BEAN ENTOMOLOGY

Activity 1. Developing germplasm with resistance to pests: *Thrips palmi*, leafhopper, pod weevil, and bruchids.

Screening for sources of resistance to major insect pests

Introduction

Identification of sources of resistance to major insect pests of beans is a continuous activity. Additional work is conducted trying to identify and characterize the mechanisms of resistance to specific major pests.

Materials and Methods: *T. palmi*, leafhopper and pod weevil nurseries are planted in the field under high levels of natural infestation, usually with 3-4 replicates per genotype in randomized complete block designs. Evaluations for resistance include damage and bean production ratings, insect counts, damage counts, and in some cases, yield components and yields. Bruchid nurseries are tested in the laboratory simulating normal storage conditions (20^{o} C, 80% R.H., and 14 % seed humidity). Genotypes are tested using 3-5 replications of 50 seeds per genotype. Evaluation units (replicates) are infested with 7 pairs of *Z. subfasciatus* per 50 seeds or two eggs per seed in the case of *A. obtectus*.

Results and Discussion: In 2002 we finished studies on the mechanisms of resistance to *T. palmi*. Main results of this work can be summarized as follows. Antixenosis and antibiosis were studied under laboratory and field conditions. Comparisons were made between a susceptible genotype (APN 18) and four moderately resistant genotypes ('Brunca', BH-130, EMP 486, FEB 115). In multiple-choice tests, antixenosis was identified in FEB 115. The antixenotic nature of FEB 115 was confirmed in dual-choice tests under field and laboratory conditions. Life table studies **(Table 1)** showed significant differences in egg duration, survivorship of adults and immature stages, female longevity, daily oviposition rates, and total fecundity among bean genotypes, meaning that antibiosis does play a role in the resistance of beans to *T. palmi*. Based on the most important demographic parameter, the intrinsic rate of natural increase, the five bean genotypes were divided into two groups: BH-130 and 'Brunca' were less favorable for the population growth of the thrips than EMP 486 and FEB 115. FEB 115 was not antibiotic. Population growth on this genotype did not differ from that on APN 18, the susceptible check.

We also finished studies on tolerance as a mechanism of resistance to *T. palmi*. As shown in **Table 2**, resistant genotypes suffered significantly lower pod and yield losses than susceptible ones. Consistent results from both field and greenhouse tests allowed us to conclude that tolerance is indeed a mechanism of resistance to *T. palmi*. In the particular case of FEB 115, tolerance is combined with antixenosis whereas antibiosis and tolerance seem to be the mechanisms responsible for resistance in Brunca, EMP 486, and BH-130.

Table 1. Demographic parameters for *Thrips palmi* reared on five bean genotypes.

Genotype	N	Net Reproductive Rate (R_0)	Generation Time (Days)	Intrinsic Rate of Natural Increase (r_m)	Doubling Time (Days)
EMP 486	74	32.6 ± 1.9c	37.8 ± 0.1c	0.092 ± 0.001a	7.5 ± 0.12b
BH-130	70	46.1 ± 2.0a	44.8 ± 0.7a	0.085 ± 0.002b	8.1 ± 0.23a
APN 18	60	46.0 ± 1.3a	41.2 ± 1.1b	0.093 ± 0.002a	7.5 ± 0.19b
Brunca	72	29.9 ± 2.7c	40.2 ± 1.6bc	0.084 ± 0.003b	8.2 ± 0.12a
FEB 115	48	39.1 ± 0.8b	39.9 ± 0.7bc	0.092 ± 0.002a	7.5 ± 0.16b

[a] For each parameter, differences among bean genotypes were determined by SNK sequential tests, based on jackknife estimates of variance for each parameter. Means (± SEM) within a column followed by the same letter are not significantly different at the 5% level.

Table 2. Percentage losses caused by *Thrips palmi* on eight bean genotypes.

Genotype	Percentage Empty Pods	Percentage Pod Losses	Percentage Yield Reduction
Brunca (R)	13.7b	21.8ab	38.5bcd
EMP 486 (R)	7.3b	5.0b	33.9bcd
FEB 115 (R)	17.0b	16.9ab	18.8d
BH-5 (I)	14.8b	20.0ab	27.9cd
BH-60 (R)	15.7b	19.9ab	34.0bcd
EMP 514 (S)	10.7b	23.1a	50.0ab
BAT 477 (S)	17.6b	23.8a	46.4abc
APN 18 (S)	50.0a	23.8a	65.9a

[a] R, resistant; I, intermediate; S, susceptible. Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's PLSD).

Work on the identification of molecular markers for thrips resistance was also terminated. For details, please see the SB-2 report.

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Bruchids:

Acanthoscelides obtectus

As indicated in our 2002 Annual Report, there is need to develop fertile *P. vulgaris* x *P. acutifolius* (common x tepary) bean hybrids using the tepary genotype NI576 (a genotype competent to *Agrobacterium*-mediated genetic transformation). Using a novel Double Congruity Backcross technique developed at CIAT, the Biotechnology Unit has been able to produce fertile interspecific hybrids involving NI576. Some of these crosses involve the tepary accession G 40199, an excellent source of resistance to the bean weevil, *Acanthoscelides obtectus*. In 2002 we identified several progenies containing both *P. vulgaris* and *P. acutifolius* cytoplasm with very high levels of antibiosis resistance to *A. obtectus*. Individual pre-selected seeds were reconfirmed in 2003 with excellent results **(Table 3)**. Six of the progenies tested showed an absolute level of antibiosis (zero adult emergence), comparable to that in G 25042, a *P. lunatus* accession known to be one of the most resistant genotypes ever tested for resistance to the bean weevil. Resistance in these progenies compared favorably with resistance in the *P. acutifolius*

resistant parent, G 40199. After multiplication of seeds in the greenhouse, further testing for resistance is in progress.

Table 3. Reconfirmation of resistance to *Acanthoscelides obtectus* in pre-selected segregating progenies derived from interspecific *Phaseolus vulgaris* x *Phaseolus acutifolius* crosses.

Code and Generation	Cross	No. of Seeds Tested[a]	Mean Percentage Emergence	Days to Adult Emergence
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. vulgaris* cytoplasm				
T7 K2 F_3	V-DCBC5 x V-DCBC4	3	0.0	-
Interspecific *P. vulgaris* x *P. acutifolius* hybrids with *P. acutifolius* cytoplasm				
GNVAV-2 F_4	{[(G40022 x N1576) x V5] x A3}x VS42-7	10	0.0	-
GVV-1 F_3	{[(G40022 x N1576) x V5] x A3}x VS42-7	14	0.0	-
GKX-6 F_2	A-DCBC8-2	3	0.0	-
GKA-12 F_2	A-DCBC7-2 x A6	14	0.0	-
ZXX-5 F_2	A-DCBC8-3	2	0.0	-
	Checks			
G 40199	Resistant *P. acutifolius* accession	26	11.2	54.0
G 40168	Susceptible *P. acutifolius* accession	22	90.1	45.7
G 25042	Resistant *P. lunatus* accession	21	0.0	-
G 25410	Susceptible *P. lunatus* accession	20	95.0	43.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	30	95.5	32.8

[a] Pre-selected seeds were tested individually using the vial technique.

We also tested 68 F_2 and F_3 progenies derived from several different interspecifc crosses as well as intraspecific *P. acutifolius* and *P. lunatus* crosses. Seeds were tested in bulk with three repetitions per entry at a level of infestation of 2-3 larvae per seed depending on seed size. Most were susceptible. However, as shown in **Table 4**, five were selected for showing acceptable levels of resistance. Selected individual seeds were multiplied and the resulting seed will be evaluated in bulk in replicated nurseries.

Table 4. Resistance to *Acanthoscelides obtectus* in selected $F_{2,3}$ progenies derived from inter and intraspecific crosses evaluated in 2003.

Code and Generation	Type of Cross	Percentage Adult Emergence	Days to Adult Emergence
GNVA21 F_3	Intraspecific *P. acutifolius* x intraspecific hybrid *P. lunatus* x intraspecific hybrid *P. lunatus*	45.5	40.7
GKA11 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	34.3	53.5
Z99ZX6 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	34.6	58.5
V5 F_2	Intraspecific *P. lunatus* hybrid	0.0	-
VS42-7 F_2	Intraspecific *P. lunatus* hybrid	34.5	67.3
	Checks		
G 40199	Resistant *P. acutifolius* accession	18.6	55.7
G 40168	Susceptible *P. acutifolius* accession	86.4	41.5
G 25042	Resistant *P. lunatus* accession	6.7	69.0
G 25410	Susceptible *P. lunatus* accession	97.8	42.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	91.1	31.4

Another set of 55 F_{2-4} progenies received from the Biotechnology Unit was tested in replicated tests for resistance to *A. obtectus* in 2003. Six double congruent hybrids with *P. acutifolius* cytoplasm and two progenies derived from intraspecific *P. lunatus* crosses showed resistance ranging from high (< 20% adult emergence) to intermediate (20-50% adult emergence) **(Table 5)**. Resistance was also expressed in terms of prolonged life cycles (up to 84 days after infestation). Reconfirmation using the individual seed testing technique is in progress.

Table 5. Resistance to *Acanthoscelides obtectus* in selected segregating progenies derived from interspecific *Phaseolus vulgaris* x *Phaseolus acutifolius* hybrids and intraspecific *Phaseolus lunatus* crosses evaluated in 2003.

Code and Generation	Type of Cross	Percentage Adult Emergence	Days to Adult Emergence
Z99ZX-1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	18.7	74.2
Z99ZX-11A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	34.9	55.7
Z99ZX-15-2 F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	36.6	59.9
ZXTG31-4-10 F_4	Double congruent hybrid with *P. acutifolius* cytoplasm	8.3	65.0
ZXTG33-3 F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	42.1	58.2
GKVGAG-1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	0.8	44.0
A6 F_2	Intraspecific *P. lunatus* hybrid	26.8	64.7
VS42-14 F_2	Intraspecific *P. lunatus* hybrid	5.6	84.0
	Checks		
G 40199	Resistant *P. acutifolius* accession	7.2	68.8
G 40168	Susceptible *P. acutifolius* accession	83.2	43.4
G 25042	Resistant *P. lunatus* accession	0.6	78.0
G 25410	Susceptible *P. lunatus* accession	90.0	43.8
ICA Pijao	Susceptible *P. vulgaris* cultivar	91.1	31.4

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Zabrotes subfasciatus

The work on the development of a molecular marker for arcelin presence and resistance to the Mexican bean weevil was terminated in 2003. In 2002, single and multiple crosses using RAZ 44 and RAZ 105 as arcelin-donor parents were made. Resulting F_5 and F_6 were used to develop microsatellites (details in SB-2 Report). The resulting 854 progenies from 29 different crosses were tested for resistance to *Z. subfasciatus* (results in the 2002 Bean Entomology Report). In 2003, we reconfirmed levels of resistance of the best progenies in replicated nurseries in the laboratory. Results **(Table 6)** confirmed previous results, with 55 of the highly resistant lines showing very high levels of resistance to the Mexican bean weevil. There was absolute correspondence between the presence of arcelin and resistance to the insect.

As part of the on-going collaborative project with the University of Ghent, we tested 11 arc-5 and six arc-1 homozygous transgenic *P. acutifolius* lines. All were very susceptible to *Z. subfasciatus* in replicated tests **(Table 7)**, possibly because the arcelin gene is not expressing itself.

Table 6. Levels of resistance to *Zabrotes subfasciatus* in selected bean lines used to develop a DNA-based molecular marker for the presence of arcelin.

Previous Rating	No. of Lines Tested	Arcelin presence	Percentage of adult emergence	Days to adult emergence	Percentage seeds damaged
Highly resistant	55	Arc +	7.4 ± 0.18d	49.9 ± 0.19b	27.1 ± 0.8c
Resistant	5	Arc +	15.5 ± 0.9c	47.2 ± 0.36c	64.1 ± 3.9b
Susceptible	2	Arc –	94.2 ± 0.7b	36.8 ± 0.15d	100 ± 0.0a
RAZ 44[a]		Arc +	4.6 ± 0.9e	52.1 ± 0.81a	15.9 ± 2.1d
ICA Pijao[b]		Arc –	97.9 ± 0.16a	37.2 ± 0.09d	100 ± 0.0a

[a] Standard improved resistant check; [b] Standard susceptible check.

Means (± SEM) of 4 replications per genotype. Means within a column followed by the same letter are not significantly different at the 5% level by LSD.

Table 7. Effect of selected bean genotypes on the biology of *Zabrotes subfasciatus.*

Genotype	Days to Adult Emergence[a]	Percentage of Emergence[b]	Percentage Seeds Damaged[c]
$5a^+$ 01-01	33.7 ± 0.11hi	92.9 ± 1.4a	100.0 ± 0.0a
$5a^+$ 01-04	34.3 ± 0.51hi	72.9 ± 3.9c	78.1 ± 5.7c
$5a^-$ negative control	32.4 ± 0.18I	97.9 ± 0.7a	100.0 ± 0.0a
$5bc^+$ 176-10	37.3 ± 0.19e	96.4 ± 0.5a	100.0 ± 0.0a
$5bc^-$ 176-10	33.7 ± 0.41hi	95.9 ± 2.1a	100.0 ± 0.0a
$5bc^+$ 176-15	35.1 ± 0.19fg	96.6 ± 1.4a	100.0 ± 0.0a
$5bc^-$ 176-15	34.4 ± 0.37hi	97.3 ± 1.5a	100.0 ± 0.0a
$5bc^+$ 186-PI-2R	39.7 ± 0.23d	95.9 ± 1.4a	100.0 ± 0.0a
$5bc^-$ 186-PI-2R	34.0 ± 0.17hi	97.9 ± 1.1a	100.0 ± 0.0a
$5bc^+$ 188-PI-2R	38.8 ± 0.23de	95.8 ± 1.6a	100.0 ± 0.0a
$5bc^-$ 188-PI-2R	34.7 ± 0.19h	95.2 ± 1.3a	100.0 ± 0.0a
$Arc1^+$ 182-11	34.1 ± 0.19hi	95.1 ± 1.5a	100.0 ± 0.0a
$Arc1^-$ 182-11	32.4 ± 0.09i	94.8 ± 0.8a	100.0 ± 0.0a
$Arc1^+$ 182-6	37.1 ± 0.28ef	82.5 ± 1.6b	100.0 ± 0.0a
$Arc1^-$ 182-6	33.8 ± 0.21hi	95.4 ± 1.6a	100.0 ± 0.0a
$Arc1^+$ 186-TR	40.4 ± 0.26d	66.0 ± 4.5d	100.0 ± 0.0a
$Arc1^-$ 186-TR	38.4 ± 0.44de	82.5 ± 6.8b	91.8 ± 1.6b
TB1 wild type	33.2 ± 0.19hi	97.5 ± 0.3a	100.0 ± 0.0a
PI440795	34.2 ± 0.18hi	96.9 ± 0.4a	100.0 ± 0.0a
EMP 175	33.0 ± 0.22hi	97.0 ± 1.2a	100.0 ± 0.0a
RAZ 2	51.7 ± 1.35c	9.8 ± 3.2e	30.5 ± 7.6e
Ica Pijao 'Ghent'	33.5 ± 0.15hi	97.1 ± 0.7a	100.0 ± 0.0a
RAZ 44	50.8 ± 1.48c	6.4 ± 0.8ef	32.1 ± 5.1e
G 12882 Arc 1	51.1 ± 0.94c	7.5 ± 1.4e	26.2 ± 5.7e
G 02771 Arc 5	56.4 ± 3.79b	1.1 ± 0.3f	4.2 ± 1.3f
G 12952 Arc 4	59.5 ± 0.55a	10.8 ± 1.5e	32.1 ± 4.6e
RAZ 136	50.3 ± 0.66c	5.4 ± 0.7ef	29.7 ± 3.3e
ICA Pijao 'CIAT'	33.3 ± 0.08hi	97.6 ± 0.3a	100.0 ± 0.0a

[a] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: $F = 113.2$; $df = 27, 107$; $P < 0.001$.

[b] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: $F = 121.3$; $df = 27, 108$; $P < 0.001$ on data transformed to arcsine square root of proportion.

[c] Means ± SEM of 5 replications per genotype. Means within a column followed by the same letter are not significantly different by LSD. ANOVA testing for differences among genotypes: $F = 193.7$; $df = 27, 108$; $P < 0.001$ on data transformed to arcsine square root of proportion.

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Leafhopper (*Empoasca kraemeri*)

In 2003 we screened a total of 867 bean germplasm accessions for resistance to the leafhopper. Those selected in 2002 (42) were reconfirmed in replicated nurseries. Of these, 21 were selected for further testing in 2004. We also gave support to the mainstream breeding activities of the Bean Project by screening a series of nurseries. These included 111 individual plant selections in Andean crosses performed with EMP 250 as a parent. Eighteen were selected for yield testing in 2004. Other tests included progeny row testing of 147 F_3 selections made among 19 populations developed for resistance to leafhopper and BGMV. Selections in Andean types also included 33 $F_{6,}$ which were tested in 2003. Of these, 16 lines are being yield-tested at present.

We will highlight the work on evaluation of interspecific *P. vulgaris* x *P. acutifolius* hybrids. Similar to the work with bruchids these progenies were obtained by means of the Double Congruity Backcross technique developed at CIAT. We received and tested 21 progenies (F_2 and F_3) of crosses made with the tepary sources of resistance to leafhopper G 40019 and G 40036. Selected progenies and their reaction to leafhopper are shown in **Table 8**. Seven were rated as intermediate, one as resistant. None was as resistant as the resistant parents. Further testing is in progress.

Table 8. Resistance to *Empoasca kraemeri* in selected F_2 and F_3 progenies derived from interespecific *Phaseolus vulgaris* x *Phaseolus acutifolius* crosses.

Code	Pedigree[a]	Mean Damage Score	Rating
A19Y-103 F_3	V-DCBC x (G40019 x A-DCBC)	6.7	Intermediate
A36Y-42 F_3	V-DCBC x (G40036 x A-DCBC)	6.0	Resistant
A99Y-86 F_2	V-DCBC x (G40199 x A-DCBC)	7.0	Intermediate
G36NGP-3 F_2	G 40036 x A-DCBC	7.0	Intermediate
KKQ-11 F_3	V-DCBC x V-DCBC	7.0	Intermediate
A99Y-15 F_2	V-DCBC x (G40199 X A-DCBC)	6.5	Intermediate
G19NGP-3 F_2	G40019 x A-DCBC	7.0	Intermediate
G36NGP-9 F_2	G40036 x A-DCBC	7.0	Intermediate
	Checks		
G 40019	Resistant P. acutifolius accession	5.0	Resistant
G 40036	Resistant P. acutifolius accession	4.5	Resistant
G 40119	Resistant P. acutifolius accession	5	Resistant
G 40016	Susceptible P. acutifolius accession	9	Susceptible
G 40056	Susceptible P. acutifolius accession	9	Susceptible
EMP 250	Resistant EMP line	5.5	Resistant
BAT 41	Standard susceptible P. vulgaris check	9	Susceptible
ICA Pijao	Standard resistant P. vulgaris check	6.5	Resistant

[a] V-DCBC, double congruent hybrid with P. vulgaris cytoplasm; A-DCBC, double congruent hybrid with P. acutifolius cytoplasm.

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Developing germplasm resistant to insects

For details of breeding activities, please refer to section 2.2.1. We will highlight results of the work trying to develop Andean type beans (crosses with PVA 773 and CAL 143) with improved tolerance to the leafhopper, *Empoasca kraemeri.* Lines selected for lower damage scores and higher reproductive adaptation scores in previous years performed relatively well under heavy (up to 7 nymphs per leaf, seasonal average) leafhopper infestation **(Table 9)**. Another set of lines derived from crosses between EMP 250 and PVA 773 or CAL 143 also performed well, certainly better than the very susceptible preferred Andean parents **(Figure 1)**. Given that susceptibility to leafhopper is usually very high in large-seeded Andean beans, these results indicate that substantial progress has been made in incorporating resistance to leafhopper in these types of beans.

Table 9. Leafhopper resistance in selected Andean bean lines.

Line	Damage scores 50 DAP[a]	Nymphs per leaf 50 DAP	Yield (kg/ha)		Percentage loss	Susceptibility index
			Unprotected	Protected		
Lines derived from the EMP 250 x PVA 773 cross						
4-1-11	5.2	3.7	1260	2102	39.9	0.81
4-11-8	5.3	2.4	522	1715	69.0	1.21
4-11-5	5.4	4.9	973	2327	58.1	0.97
4-11-14	5.3	3.0	854	2980	71.3	1.02
4-12-9	5.7	6.8	733	2168	65.9	1.10
4-12-10	5.7	4.7	708	1378	48.5	1.12
4-17-18	5.6	2.9	909	2039	55.3	0.99
4-18-1	5.3	2.8	989	2089	52.8	0.95
4-18-5	5.1	3.3	947	1668	43.2	0.96
Lines derived from the EMP 250 x CAL 143 cross						
5-9-1	5.2	2.8	750	1930	60.9	1.10
5-14-4	5.4	5.0	645	2167	70.2	1.12
5-25-3	5.0	3.5	894	2356	61.9	1.00
5-25-6	5.4	4.7	927	2194	57.9	0.99
5-26-9	5.4	3.8	889	2041	56.3	1.00
			Checks[b]			
CAL143 (S)	6.0	1.0	370	2215	83.3	1.24
PVA773 (S)	5.9	1.4	379	2028	81.1	1.26
EMP250 (R)	5.1	3.0	1486	2847	47.6	0.79
EMP228 (R)	5.6	3.7	1123	1889	40.3	0.86
PIJAO (T)	5.0	5.6	1483	2473	40.0	0.76
LSD 5%	0.25	0.52	290.6	491.8		

[a] DAP, days after planting; [b] S, susceptible, T, tolerant, R, resistant.

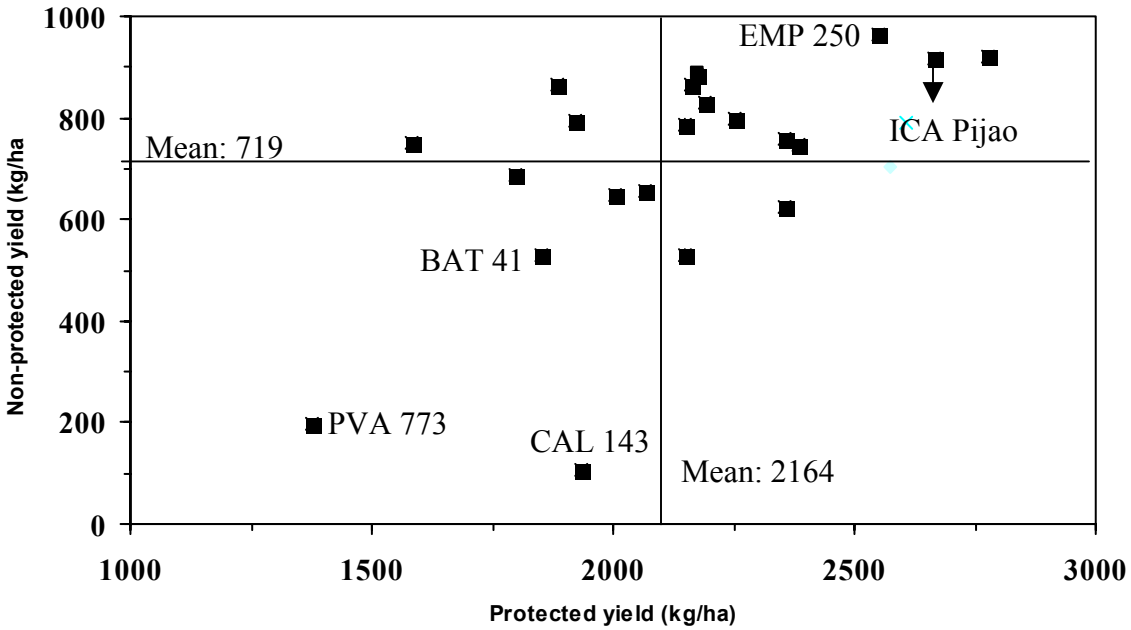

Figure 1. The relationship between protected and non-protected yield in selected Andean bean lines bred for tolerance to leafhopper. PVA 773 and CAL 143 are susceptible parents. EMP 250 is the tolerant parent. BAT 41 and Pijao (both Mesoamerican) are susceptible, and tolerant checks, respectively.

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Tolerance to leafhopper studies

Studies aimed at measuring progress in incorporating tolerance to leafhopper continued in 2003. On this occasion we measured the response of selected EMP lines (bred for leafhopper resistance) to two levels of infestation (3 and 6 nymphs per leaf) obtained by exercising chemical control at pre-established action levels. In terms of percentage yield losses, new bred lines (the EMP 500 series) performed better at all levels of infestation than the improved checks EMP 124 and EMP 250, and better than the standard tolerant check, ICA Piajo **(Figure 2)**. At very high levels of infestation (6 nymphs per leaf) average yield losses in EMP lines was above the 30% level, meaning that even tolerant materials would benefit from integration with chemical control exercised at pre-established action levels.

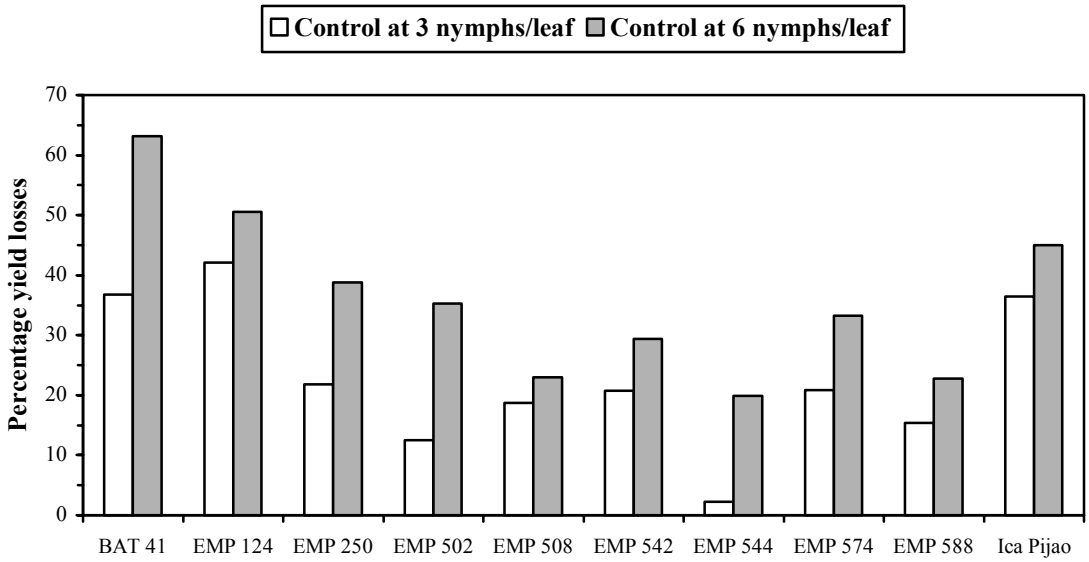

Figure 2. Yield losses in selected EMP lines and checks (BAT 41, ICA Pijao) at two levels of infestation with the leafhopper *Empoasca kraemeri.*

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BSc, MSc or PhD candidates supervised in 2003

Juan Miguel Bueno (National University), M.Sc. Thesis on Sampling methods for whiteflies.
Maria Fernanda Montenegro (National University), B. Sc. Thesis on Management of whiteflies.
Sergio Prieto (National University), B. Sc. Thesis on resistance to Mexican bean weevil.
Andrea Frei (ETH University, Switzerland), Ph.D. Thesis on resistance to *Thrips palmi* (terminated*).*

Progress toward achieving output milestones

- Identification of sources of resistance, understanding of mechanisms of resistance to insects, and development of insect resistant bean lines contribute to the mainstream breeding objectives of the Bean Project.
- Insect resistant beans may be basic components for management of insect pests in beans
- The development of molecular markers for pod weevil, thrips, and bruchids should facilitate breeding for resistance.

Publications

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