CROP PROTECTION

Antibiosis Effects of Wild Bean Lines Containing Arcelin on *Bemisia tabaci* (Genn.) Biotype B (Homoptera: Aleyrodidae)

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An. Soc. Entomol. Brasil 29(3): 573-582 (2000)

Efeitos Antibióticos de Genótipos de Feijoeiros Portadores de Arcelina nas Sementes sobre *Bemisia tabaci* (Genn.) Biótipo B (Homoptera: Aleyrodidae)

RESUMO – A biologia de *Bemisia tabaci* biótipo B (Genn.) foi avaliada em genótipos de feijoeiro (*Phaseolus vulgaris* L.) que contêm arcelina em suas sementes. Foi também realizada análise bioquímica de proteínas, em sementes e em folhas dos genótipos de feijoeiro, a fim de verificar se haveria traços de arcelina nas folhas dos materiais a serem avaliados. Os testes foram conduzidos em condições de casa de vegetação, nas épocas "das águas" e "da seca", em dois anos consecutivos, com os seguintes genótipos: ARC 3s, ARC 5s (genótipos selvagens portadores de arcelina); ARC 1, ARC 2, ARC 3, ARC 4 (linhagens quase-isogênicas portadoras de arcelina - EMBRAPA), Porrillo 70, Bolinha e IAPAR MD 808 (genótipos sem arcelina). Os genótipos selvagens, ARC 3s e ARC 5s, apresentaram altos níveis de antibiose, com ênfase para o ARC 5s (as ninfas tiveram alta mortalidade, em torno de 90%). O prolongamento do ciclo de desenvolvimento dos insetos provenientes do genótipo ARC 5s podem sugerir uma resistência do tipo antibiose e/ou não-preferência para alimentação. A resistência dos genótipos selvagens não está relacionada com a presença de arcelina nas sementes, já que nenhum traço dessa proteína foi encontrado nas folhas destes.

PALAVRAS-CHAVE: Insecta, *Phaseolus vulgaris*, mosca branca, biologia, resistência de plantas.

ABSTRACT - The biology of *Bemisia tabaci* biotype B (Genn.) on bean (*Phaseolus vulgaris* L.) genotypes containing arcelin in the seeds was evaluated. Also, biochemical screening of seeds and leaves material of these genotypes were carried out in order to verify if traces of arcelin could be found in its leaves. The tests were conducted under greenhouse conditions, in the dry and wet seasons, with the following genotypes: ARC 3s, ARC 5s (wild genotypes containing arcelin in the seeds); ARC 1, ARC 2, ARC 3, ARC 4 (near isogenic lines containing arcelin in the seeds - EMBRAPA) and Porrillo 70, Bolinha, IAPAR MD 808 (commercial genotypes without arcelin). The wild genotypes,

ARC 3s and ARC 5s, showed high levels of antibiosis resistance type, mainly for ARC 5s which presented the highest nymphs mortality rates, approximately 90%. Also, the longest development time for nymphs fed on ARC 5s genotype suggest antibiosis and/or feeding nonpreference resistance type. The wild genotype resistance is not related with arcelin presence in the seeds, since no trace of this protein was found in its leaves.

KEY WORDS: Insecta, *Phaseolus vulgaris*, whitefly, biology, host plant resistance.

The sweetpotato whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), is supposed to be one of the most harmful pests that attacks bean crops, which are primary sources of protein for many people in the Tropics. Until recently, whitefly populations in Brazil comprised the biotype A, occasionally reported infesting soybean, bean and cotton crops, in some localities of São Paulo and Paraná States (Costa *et al.* 1973). These insects damage the plants by extracting large quantities of phloem sap and transmitting the bean golden mosaic virus. The disease can induce bean yield losses ranging from 40% to 100% (Faria & Zimmermann 1987, Faria *et al.* 1994), depending on the time plants are infected. Recently, a new biotype, *B. tabaci* biotype B, also referred to as *B. argentifolli* (Bellows & Perring) has been associated with high yield losses in several crops. In Brazil, the biotype B was first reported in 1991, infesting crops such as: tomato, broccoli, eggplant, and pumpkin crops, as well as ornamental plants, and some weeds and other wild plants (Lourenção & Nagai 1994). During late 90s, the whitefly population has exploded to outbreak levels and the biotype B has almost completely displaced biotype A. This displacement is associated with the wider host range of B biotype, allowing it to colonize a larger number of agricultural, ornamental, weedy and wild plants (Bedford *et al.* 1994). Besides, biotype B lays significantly higher number of eggs (Bethke *et al.* 1991, Costa & Brown 1991), ingests greater quantities of plant sap during feeding and, consequently, excretes greater volumes of honeydew (Byrne & Miller 1990). Furthermore, biotype B induces phytotoxic disorders, such as: squash silverleaf on cultivars of Cucurbitaceae (Costa & Brown 1991, Costa *et al.* 1993, Jiménez *et al*. 1995), uneven ripening on tomato (Schuster *et al.* 1990) and white stem streaking on cole crops (Brown *et al.* 1991). Although polyphagous, *B. tabaci* shows great variation in developmental rates on different host plants (Coudriet *et al.* 1985), at different temperatures (Butler *et al.* 1983, Wang & Tsai 1996), and on leaves at different ages (Arx *et al.* 1983). Therefore, the aim of this study was to determine the effects of bean genotypes containing arcelin in the seeds on *B. tabaci* biotype B, in the dry and wet seasons. Also, biochemical screening of seeds and leaves material were carried out in order to verify if traces of arcelin could be found in its leaves.

Material and Methods

All trials were carried out in the Laboratories of Host Plant Resistance to Insects, Department of Entomology, and in an experimental area of Faculdade de Ciências Agrárias e Veterinárias/Universidade Estadual Paulista. The following genotypes of *Phaseolus vulgaris* L. were used: ARC 3s and ARC 5s (wild genotypes containing arcelin 3 and 5, respectively); ARC 1, ARC 2, ARC 3 and ARC 4 (genotypes bred by EMBRAPA containing arcelin 1, 2, 3 and 4, respectively); Porrillo 70, IAPAR MD 808 and Bolinha. The genotypes containing arcelin were obtained from the Centro Nacional de Pesquisa do Arroz e do Feijão/EMBRAPA. The seeds were grown

in plastic pots containing three parts of soil, one part of sand and one part of organic compound. The plants were watered daily and fertilized as recommended for the crop. When tests were set up, adults of *B. tabaci* biotype B, reared on broccoli, were collected by mouth vacuum apparatus. The infestation took place when bean plants were 21 day-old, characterized by Azael (1976) as stages IV-2 and/or IV-3. All experiments were carried out under greenhouse conditions with 10 replications, in the wet and dry seasons. The trials were set up in a completely randomized design. Data were submitted to ANOVA test and the means were compared by Tukey test ($P \leq 0.05$). When necessary, the original data were transformed to arcsen $x^{1/2}$.

Feeding Nonpreference and/or Antibiosis. One-day-old whitefly's eggs were obtained after infestation of two bean plants with 200 insects during 24 hours. After this period, the number of egg was counted on the four top leaves with the aid of a hand magnifying glass (20x). Each leaf was labeled with the number of eggs, being 10 the minimum number per leaf. The number of nymphs, fourth nymphal instar (referred as pupa), and empty pupal cases were counted daily, until the emergence of all adults.

Since the data reported in the wet and dry seasons were slightly contradictory, another trial was set up in another wet season, to confirm the resistance index of bean genotypes previously tested. It was selected two resistant (ARC 3s and ARC 5s) and one susceptible genotype (Porrillo 70).

Biochemical Screening of Seeds and Leaves Material. These analyses took place in the Department of Biology, University of Durham, England, in order to verify if the presence of arcelin in bean seeds was affecting the whitefly feeding. Electrophoresis and Western blotting analyses of total seed and leaf proteins were carried out for bean genotypes. Seed and leaf samples were run on SDS-polyacrilamide gel electrophoresis (12.5% acrylamide, 0.5% bis-acrylamide, containing 0.1% SDS - sodium dodecyl sulphate), as described by Hames (1981), and electrophoresed in a vertical gel electrophoresis apparatus (ATTO, Genetic Research Instruments Ltd, Dunmow, Essex, England). Afterwards, the proteins were transferred from SDS-polyacrilamide gels to nitrocellulose by electroblotting (ATTO Corp Semi-dry Electroblotter) (Towbin *et al.* 1979) and then reacted with polyclonal LLP antibodies raised in mice (Western blotting).

Results and Discussion

Biochemical Screening of Seeds and Leaves Material. Western blotting positive antigenic response was observed for bean seeds of ARC 1, ARC 3, ARC 4, ARC 5s (very weak band) and for arcelin patterns (G12953 and LLP1), as shown in Fig. 1. Regarding the other genotypes, although the referred protein is part of its genotypic constitution (exception for Porrillo 70), positive response was not visualized, probably due an arcelin antibody nonespecificity. This can be explained by the existence of five different arcelin variants. If more specific antibodies were used, perhaps this reaction could be visualized.

There was no arcelin band detected in bean leaves for all tested genotypes by Western blotting (Fig. 2). When seed extracts were electroblotted (Fig. 1), the genotypes ARC 1, ARC 3, ARC 4 and ARC 5s had presented specificity for the antibody used. In this way, it was also expected positive antigenic reaction for those genotypes if traces of arcelin were present in its leaves. This suggests that there are no arcelin traces in bean leaves of all genotypes analysed.

Feeding Nonpreference and/or Antibiosis. Data on the time required for *B. tabaci* to complete the development from egg to adult on nine bean genotypes in the wet season is presented in Table 1. The egg incubation period varied from 7.5 to 8.2 days and differences among all genotypes were not observed. The nymphal development period was significantly longer on ARC 2 (15.4 days) and ARC

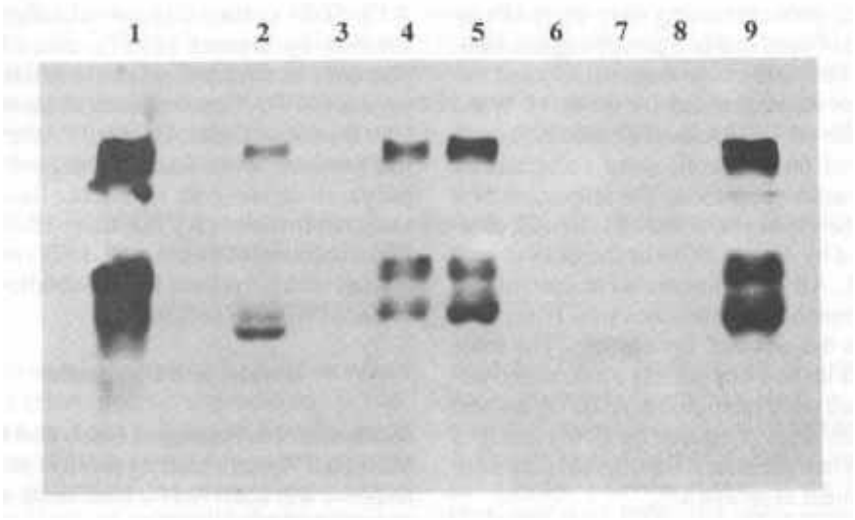

Figure 1. Western blotting analysis of bean seeds samples run on SDS-PAGE after transfer to nitrocellulose, reacted with polyclonal mouse anti-LLP antibodies. Track: 1) LLP1 (purified arcelin pattern); 2) ARC 1; 3) ARC 2; 4) ARC 3; 5) ARC 4; 6) ARC 3s; 7) ARC 5s; 8) Porrillo 70; 9) G12953 (arcelin pattern). Durham, England, 1996.

1 (15 days) than IAPAR MD 808, Porrillo 70, ARC 3 and ARC 3s (values varying from 11,0 to 11.5 days). Consequently, the total developmental time was significantly longer for nymphs reared on ARC 2 and ARC 1 (23.1 and 22.6 days, respectively) and faster on

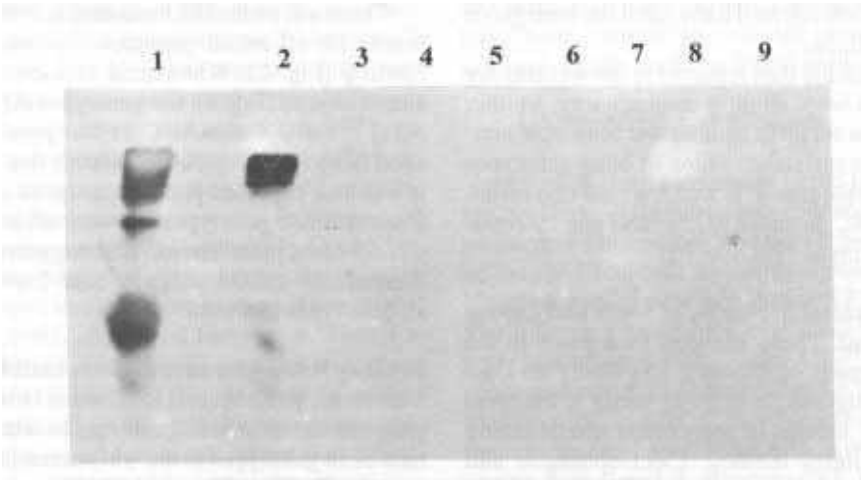

Figure 2. Western blotting analysis of bean leaves samples run on SDS-PAGE after transfer to nitrocellulose, reacted with polyclonal mouse anti-LLP antibodies. Track: 1) LLP1 (purified arcelin pattern); 2) G12953 (arcelin pattern); 3) ARC 1; 4) ARC 2; 5) ARC 3; 6) ARC 4; 7) ARC 3s; 8) ARC 5s; 9) Porrillo 70. Durham, England, 1996.

Table 1. Mean developmental periods ± SE (days) of immature stages of *B. tabaci* biotype B on nine bean (*P. vulgaris*) genotypes, in a wet season. Jaboticabal, SP, 1996/97.

Genotypes	(*n*)	Egg stage	Nymphal stage	Life cycle
		(days)		
ARC 2	530	7.7 ± 0.15	15.4 ± 0.81a	23.1 ± 0.86a
ARC 1	732	7.5 ± 0.10	15.0 ± 0.85ab	22.6 ± 0.84ab
ARC 4	546	7.7 ± 0.12	13.4 ± 0.38abc	21.0 ± 0.36abc
Bolinha	1.358	7.9 ± 0.24	13.0 ± 0.41abc	20.9 ± 0.37 bc
ARC 5s	530	7.5 ± 0.11	12.5 ± 0.65 bc	20.1 ± 0.62 c
ARC 3s	646	7.7 ± 0.17	11.5 ± 0.80 c	19.1 ± 0.77 c
ARC 3	457	7.8 ± 0.19	11.5 ± 0.71 c	19.3 ± 0.73 c
Porrillo 70	819	7.7 ± 0.23	11.4 ± 0.37 c	19.1 ± 0.40 c
IAPAR MD 808	548	8.2 ± 0.33	11.0 ± 0.46 c	19.2 ± 0.33 c
F		1.39^{NS}	7.78*	7.26*
CV %		7.36	14.45	8.75

Original data; means followed by the same letters within columns are not significantly different by Tukey's test ($P \leq 0.05$).

Porrillo 70 and ARC 3s (19.1 days).

In the dry season, there was also no significantly difference on egg incubation period which was approximately 13 days for all genotypes tested (Table 2), and were almost two-fold longer when compared with wet season (approximately eight days, Table 1). These longer periods must be due to the low temperature and humidity conditions when the trials were set up. According to Azab *et al.* 1971, El-Helaly *et al.* 1971, Hendi *et al.* 1985, the egg incubation period can vary between three and 29 days, depending on the whether conditions.

Insects reared on ARC 5s (27.4 days) showed significantly longer nymphal development period than those reared on IAPAR MD 808, ARC 1, ARC 4, ARC 3, Porrillo 70 and ARC 3s (values varying from 19.6 to 21 days), which had faster cycles. The nymphal development periods of insects reared on Bolinha and ARC 2 genotypes (23 and 22 days, respectively) did not differ significantly to those observed on ARC 5s. The maximum developmental time was 41 days for insects fed on ARC 5s genotype, statistically differing from all tested genotypes. Insects developed on Bolinha genotype showed an identical developmental cycle (36.4 days) as that reported by Boiça Júnior & Vendramim (1986), who suggested the existence of antibiosis resistance type for the referred genotype. Therefore, the ARC 5s genotype also affected the *B. tabaci* developmental time, delaying its cycle in nine days when compared with ARC 1 (Table 2), suggesting thereby, antibiosis and/or feeding nonpreference resistance type.

High egg viability percentage was observed, with values varying from 90.7 ± 3.16 % (ARC 2) to 98.5 ± 2.50 % (ARC 1). These data are in agreement with those observed by Hendi *et al.* (1985), Verma *et al.* (1990), Wagner (1995) and Simmons (1999). Generally, a higher nymphal mortality rate was observed in the first two nymphal stages and lower mortality rates in the third and forth instars, fact also verified by Hendi *et al.*

Table 2. Mean developmental periods ± SE (days) of immature stages of *B. tabaci* biotype B on nine bean (*P. vulgaris*) genotypes, in a dry season. Jaboticabal, SP, 1997.

Genotypes	(*n*)	Egg stage	Nymphal stage	Life cycle
		(days)		
ARC 5s	1,751	13.7 ± 0.38	27.4 ± 1.78a	41.0 ± 1.68a
Bolinha	2,599	13.4 ± 0.40	23.0 ± 1.13ab	36.4 ± 1.26 b
ARC 2	1,987	12.8 ± 0.31	22.0 ± 1.34ab	34.8 ± 0.55 b
ARC 3s	1,618	12.8 ± 0.41	21.0 ± 1.32 b	33.7 ± 1.46 b
Porrillo 70	2,217	12.7 ± 0.40	20.2 ± 0.68 b	32.9 ± 0.54 b
ARC 3	2,765	13.2 ± 0.37	20.0 ± 0.84 b	33.1 ± 0.80 b
ARC 4	1,885	12.7 ± 0.59	19.7 ± 0.91 b	32.5 ± 0.80 b
ARC 1	2,383	12.6 ± 0.34	19.7 ± 0.85 b	32.3 ± 0.78 b
IAPAR MD 808	1,765	13.5 ± 0.31	19.6 ± 1.01 b	33.1 ± 0.94 b
F		2.06^{NS}	6.71*	9.29*
CV %		7.22	14.78	8.71

Original data; means followed by the same letters within columns are not significantly different by Tukey's test ($P \leq 0.05$).

(1985) and Drost *et al.* (1998). The total nymphal mortality percentage varied from 65.2 ± 0.04 % (IAPAR MD 808) to 91.3 ± 0.02 % (ARC 3s), in the wet season and from 47.5 ± 0.06 % (Porrillo 70) to 95.4 ± 0.01 % (ARC 3s), in the dry season (Fig. 3). In both sea-

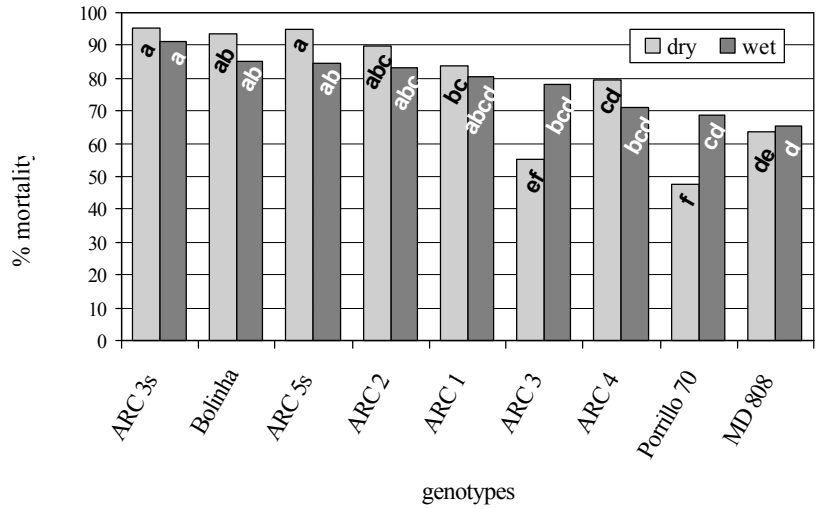

Figure 3. Percentage of *B. tabaci* biotype B nymphs mortality on nine bean genotypes in a dry and wet season. Jaboticabal, 1996/97.

sons, the highest nymphal mortality rates occurred on ARC 3s, Bolinha and ARC 5s genotypes that differed from the ones with the lowest mortality (IAPAR MD 808 - wet season and Porrillo 70 - dry season). The very low percentage of emerged adults from wild genotypes confirms that antibiotic and/or feeding nonpreference factors are acting on whitefly development, increasing though its mortality rates.

Regarding the feeding nonpreference and/or antibiosis test with three selected bean genotypes, the whitefly egg incubation period was approximately 8.5 days (Table 3). The nymphal and total developmental cycle were significantly shorter for insects fed on Porrillo 70 genotype (11.9 and 20.4 days, respectively) and longer on ARC 3s and ARC 5s, which showed similar values for both cycles (around 13.6 and 22.1 days, respectively).

In addition, higher mortality rates were observed for insects reared on wild genotypes,

Table 3. Mean developmental periods ± SE (days) of immature stages of *B. tabaci* biotype B on three bean (*P. vulgaris*) genotypes, in a wet season. Jaboticabal, SP, 1997/98.

Genotypes	(*n*)	Egg stage	Nymphal stage	Life cycle
		(days)		
ARC 5s	1,331	8.4 ± 0.13	13.7 ± 0.39a	22.1 ± 0.48a
ARC 3s	1,288	8.6 ± 0.18	13.6 ± 0.25a	22.1 ± 0.26a
Porrillo 70	1,013	8.5 ± 0.12	11.9 ± 0.26 b	20.4 ± 0.30 b
F		0.29^{NS}	10.47*	7.91*
CV %		2.56	7.38	5.24

Original data; means followed by the same letters within columns are not significantly different by Tukey's test ($P \leq 0.05$).

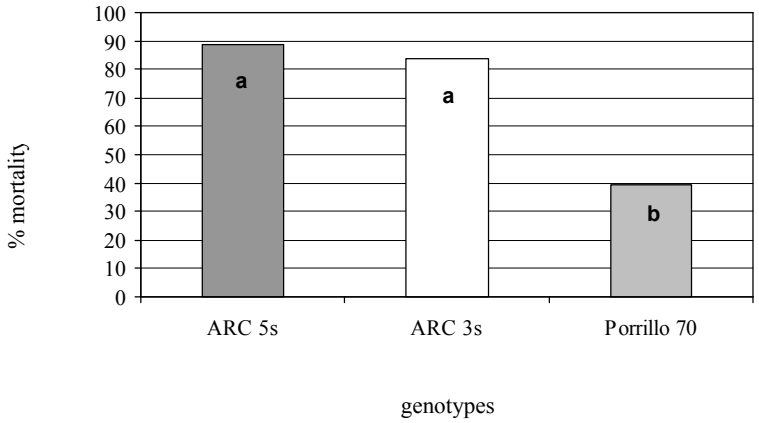

Figure 4. Percentage of *B. tabaci* biotype B nymphs mortality on three bean genotypes in a wet season. Jaboticabal, 1997/98.

ARC 3s (83.7 ± 0.04 %) and ARC 5s (88.8 ± 0.03 %) and significantly smaller on Porrillo 70 (39.2 ± 0.06 %) (Fig. 4). Therefore, all these data confirm that wild genotypes (ARC 3s and ARC 5s) have high levels of antibiosis and/or feeding nonpreference resistance type. Since the mortality was very high, it is likely to be the antibiosis type because the feeding nonpreference usually does not cause such hard effects (Fig. 4). Furthermore, M.A.G. Oriani (unpublished data) also verified that ARC 3s and ARC 5s showed oviposition nonpreference resistance type towards *B. tabaci*. The resistance of wild genotypes is not related with arcelin presence in the seeds, since no trace of this protein was found in its leaves (Fig. 2). Besides, the ARC 1, ARC 3 and ARC 4 genotypes (bred genotypes) also contain arcelin in their seeds, detected in the Western blotting trial (Fig. 1) and have not manifested such resistance. Other resistant factors must be associated to the wild genotypes resistance (ARC 3s and ARC 5s), which had not been transferred to the bred genotypes. In this way, the ARC 5s and ARC 3s should be used for breeding program towards *B. tabaci* control.

Acknowledgments

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a research fellowship; to EMBRAPA, which provided the bean seeds; to Dr. Maria Regina V. de Oliveira, who identified the insect species, and to Dr. Angharad M. R. Gatehouse from University of Durham, who supervised the biochemical analyses of bean seeds and leaves.

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Accepted 15/V/2000.