods with abundant sporulation, extensive defoliation, stem girdling, and death of most plants.

^w Field evaluations in Syria in 1980 were made with inoculum IA, composed of a wide range of isolates from susceptible local faba bean landraces, whereas evaluations in 1981, 1983, 1984, and 1985 were made with inoculum IB which consisted of a mixture of selected virulent isolates from resistant lines only.

^x Evaluations in Canada were made with two different isolates, A and Y, from Manitoba.

^y NT = not tested.

^z Local faba bean susceptible cultivars in respective countries.

pathogen interactions are presented in Table 3. The significant ($P = 0.01$) differential interaction between isolates

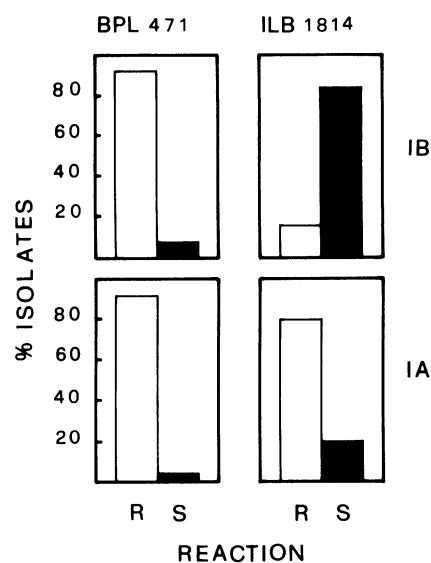


Fig. 1. The percentage of pathogenic (black columns) and nonpathogenic (blank columns) isolates in the mixed inocula IA and IB of *Ascochyta fabae* on the faba bean lines BPL 471 and ILB 1814 (R and S denote resistant and susceptible disease reactions, respectively, as shown under Table 1).

Table 2. Pathogenic differences between inoculum of the first screening cycle (IA) and inoculum of the second screening cycle (IB) of *Ascochyta fabae* on the faba bean lines BPL 471 and ILB 1814 in isolation chambers

Host-pathogen combinations	Disease severity (% necrosis) ^x				Pathogenic isolates ^z (%)
	Mean ^y	SD	Range about mean ($P = 0.01$)	Variance	
BPL 471-IA	11.36 a	7.62	9.36-13.35	58.09	4.0
BPL 471-IB	16.00 b	5.41	15.58-17.41	29.30	8.0
ILB 1814-IA	29.55 c	16.68	25.19-33.91	278.33	20.0
ILB 1814-IB	47.25 d	10.26	44.57-49.93	105.24	84.0

^x0 = No necrosis and 100 = 100% of leaf tissue necrotic.

^yPairs of means for each line followed by different letters are significantly different at $P = 0.01$ ($t = 4.9$ for BPL 471 and 9.03 for ILB 1814).

^zInducing any of the susceptible type reactions, 7 or 9, where 7 = lesions coalesced covering 25.1-50% of leaf surface, sunken on stems and pods with intermediate sporulation and some defoliation; and 9 = lesions large and coalesced covering more than 50% of leaf surface, deeply sunken on stems and pods with abundant sporulation, extensive defoliation, stem girdling, and death of most plants.

Table 3. *Ascochyta* blight reactions of five faba bean lines to eight isolates of *Ascochyta fabae* from Syria (in isolation chambers in the field)

Line	Accession	Disease reaction ^z							
		Race 1			Race 2		Race 3	Race 4	
		IA ₅	IA ₉	IA ₁₃	IA ₂	IB ₃	IB ₂	IB ₂₅	IB ₄
7	BPL 818	R	R	R	R	R	S	S	S
8	ILB 1814	R	R	R	S	S	R	S	S
1	BPL 471	R	R	R	R	R	R	R	R
5	BPL 2485	R	R	R	R	R	R	R	R
6	Giza 4	S	S	S	S	S	S	S	S

^zResistance (R) denotes 1, 3, or 5 and susceptibility (S) denotes 7 or 9 on a disease rating scale of 1-9, where 1 = no lesions or very small nonsporulating flecks covering up to 1% of leaf surface, with little or no stem bronzing; 3 = few small localized nonsporulating lesions covering 1.1-5% of leaf surface, with moderate stem bronzing; 5 = lesions common covering 5.1-25% of leaf surface, with poor sporulation and high level of stem bronzing; 7 = lesions coalesced covering 25.1-50% of leaf surface, sunken on stems and pods with intermediate sporulation and some defoliation; and 9 = lesions large and coalesced covering more than 50% of leaf surface, deeply sunken on stems and pods with abundant sporulation, extensive defoliation, stem girdling, and death of most plants.

of *A. fabae* and lines of faba bean demonstrated physiological specialization in the pathogen. Based on their pathogenicity on five faba bean lines, the eight isolates of *A. fabae* were separated into four distinct races. The pathogenicity varied from one race to another depending on faba bean differentials. Isolates IA₅, IA₉, and IA₁₃, which induced susceptible reactions only on Giza 4, are designated race 1. Isolates IA₂ and IB₃, which were pathogenic on ILB 1814 and Giza 4, are designated race 2. The IB₂ isolate, which induced a susceptible reaction on BPL 818 and Giza 4, is designated race 3. Isolates IB₂₅ and IB₄, which were pathogenic on BPL 818, ILB 1814, and Giza 4, are designated race 4. Although all of the eight isolates included in this test induced susceptible reactions on Giza 4, none of them was pathogenic on BPL 471 or BPL 2485.

DISCUSSION

This study demonstrated the presence of considerable heterogeneity in *V. faba* for resistance to *A. fabae*, and further confirmed the great pathogenic variability of the pathogen reported by other workers (4,11). This variability should be considered in breeding for disease resistance, inheritance of resistance,

disease management, and other aspects of blight on faba bean.

Most plant breeders and pathologists use narrow-based specific resistance with very little attention paid to broad-based general resistance (3). Because specific resistance has frequently been eroded by the evolution of virulent forms (3,15,18), the identification and use of broad-based resistance should constitute the backbone of international disease-resistance breeding programs.

The application of the two-cycle screening technique in Syria demonstrated that the use of different types of mixed inocula favored the detection of different types of resistance. This is true because the use of inoculum IA, with a wide range of virulence and low percentage of pathogenic isolates, confounded broad and narrow types of resistance, whereas the use of inoculum IB, with a narrow range of virulence and high percentage of pathogenic isolates, suppressed narrow-based resistance while favoring the expression of broad-based resistance to the pathogen.

Of the 19 lines rated as resistant in the first cycle, only seven lines remained resistant and 12 became susceptible in the second cycle. The seven lines in the first group were believed to have a broader-based resistance compared with the 12 lines in the second group.

In these evaluations, faba bean lines were inoculated with a mixture of equal amounts of spores of inoculum IA in the first cycle and IB in the second cycle. Therefore, the change in the disease reaction of the 12 lines, from resistant in the first cycle to susceptible in the second cycle, was apparently due to the presence of a greater percentage of some highly virulent forms in inoculum IB compared with inoculum IA and to variations in the range of resistance of the 12 lines as compared with the seven faba bean lines.

The first test on host-pathogen interactions demonstrated the presence of qualitative pathogenic differences between isolates of inoculum IA compared with those of inoculum IB (susceptible reactions can only be induced as a result of compatible host-pathogen interactions). On ILB 1814, 20% of the isolates of inoculum IA induced susceptible reactions compared with 84% of those of inoculum IB, whereas on BPL 471, only 4% of the isolates of inoculum IA were pathogenic compared with 8% of those of inoculum IB. Therefore, it should be reasonable to conclude that the occurrence of a greater level of compatible host-pathogen interactions with isolates of inoculum IB compared with those of inoculum IA was responsible for the large difference in the disease reaction, which changed the host status of the 12 lines from resistant in the first cycle to susceptible in the second cycle. However, pathogenic differences between inocula IA and IB were apparently insufficient to

change the disease reaction of the remaining seven lines that were rated as resistant in both the first and second screening cycles.

Because ILB 1814 was exposed at the same time as BPL 471 to isolates of *A. fabae* in isolation chambers, yet exhibited susceptible reactions to a broader range of those isolates, most of the isolates that were pathogenic on ILB 1814 apparently failed to induce susceptible reactions on BPL 471. The decrease in the percentage of the pathogenic isolates of inoculum IB from 84% on ILB 1814 to only 8% on BPL 471 clearly indicates that lines such as BPL 471 have a broader range of resistance to isolates of *A. faba* compared with lines such as ILB 1814. Therefore, the seven lines in the first group seemed to have a broader range of resistance compared with the 12 lines of the second group. These results agree with those reported by other workers who showed that the use of mixtures with a wide range of virulence tend to confound vertical with horizontal resistance (14), and that the use of virulent pathogen races for screening the most resistant host material helps the detection of new sources of horizontal resistance (3,7).

Findings from local evaluations in Syria were in close agreement with those obtained from the FBIABN. Lines BPL 471, 460, 646, 74, and 2485, with resistance to inocula IA and IB in Syria, were consistently rated as resistant at all locations, whereas ILB 1814, with resistance only to inocula IA, revealed a resistant reaction at some, but not all, locations.

In naturally occurring populations of *A. fabae*, great pathogenic variabilities may exist (4,11). The second test on host-pathogen interactions demonstrated the presence of at least four races of the pathogen in Syria. On a susceptible line such as Giza 4, the great majority of spores of these races induce susceptible reactions under suitable conditions, whereas on a line such as ILB 1814 with an intermediate range of resistance, a moderate number of spores of certain races could cause susceptible reactions. However, on lines such as BPL 471, with a broad range of resistance, very few, if any, of the spores of *A. faba* could cause lesions.

The exposure of five representative lines to four races of *A. fabae* further demonstrated the presence of broad and narrow types of resistance to *A. fabae*. BPL 471 and 2485, with resistance to inocula IA and IB in Syria, and rated as resistant at all locations in the FBIABN, were consistently rated as resistant to all of the four races with no significant ($P = 0.01$) differential interactions.

Although BPL 818 was rated as resistant to inocula IA and IB in Syria, it revealed a susceptible reaction in France

and also exhibited significant differential interactions with some races of *A. fabae* in Syria. This was probably due to the wide prevalence of some pathogenic races in France that were apparently present at very low frequencies in local populations in Syria or other locations where BPL 818 was also rated as resistant. However, ILB 1814, with resistance only to inoculum IA, revealed a resistant reaction at some, but not all, locations in the FBIABN and consistently exhibited significant ($P = 0.01$) differential interactions with different races of *A. fabae* in Syria.

Although the experiments on host-pathogen interactions reconfirmed the existence of great pathogenic variabilities in *A. fabae* (4,11) and established the presence of at least four races of the pathogen in Syria, the application of the two-cycle screening technique and international evaluations resulted in the detection of lines with broad-based resistance, despite the presence of those with narrow-based resistance to the pathogen.

The disease reaction of certain lines in the FBIABN revealed important information about the geographical distribution of different races of the pathogen. The susceptible reaction of BPL 818 in France and its resistance at all other locations suggested that races 3 and 4 are probably more common in France compared with other locations. Similarly, the susceptible reaction of ILB 1814 in France and Tunisia suggested that races 2 and 4 are probably more common in these countries compared with other locations.

The faba bean lines BPL 471, 818, ILB 1814, and Giza 4 can be used as a host differential set to check the presence or absence of races 1, 2, 3, and 4 of *A. fabae* in major faba bean production regions. However, an in-depth race survey is needed within each region to determine what genes for resistance are more effective in what regions.

Our survey in the coastal area in Syria indicated that races 2, 3, and 4 are apparently more common than race 1. Therefore, genes for resistance from BPL 471, 460, 74, and 2485 are being exploited in ICARDA's breeding program to develop faba bean blight-resistant cultivars for that area.

Although this study resulted in the identification of some faba bean lines with broad-based resistance to *A. fabae*, these sources may not have the absolute horizontal resistance to all of the virulent forms that may occur at locations other than those mentioned in this report. Therefore, efforts should continue to test these lines in the FBIABN at as many new locations as possible to determine their true range of resistance to other possible virulent forms not included in

this work.

This is the first report on the detection of useful sources of faba bean with resistance to *A. fabae*. These sources have been provided by ICARDA to several national programs and institutions in the Middle East, North Africa, Europe, and Canada to help stabilize faba bean production in areas where *A. fabae* has long been known to be serious.

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