Output 3. Strategies developed for management of diseases and pests in bean-based cropping systems

Activity 3.1 Characterizing and monitoring pathogen and insect diversity

Highlights:

- 55 *Phaeoisariopsis griseola* and *Colletotrichum lindemuthianum* isolates were characterized using host differential interactions.

- 400 isolates of *Xanthomonas campestris* pv. *phaseoli* (XCP) and *X. campestris* pv. *phaseoli* var. *fuscans* (XCPF) have been characterized using repetitive extragenic palindromic polymerase chain reaction (REP-PCR) and RFLP of the 26S ribosomal genes. Results show that XCP is very distinct from XCPF.

- 125 *C. lindemuthianum* isolates were characterized using microsatellites and REP-PCR.

- Polymorphic RAMS fragments were cloned to develop locus specific markers.

- 400 *P. griseola* isolates were characterized using RAMS and all isolates were put into Andean and Mesoamerican groups.

- The "Afro-Andean" group is part of the Andean group that represents isolates resulting from point mutations in genes for virulence.

- The presence of BGYMV virus in Colombia was confirmed.

3.1.1 Characterizing pathogen diversity

Rationale: Genetic diversity and pathogen population structure information is useful in identifying sources of disease resistance and deployment of the resistance genes in ways that prolong their durability. The breakdown of resistance has been attributed to the change in the population structure. Understanding the amount of genetic diversity that exists within populations helps us predict the rate at which a resistant variety will break down, understand factors that contribute to the breakdown of resistance, and predict how long a resistant variety will last before being overcome by new pathotypes. Therefore, monitoring the pathogen for emergence of new and more virulent (aggressive) races should be a continuous activity in a disease management program.

***Phaeoisariopsis griseola* (PG):** Thirty-five *P. griseola* isolates were characterized on a set of 12 host differential cultivars. Six of the isolates were from Bolivia, one from Colombia, two from El Salvador, seven from Haiti, three from Malawi, 11 from Nicaragua, and five from Tanzania (Table 35). Twenty-one races were defined. However, all these have been described before. This brings the total of isolates characterized using virulence markers to 446.

Table 35. Virulence phenotypes of 35 *Phaeoisariopsis griseola* isolates characterized during 2000.

	Isolate[a]	Differential cultivars[b]												Race
		A	B	C	D	E	F	G	H	I	J	K	L	
1	Pg 307 COL		b	c	d	e	f							62-0
2	Pg 5 TZA	a	b	c	d	e	f						l	63-32
3	Pg 6 TZA	a	b	c	d		f	g	h	i		k		47-23
4	Pg 9 TZA	a	b	c	d	e	f	g					l	63-0
5	Pg 10 TZA	a	b	c				g	h	i			l	7-39
6	Pg 611TZA			c	d									12-0
7	Pg 32 BOL	a	b	c	d	e	f	g	h	i		k		63-23
8	Pg 33 BOL	a	b	c	d	e	f	g	h	i		k		63-23
9	Pg 34 BOL	a	b	c				g	h	i		k		7-23
10	Pg 35 BOL	a	b	c		e		g	h	i		k		23-23
11	Pg 36 BOL	a	b	c	d	e	f	g	h	i		k		63-23
12	Pg 38 BOL	a	b	c	d			g	h	i		k		31-23
13	Pg 33 MWI	a	b	c	d			g	h	i			l	31-39
14	Pg 34 MWI	a	b	c	d			g	h	i			l	31-39
15	Pg 35 MWI	a	b	c	d			g	h	i			l	31-39
16	Pg 19 ELS	a	b	c				g	h		J	k		7-27
17	Pg 20 ELS	a	b	c	d	e	f	g	h		J	k		63-27
18	Pg 11 NIC	a	b	c	d	e		g	h	i		k	l	31-55
19	Pg 12 NIC	a	b	c	d	e	f	g	h		J	k		63-27
20	Pg 13 NIC	a	b	c	d	e	f	g	h	i	J	k		63-31
21	Pg 14 NIC	a	b	c	d	e	f	g	h		J	k		63-27
22	Pg 15 NIC	a	b	c		e		g	h		J	k		23-27
23	Pg 16 NIC	a	b	c	d	e		g	h			k	l	31-51
24	Pg 17 NIC	a	b	c	d	e	f	g	h	i	J	k		63-31
25	Pg 18 NIC	a	b	c		e		g	h		j	k		23-27
26	Pg 19 NIC	a	b	c	d	e	f	g	h		j	k		63-27
27	Pg 20 NIC	a	b	c	d	e	f	g	h		j	k	l	63-59
28	Pg 21 NIC	a	b	c	d	e		g	h	i	j	k	l	31-63
29	Pg 1 HTI	a	b	c	d	e		g	h	i			l	31-39
30	Pg 2 HTI	a	b	c	d	e	f	g	h	i			l	63-39
31	Pg 3 HTI	a	b	c	d	e		g	h	i			l	31-39
32	Pg 4 HTI	a	b	c	d	e	f	g	h	i			l	63-39
33	Pg 5 HTI	a	b	c	d	e		g	h	i			l	31-39
34	Pg 6 HTI	a	b	c	d	e		g	h	i				31-7
35	Pg 7 HTI	a	b	c	d	e		g	h	i				31-7

a. Pathogen identification: the last three letters represent the country of origin. HTI = Haiti, NIC = Nicaragua, COL = Colombia, BOL = Bolivia, TZA = Tanzania, and MWI = Malawi.

b. Andean differential cultivars: A = Timoteo, B = G 11796, C = Bolón Bayo, D = Montcalm, E = Amendoim, F = G 5686. Mesoamerican differential cultivars: G = PAN 72, H = G 2858, I = Flor de Mayo, J = MEX 54, K = BAT 332, L = Cornell 49242.

***Colletotrichum lindemuthianum* (CL):** Twenty isolates collected from Ecuador were characterized using host differential interactions. Seven races were identified among the 20 isolates (Table 36). An interesting observation was the incidence of simple races, race 0 (six isolates), race 1, race 4, race 6, and race 7. Only race 256 was the most complex. These results show that the *C. lindemuthianum* population pathogen structure in Ecuador is predominantly Andean and composed of relatively simple races. Introgressing resistance genes found in the differential cultivars Cornell 49242, MEX 222, PI 207262, Tu, AB 136, and G 2333 will be sufficient to manage anthracnose in this country.

Table 36. Virulence phenotype of 20 *Colletotrichum lindemuthianum* isolates collected from Ecuador.

Isolate	Differential cultivars[a]												Race
	A	B	C	D	E	F	G	H	I	J	K	L	
	1	2	4	6	18	32	64	128	256	512	1024	2048	
CL 163		b	c										6
CL 164													0
CL165		b	c										6
CL166		b	c										6
CL167	a	b	c		e								23
CL168			c										4
CL169	a	b	c										7
CL170		b	c										6
CL171		b	c										6
CL172													0
CL173													0
CL174													0
CL175													0
CL176	a												1
CL177													0
CL178			c										4
CL179									i				256
CL180	a												1
CL181	a												1
CL182													0

a. Differential cultivars: A = Michelite, B = Michigan Dark Red Kidney, C = Perry Marrow, D = Cornell 49242, E = Widusa, F = Kaboon, G = MEX 222, H = PI 207262, I = To, J = Tu, K = AB 136, and L = G 2333. The binary value used to designate race of the isolate is given underneath the letters.

Contributors: G Mahuku, C Jara, G Castellanos, J Cuasquer (IP-1); R Buruchara (IP-2)

3.1.2 Molecular characterization of *Xanthomonas campestris* pv. *phaseoli,* causal agent of common bacterial blight

Rationale: A clear understanding of the genetic variation that exists in pathogen populations is essential not only in formulating appropriate management strategies, but also in understanding the origin and maintenance of genetic variation and the emergence of new pathogen variants and/or races. Common bacterial blight is a major disease of common bean in temperate and tropical countries, and yield losses can be as high as 60%. Two phytogenetically distinct *Xanthomonas*, XCP and XCPF, cause the disease. Variation in pathogenicity occurs among XCP isolates. This variation is mainly quantitative and differential interactions between host and isolate are not clearly exhibited. Controversy surrounds the existence of genetic diversity within XCP, with some researchers reporting the existence of host differential interactions in common bean, while others have disputed this report. This study is aimed at using molecular techniques to elucidate the amount of genetic diversity that exists within and between XCP and XCPF. This information will be used to select strains to screen a range of *Phaseolus* genotypes and establish if host differential interaction occurs within CBB.

Materials and methods: DNA was extracted from 450 CBB pathogens and amplified using three enterobacterial repetitive intergenic consensus (ERIC) REP-PCR primers, REP-PCR sequences, and BOX elements (BOX PCR). In addition, restriction fragment length polymorphisms (RFLPs) of the 26S ribosomal gene were obtained using conserved primers and digestion with restriction endonucleases. We also made bacterial isolations and pathogenicity tests for 20 samples: seven from Malawi, one from Colombia, one from Puerto Rico, one from Honduras, three from Nicaragua, and seven from Iran. These isolates were pathogenic on BAT 41, the susceptible check.

Results and discussion: Preliminary analysis of the results has shown that XCP and XCPF are distinct at the molecular level and should be separated into different groups (Figure 46). However, very little genetic diversity exists for the 26S ribosomal gene. Only three restriction enzymes (*RSA* 1, *MBO* 1, and *HAE* III) showed polymorphisms that separated XCP from XCPF and showed no within-group polymorphisms. Figure 47 shows profiles generated after digestion with RSA 1. The REP-PCR revealed greater genetic diversity that was correlated to geographical origin of the isolates. We are analyzing all the molecular data and generating molecular genetic groups to use for host differential analysis.

Conclusions: Preliminary analysis has shown that XCP and XCPF are distinct. However, more information will be obtained following completion of this study.

Contributors: G Mahuku, C Jara

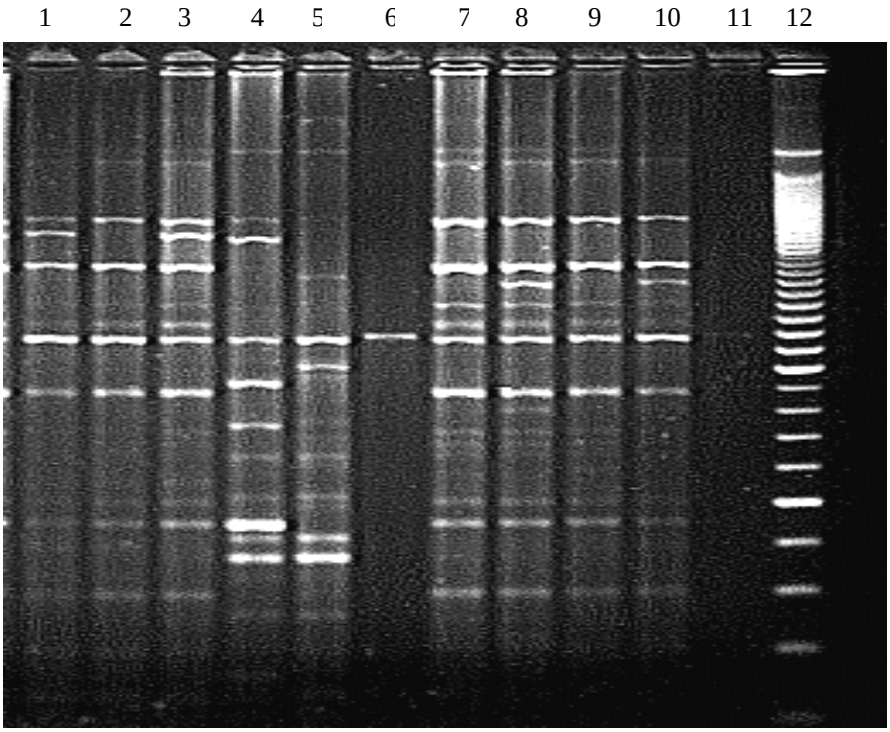


Figure 46. Enterobacterial repetitive intergenic consensus (ERIC) – polymerase chain reaction (PCR) profiles for some *Xanthomonas campestris* pv. *phaseoli* (XCP) and XCP var. *fuscans* (XCPF) strains. Lanes 1, 2, 3, 6, 7, 8, 9, and 10 are XCP, while lanes 4 and 5 are XCPF strains. Lane 11 is negative control and lane 12 is the 100 bp DNA ladder.

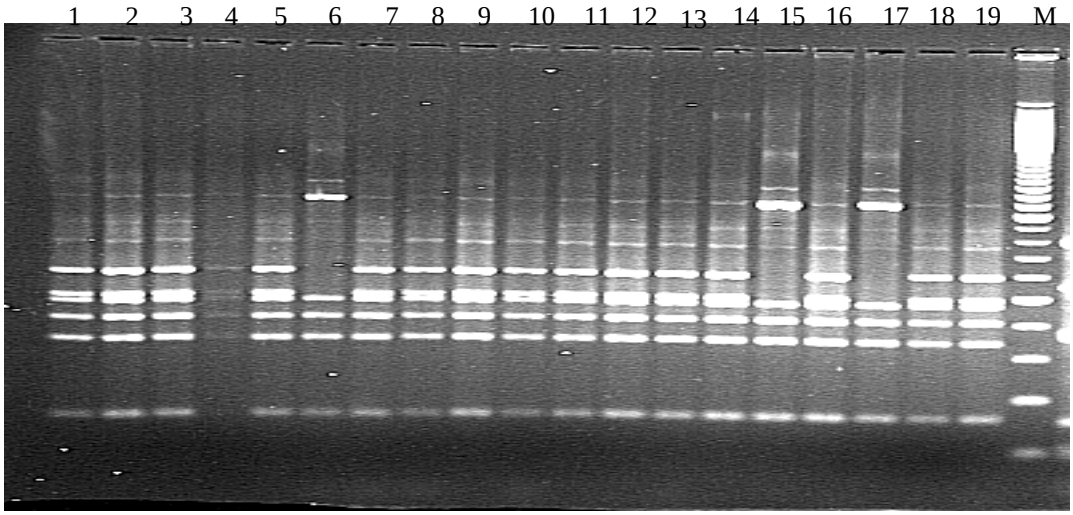


Figure 47. Profiles generated by digesting the amplified 26S ribosomal gene with the restriction enzyme *RSA*1. Lanes 6, 15, and 17 are *Xanthomonas campestris* pv. *fuscans*, while the other lanes are *Xanthomonas campestris* pv. *phaseoli.* Lane M is the 100 bp DNA molecular marker.

3.1.3 Molecular and virulence characterization of *Colletotrichum lindemuthianum*, causal agent of anthracnose of common bean

Rationale: Anthracnose of bean, caused by *C. lindemuthianum,* is an important disease in the highlands of Africa and Latin America. Yield losses from this pathogen can be as high as 90%. It has been shown to exist in many different forms (races), and the major reason for the continued susceptibility of bean to anthracnose is pathogen variation. By analyzing the structure of pathogen populations and the ways in which populations respond to experimental, agricultural, and natural constraints, the mechanisms by which pathogen population change can be understood. This understanding can provide the basis for formulating disease support systems that lead to effective disease management. This project seeks to develop molecular markers (microsatellites) for genetic characterization and differentiation of *C. lindemuthianum* races.

Materials and methods: To check if the isolates maintained their virulence during long periods of storage, 12 isolates from different countries and representing different races were tested in the greenhouse on a set of host differential genotypes. From our collection of 1050 isolates, 125 *C. lindemuthianum* isolates were selected and DNA extracted from them. The isolates were selected to represent the greatest genetic diversity possible (i.e., the most widely distributed and frequently occurring *C. lindemuthianum* race from different geographical origin). We took into account the geographical origin of the isolates and the race designation based on host differential interaction on 12 bean genotypes.

Molecular characterization of the isolates was done using RAMS and REP-PCR primers, ERIC-PCR, REP-PCR sequences, and BOX-PCR, originally developed for amplifying conserved repetitive sequences in the bacterial genome.

Results and discussion: Evaluation of the 12 isolates on a set of host differentials showed that the isolates had maintained their virulence despite long periods of storage (Table 37). Initial race designations were in agreement with our current test characterization, showing that the storage conditions did not influence the virulence of the pathogen.

Preliminary results obtained from characterization of isolates using RAMS have shown high levels of polymorphism among and within *C. lindemuthianum* races (Figure 48). No clear correlation between race designation and molecular phenotype were apparent (Figure 49). However, we are in the process of correlating individual RAMS bands for each of the RAMS primers used in this study in order to identify race-discriminating bands.

Table 37. Virulence phenotype and race designation of 12 *Colletotrichum lindemuthianum* isolates evaluated on 12 differential varieties 7 years after storage.

Isolates[a]	Differential varieties[b]												Race
	A	B	C	D	E	F	G	H	I	J	K	L	
CL11CRI*	a	.	.	d	.	.	g	h	i	j	k	.	1993
CL11CRI	a	.	.	d	.	.	g	h	i	j	k	.	1993
CL82CRI*	a	.	.	d	.	.	g	h	i	.	.	.	457
CL82CRI	a	.	.	d	.	.	g	h	i	.	.	.	457
CL41CRI*	a	.	.	d	e	.	g	h	i	.	k	.	1497
CL41CRI	a	.	.	d	e	.	g	h	i	.	k	.	1497
CL7CRI*	a	b	c	.	.	.	.	.	.	.	.	.	7
CL7CRI	a	b	c	.	.	.	.	.	.	.	.	.	7
CL96BRA*	a	b	c	.	e	.	.	.	.	.	.	.	23
CL96BRA	a	b	c	.	e	.	.	.	.	.	.	.	23
CL114CRI*	a	.	.	.	.	.	.	h	.	.	k	.	1153
CL114CRI	a	.	.	.	.	.	.	h	.	.	k	.	1153
CL93CR*	a	.	.	d	.	.	.	.	i	.	.	.	1033
CL93CR	a	.	.	d	.	.	.	.	i	.	.	.	1033
CL5CRI*	a	.	.	d	.	.	.	.	.	j	.	.	521
CL5CRI	a	.	.	d	.	.	.	.	.	j	.	.	521
CL154CRi*	a	.	.	d	.	.	.	h	.	.	.	.	137
CL154CR	a	.	.	d	.	.	.	h	.	.	.	.	137
CL72CRI*	a	.	.	d	e	.	.	h	i	.	k	l	3481
CL72CRI	a	.	.	d	e	.	.	h	i	.	k	l	3481
CL106CRI*	a	.	.	.	.	.	.	.	.	.	k	.	1025
CL106CRI	a	.	.	.	.	.	.	.	.	.	k	.	1025
CL90CRI*	a	.	.	d	.	.	g	.	.	.	.	.	73
CL90CRI	a	.	.	d	.	.	g	.	.	.	.	.	73

a. * First evaluation of *C. lindemuthianum* isolates evaluated previously by the Bean Pathology section.

b. Anthracnose differential cultivars: A = Michelite, B = Michigan Dark Red Kidney, C = Perry Marrow, D = Cornell 49242, E = Widusa, F = Kaboon, G = MEX 222, H = PI 207262, I = To, J = Tu, K = AB 136, and L = G 02333.

We have started cloning polymorphic and race-discriminating bands for subsequent sequencing and development of locus-specific microsatellite markers for *C. lindemuthianum* that can be used for population genetic studies and to elucidate the mechanisms leading to the high genetic diversity and distribution observed in this fungus. The wide genetic polymorphism among *C. lindemuthianum* races revealed by microsatellite markers shows the great potential of this type of marker for population genetic studies of this fungus.

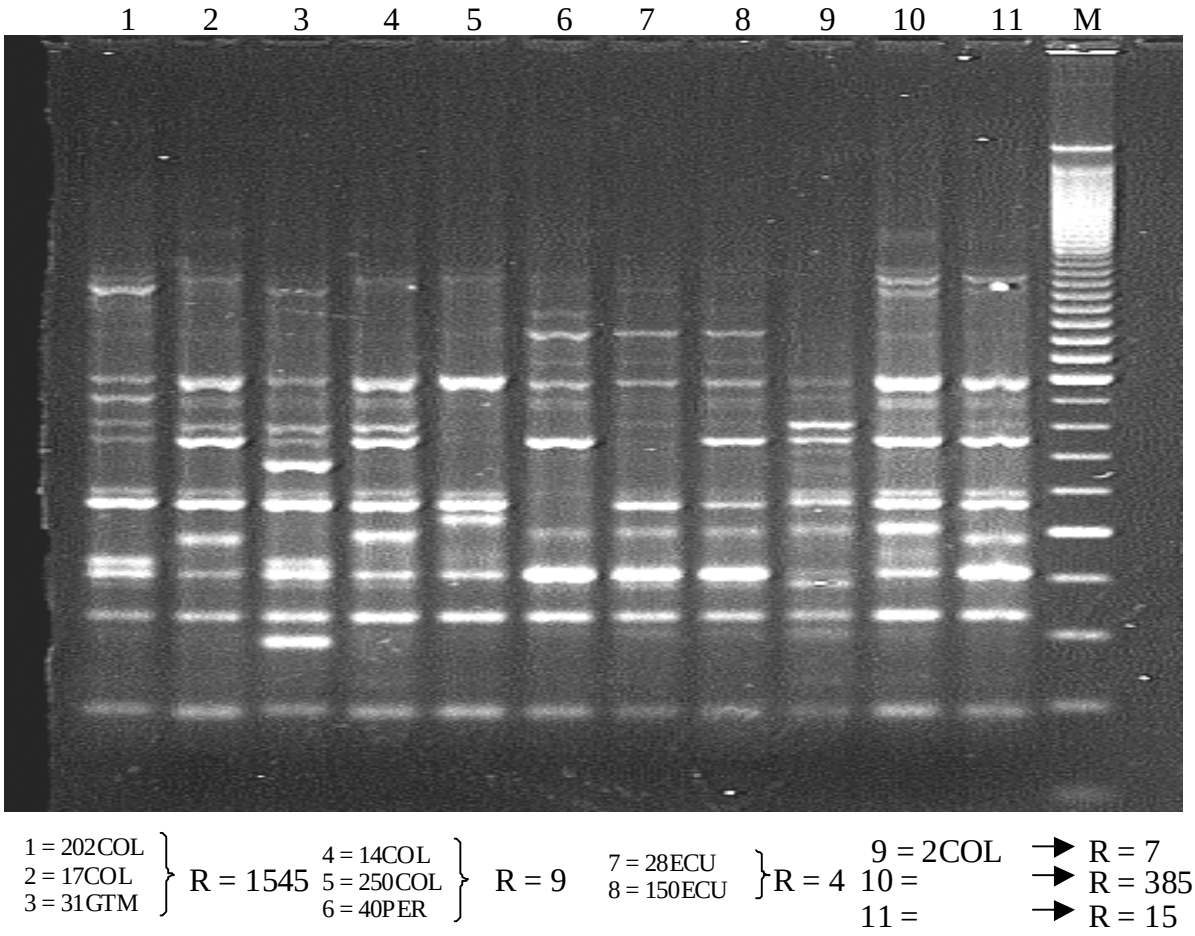


Figure 48. Profiles generated by microsatellite primers (GT)n for different races of *Colletotrichum lindemuthianum*. Lanes 1, 2, and 3 represents race 1545, lanes 4–6 = race 9, lanes 7 and 8 = race 4, lane 9 = race 7, lane 10 = race 385, and lane 11 = race 15. Lane M represents the 100 bp DNA molecular marker.

Contributors: JJ Riascos, G Mahuku

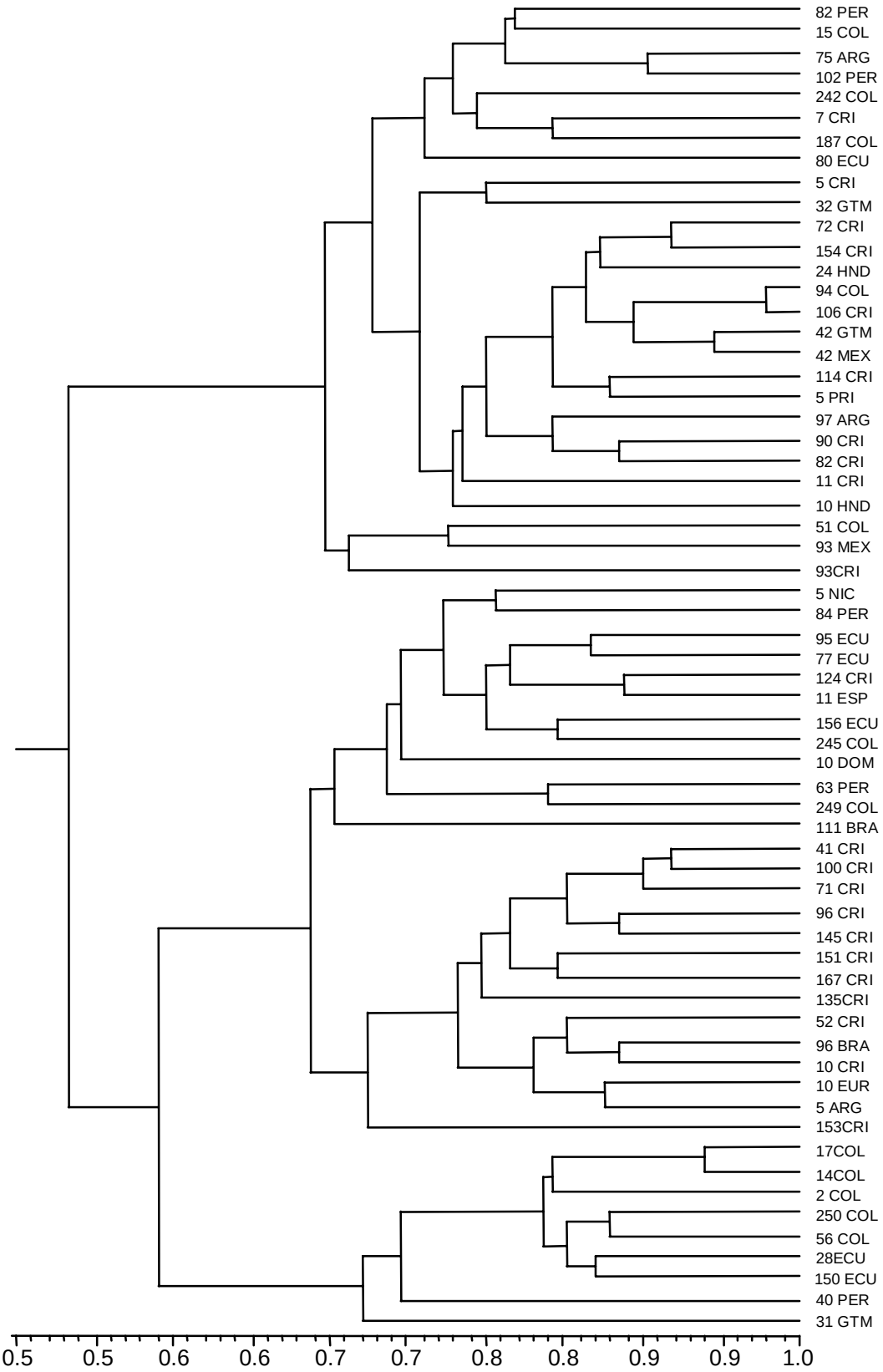


Figure 49. Dendrogram of *Colletotrichum lindemuthianum* isolates based on unweighted pair-group distance method of averaging (UPGMA) analysis of random amplified microsatellite data using numerical taxonomy system (NTSYS) program Version 1.8.

3.1.4 Molecular characterization of the angular leaf spot pathogen, *Phaeoisariopsis griseola*

Rationale: Two major groups of the ALS pathogen, *P. griseola*, were described using virulence and molecular markers. Isolates belonging to the Andean group were shown to infect and colonize bean varieties belonging to the Andean gene pool, while Mesoamerican isolates have a broader virulence spectrum, although preferring Mesoamerican beans. Recently, a group of typical Andean isolates, designated "Afro-Andean", which are able to infect one or two Mesoamerican differential cultivars, have been identified and only from Africa. This study was conducted to elucidate the relationship between the Afro-Andean, Andean, and Mesoamerican PG groups, and to establish if the Afro-Andean group represented a new group of isolates within *P. griseola*.

Materials and methods: *P. griseola* isolates were divided into three groups: Andean, Mesoamerican, and Afro-Andean, based on differential interaction on 12 bean cultivars. The molecular profiles of these isolates were assessed using RAPD, RAMS, and restriction digestion of the amplified ribosomal intergenic spacer region (IGS-RFLP). Analysis of molecular variance (AMOVA) and cluster and multiple correspondence analysis were used to determine the relationship existing between *P. griseola* groups. In addition, statistical support for phenogram branching in qualitative analyses was obtained using bootstrapped analysis.

Results and discussion: Polymorphic and easily scorable banding patterns were obtained using RAMS, RAPDs, and IGS-RFLP. Figure 50 shows comparative banding patterns obtained using the RAMS primer (CA)n for some *P. griseola* isolates.

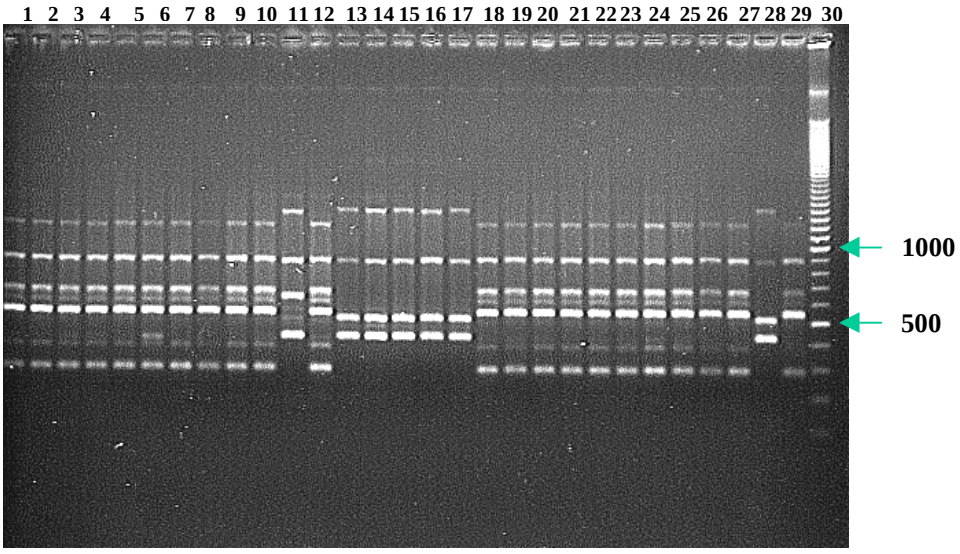


Figure 50. Band profiles generated for *Phaeoisariopsis griseola* isolates using RAMS primer (CA)n. The isolates in lanes 1-10, 12, 18-27, and 29 belong to the Andean group, while isolates in lanes 11, 13-17, and 28 are Mesoamerican. Lane 30 represents the 100 bp molecular DNA step marker.

Figure 51 shows the size of IGS products and the profiles obtained following digestion with the restriction enzyme *RSA* 1. No differences were obtained between groups in the size of the IGS fragment.

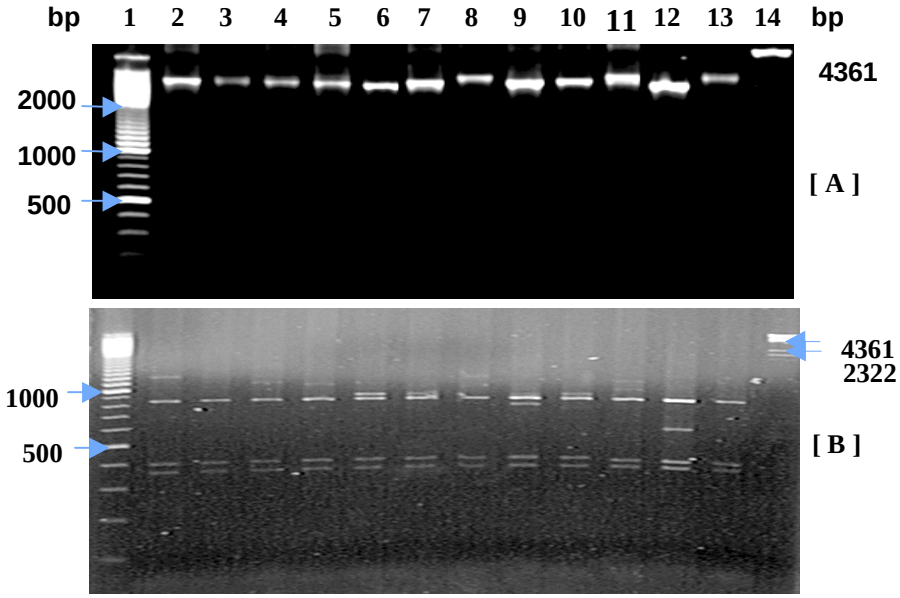
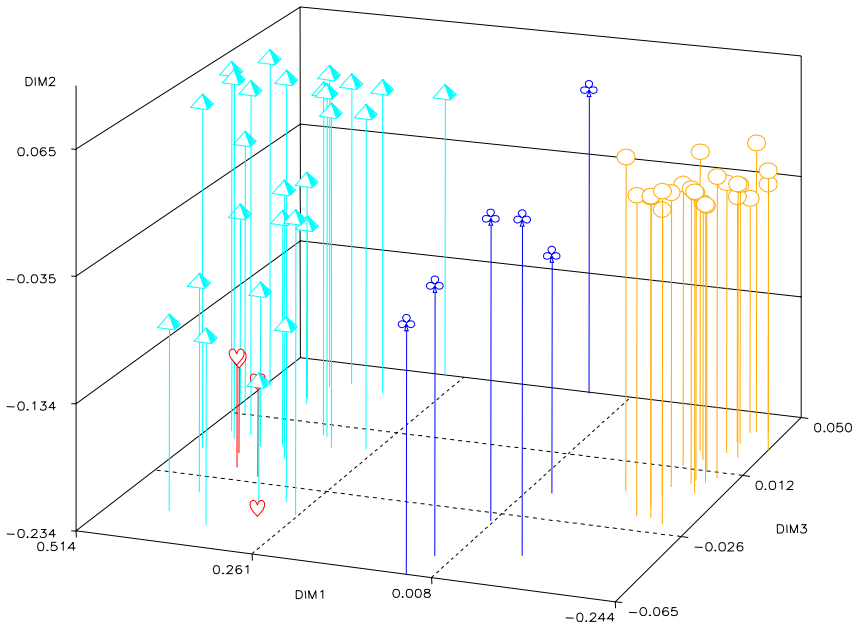


Figure 51. [A] Polymerase chain reaction (PCR) product following amplification with the intergenic spacer region (IGS) primers CLN12 and CLNTS1. [B] IGS profiles of *Phaeoisariopsis griseola* isolates resulting from restriction digestion of the IGS PCR product with the restriction enzyme *Rsa*I. Isolates in lanes 2-6 are Andean isolates, lanes 7-10 are Mesoamerican, and lanes 11-13 are Afro-Andean. Lane 1 corresponds to the molecular weight marker, the 100 bp DNA stepladder, and lane 14 to HIND III digested Lambda DNA.

Analysis of genetic differentiation showed that most of the genetic diversity was distributed within groups (81%) rather than between groups (19%). The AMOVA analysis showed that Andean and Afro-Andean *P. griseola* isolates belonged to the same group (Gst = 0.067), whereas the Mesoamerican group was distinct from both the Andean group (Gst = 0.41) and the Afro-Andean (Gst = 0.54) (Figure 52). These high levels of genetic differentiation signify the distance between the Andean and Mesoamerican group of *P. griseola*, further confirming the co-evolution concept. However, the low levels of genetic differentiation between the Andean and Afro-Andean groups show that these isolates represent recent events of genetic separation that has not occurred long enough to significantly separate them into two groups.

Similarly, cluster analysis separated isolates into two major groups, corresponding to Andean and Mesoamerican groups, and all isolates classified as Afro-Andean separated with the Andean group (Figure 53).



△ Mesoamerican isolates
♡ Mesoamerican isolates
♧ Andean isolates
○ Andean and “Afro-Andean” isolates

Figure 52. Multiple correspondence analysis of RAPD profiles of *Phaeoisariopsis griseola* isolates representative of Andean, Mesoamerican, and “Afro-Andean” groups.

Contributors: MA Henríquez, G Mahuku (IP-1); R Buruchara (IP-2)

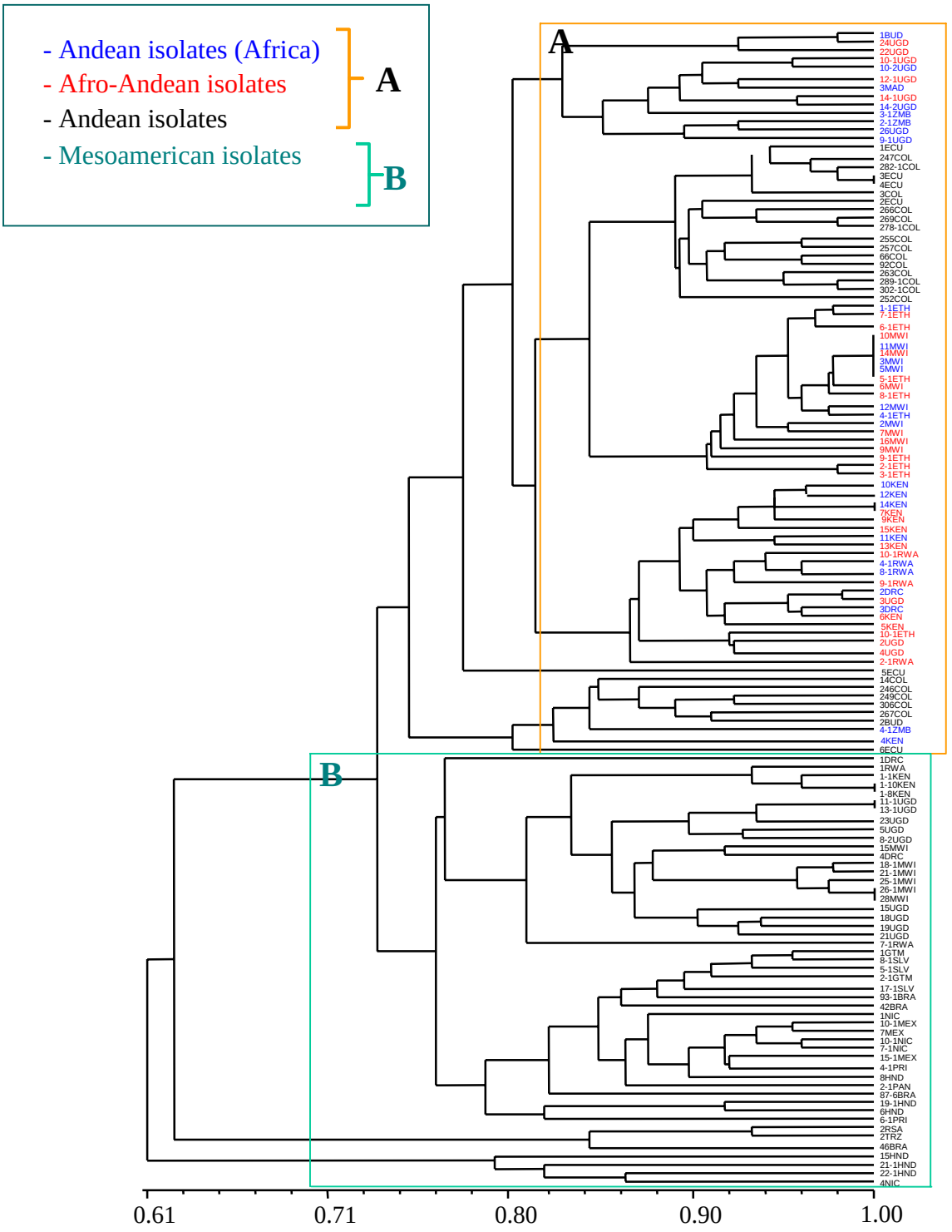


Figure 53. Dendrogram of *Phaeoisariopsis griseola* isolates based on UPGMA methods using the SAHN and TREE programs in NTSYS program using similarity coefficient calculated from combined IGS-RFLP data for five restriction enzymes. Dice's coefficient within the SimQual program of NTSYS-pc Version 1.8 (Exeter Software, Setauket, NY, USA) was used to calculate genetic similarities between isolates. (Note: acronyms are given in full at the end of this report.)

3.1.5 Characterization of whitefly-transmitted geminiviruses

Rationale: Whitefly-transmitted viruses, currently known as begomoviruses, are rapidly expanding their geographic range. Originally important only in lowland tropical regions with a prolonged dry season (particularly in Mexico, northern Central America, and the Caribbean) begomoviruses have been progressively impacting bean-producing regions in the humid tropics of the Americas. Costa Rica and Panama are good examples of regions where BGYMV has emerged in past years, despite the apparently unsuitable climatic conditions that affect the biology of the whitefly vector, *Bemisia tabaci*, in those countries. In South America, only the related species, BGMV, has been reported. This virus induces similar yellowing symptoms in common bean varieties, but it is genomically different to BGYMV.

The existence of whitefly-transmitted viruses in common bean has been known for at least two decades in Colombia. Galvez and co-workers reported on the occurrence of bean golden mosaic and bean dwarf mosaic in the municipality of El Espinal, department of Tolima[5].

Materials and methods: Golden mosaic-like symptoms have also been sporadically observed at CIAT at a very low incidence since the early 1980s. Recently, in CIAT fields, a few bean plants showing intense yellowing were tested with a monoclonal antibody that detects begomoviruses in general, confirming the presence of a whitefly-transmitted virus in the affected common bean plants. This virus was characterized at CIAT at the molecular level, as an isolate of BGYMV.

Results: The Colombian isolate had over 90% amino acid and nucleotide sequence homology with the known BGYMV isolated from Central America and the Caribbean, at the coat protein, replicase, and common region levels.

Conclusions: This is the first report of the presence of BGYMV in South America. However, it is unlikely that the virus will become economically important in the Cauca Valley of Colombia because of the marginal climatic conditions that predominate in this region for most of the year. Nevertheless, the virus may appear sporadically during the dry months of the year when *B. tabaci* populations tend to increase.

Contributors: C Muñoz (Instituto Colombiano para el desarrollo de la Ciencia y la Tecnología "Francisco José de Caldas" [COLCIENCIAS]);
FJ Morales, M Castaño, CJ Alvarez

[5] Galvez GE, Castaño M, Belalcazar S. 1975. Presencia de los virus del mosaico dorado y del moteado clorótico del frijol en Colombia. Ascolfi Informa 1:3-4.

Progress towards achieving output milestones:

- 400 isolates of the ALS pathogen have been characterized using molecular and virulence markers and pathogen structure. This information is being used in looking for sources of resistance and developing gene deployment strategies.

- 400 isolates of *Xanthomonas campestris* pv. *phaseoli* were characterized using REP-PCR and RFLP of the 26S ribosomal genes and results show that XCP is highly distinct from XCPF. This information will contribute to the elucidation of races in this pathogen, and the development of resistant varieties.

- The "Afro-Andean" group is part of the Andean group that represents isolates resulting from point mutations in genes for virulence. This information contributes to the understanding of the mechanisms underlying emergence of virulence in *P. griseola*.

Activity 3.2 Characterizing disease and insect resistance genes

Highlights:

- 150 RILs were identified as having high levels of CBB resistance under both field and greenhouse conditions.

- 16 lines were identified that combine Andean and Mesoamerican ALS-resistance genes.

- Inheritance of ALS resistance to Andean and Mesoamerican *P. griseola* races in G 19833 and DOR 364 is different and complex being recessive for one race and dominant for another, depending on the source of resistance.

- Resistance to *Ascochyta* blight was found to be largely under additive gene control in *P. coccineus* although it tended to be recessive in *P. polyanthus*. From two to four genes appeared to control resistance.

- QTL mapping of resistance to *Thrips palmi* shows few genes controlling resistance to insect damage and reproductive adaptation under high infestation pressure.

- QTL mapping of ALS and anthracnose disease resistance shows clustering of resistance genes in the bean genome.

- Populations were developed to study the inheritance of ALS resistance.

3.2.1 Screening of a population derived from VAX 6 for resistance to common bacterial blight (X*anthomonas campestris* pv. *phaseoli*)

Rationale: Common bacterial blight (CBB) is the most widely distributed disease of common bean that can cause yield losses of more than 40%. High levels of resistance to CBB have been introgressed from tepary bean, *P. acutifolius*, to develop a series of highly CBB-resistant *P. vulgaris* VAX lines, of which VAX 6 is especially resistant. However, its seed type and color is not desirable and resistance must be transferred to preferred grain and market class type. In order to maintain the same level of resistance as currently found in VAX 6, all the genes must be transferred to the same cultivar, and markers that are tightly linked to these genes are indispensable. Our objective was to screen RILs developed by crossing MAR 1 (CBB susceptible) x VAX 6 (CBB resistant) under field and greenhouse conditions to generate phenotypic data needed for QTL analysis and subsequent tagging of CBB resistance genes in VAX 6.

Materials and methods: VAX 6, MAR 1, and 223 RILs that were developed from a cross between VAX 6 (CBB resistant) and MAR 1 (CBB susceptible) were planted at Santander de Quilichao and inoculated three times using a local isolate of XCP. The first inoculation was done 25 days after planting and at weekly intervals thereafter. Four evaluations were taken starting 10 days after the first inoculation and using a 1 to 9 scale, where 1 represents no symptoms and 9 represents severe symptoms. Ratings of 1–3 are considered resistant, 4-6 intermediate, and >6 susceptible response. Similarly, the same RILs were screened in the greenhouse with the same XCP strain and using the same evaluation scale.

Results and discussion: To select the best XCP strain to use in field and greenhouse evaluations, the parents, MAR 1 and VAX 6, were screened with isolates collected in Santander de Quilichao. No differences were obtained in the levels of infection, but the *fuscans* variant of XCP consistently gave high disease ratings. Therefore XCP isolates were selected that are normally used for all inoculations in Santander de Quilichao. Most of the MAR 1 x VAX 6 RILS (76%) were resistant to XCP under field conditions, 21% were intermediate, and 3% susceptible (Figure 54). As expected, MAR 1 had a susceptible reaction (rating 7) while VAX 6 had a resistant reaction (rating 2). Under greenhouse conditions 78% of the RILS were resistant, 20% intermediate, and 2% susceptible (Figure 55). The field and greenhouse results were highly correlated (r = 0.94) showing the high levels of resistance that were introgressed into VAX 6. We are re-evaluating the RILS in the field with the same isolate, and making greenhouse evaluations with the *fuscans* variant of XCP. In addition, the parents are being screened with RAPD and microsatellite markers in order to identify polymorphic markers that can be used to screen the RILS to identify QTLs linked to resistance genes.

Contributors: G Mahuku, C Jara, H Terán, S Beebe

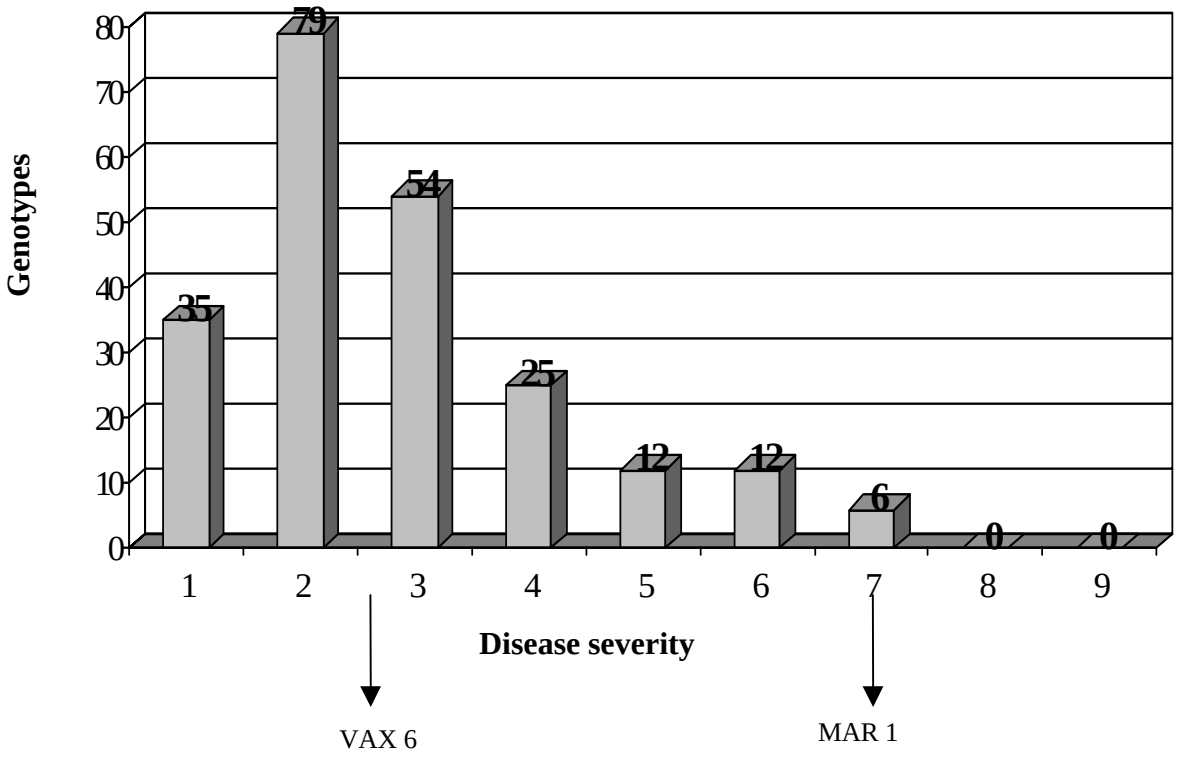


Figure 54. Response of 223 recombinant inbred lines of VAX 6 x MAR 1 to inoculation with *Xanthomonas campestris* pv. *phaseoli* under field conditions. Disease severity ratings of 1–3 are considered resistant, 4-6 intermediate, and >6 a susceptible response.

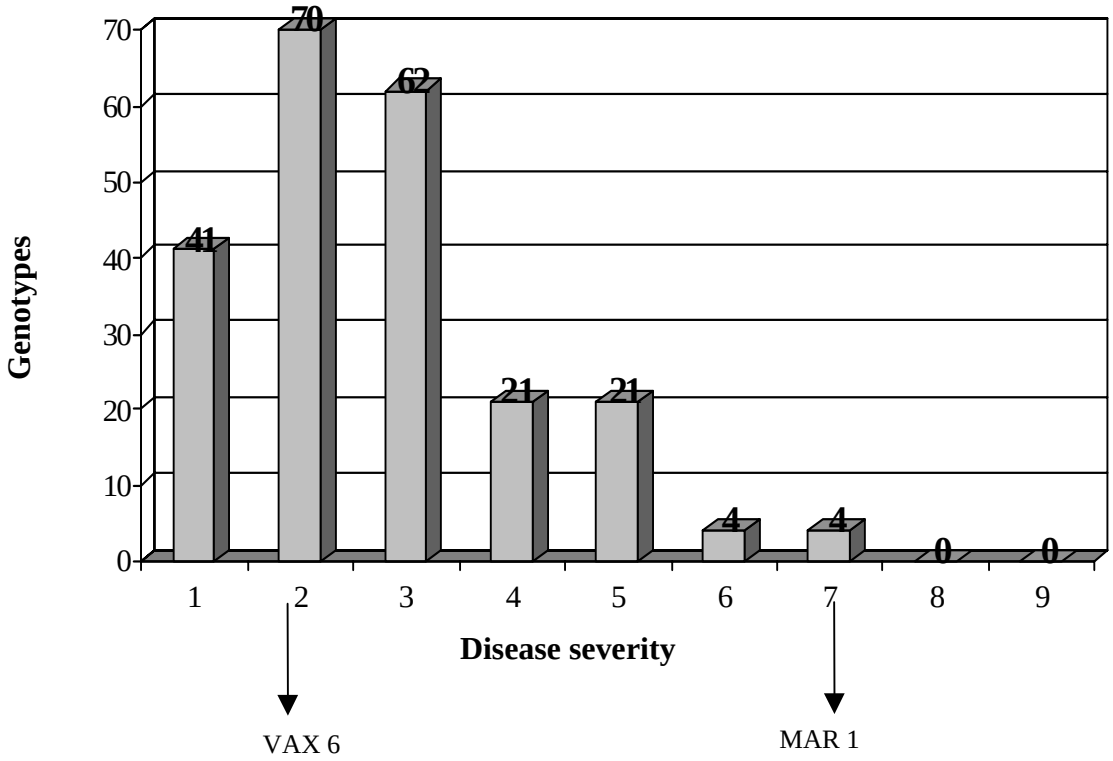


Figure 55. Response of 223 recombinant inbred lines of VAX 6 x MAR 1 to inoculation with *Xanthomonas campestris* pv. *phaseoli* under greenhouse conditions. Disease severity ratings of 1–3 are considered resistant, 4-6 intermediate, and >6 a susceptible response.

3.2.2 Inheritance of angular leaf spot resistance in the population VAX 6 x MAR 1

Rationale: Previous studies have shown that the variety MAR 1 is resistant to several ALS races including race 31-55, while the variety VAX 6 is not. In addition, MAR 1 has been shown to yield moderately well under low soil fertility, especially low P, and yields reasonably well under drought conditions although it has low levels of tolerance to *Macrophomina phaseolina*. We screened RILs that were developed by crossing MAR 1 (ALS resistant, but susceptible to CBB) and VAX 6 (susceptible to ALS, but highly resistant to CBB) for resistance to race 31-55 of ALS. These RILs present a very interesting population from which materials with resistance to multiple constraints (e.g., CBB, ALS, and low soil fertility) can be identified. This study was initiated to look at the nature and inheritance of resistance to ALS that is in MAR 1, develop markers that are tightly linked to the resistance genes, and identify materials that are resistant both to CBB and ALS.

Materials and methods: The parents, VAX 6 and MAR 1, and 233 RILS, which were derived from a cross between VAX 6 (ALS susceptible) and MAR 1 (ALS resistant), were planted at Santander de Quilichao and inoculated three times using the ALS race 31-55 local. The first inoculation was done 25 days after planting and further inoculations at weekly intervals thereafter. Evaluations for disease severity were assessed four times, starting 2 weeks after inoculation, using a CIAT 1–9 scale, where 1 represents no visible symptoms and 9 represents severe symptoms and disease expression. Plants that had a rating of 3 or less were considered resistant, 4-6 intermediate, and a rating greater than 6, susceptible.

Results and discussion: Figure 56 shows the response of the 233 RILs to inoculation with *P. griseola*. Preliminary results showed that 40% of the plants were resistant, while 60% had a susceptible response. These materials will be screened again under field and greenhouse conditions using the same ALS race. We are generating molecular profiles of these materials for QTL analysis and identification of markers for MAS breeding.

Conclusions: Pending completion of study.

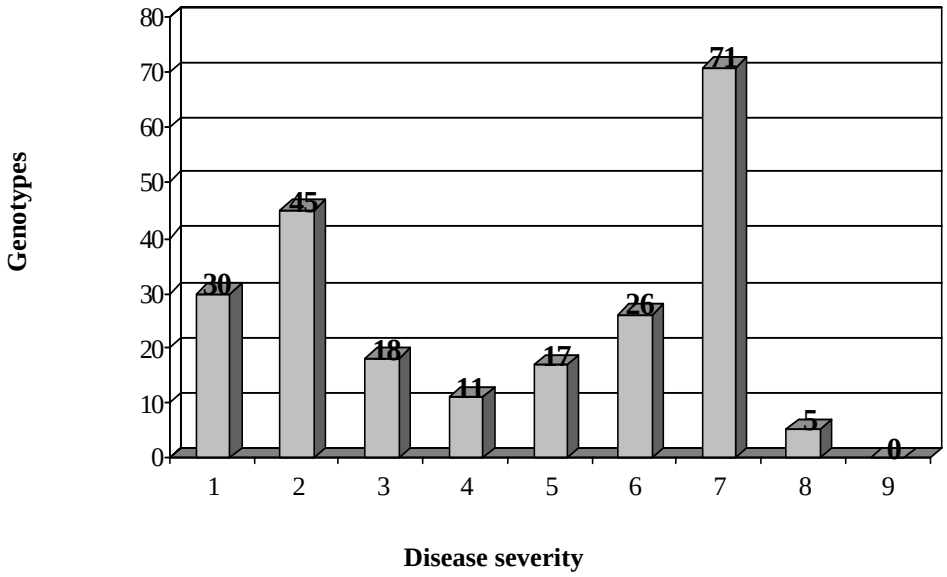


Figure 56. Response of 233 recombinant inbred lines derived from crossing MAR 1 x VAX 6 following inoculation with *Phaeoisariopsis griseola* under field conditions. Plants that had a rating of 3 or less were considered resistant, 4-6 intermediate, and a rating greater than 6, susceptible.

Contributors: G Mahuku, C Jara

3.2.3 Inheritance of angular leaf spot resistance in a population derived from DOR 364 x G 19833

Rationale: Pathogen characterization studies have shown that all *P. griseola* races can be separated into two major groups that correspond to the common bean gene pools. A unique opportunity exists of combining Andean and Mesoamerican genes to have broad spectrum and durable resistance to this pathogen. However, a clear understanding of the nature and inheritance of the identified sources of resistance (resistance genes) is necessary to fully take advantage of this host pathogen co-evolution to manage ALS of common bean. This is an ongoing study to understand the nature of inheritance of ALS resistance in common bean, with the ultimate objective of developing molecular markers that can be used to aid in transferring this resistance to well-adapted, market-class type bean.

Materials and methods: Eighty-seven RILs derived from a cross of DOR 364 (Mesoamerican variety) x G 19833 (Andean variety) were screened under greenhouse conditions using four *P. griseola* isolates. Two Andean and two Mesoamerican isolates were used in this study. With regard to these four isolates, DOR 364 is resistant to the Andean races of *P. griseola,* but highly susceptible to Mesoamerican races, while

G 19833 is highly resistant to the Mesoamerican races that were used, but susceptible to Andean races. The same isolates were used to screen F_1 (100 plants) and F_2 (100 plants) derived from DOR 364 x G 19833 crosses. Evaluations for disease severity were assessed using a CIAT 1–9 scale, where 1 represents no visible symptoms and 9 = severe symptoms and disease expression. Ratings of 1 to 3 were considered resistant and ratings >4 as susceptible.

Results and discussion: Sixteen of the RILS were resistant to the four isolates representing Mesoamerican and Andean races of *P. griseola* (Figure 57). These materials represent interesting sources of ALS resistance that combine Andean and Mesoamerican resistance genes that have been pyramided in the same material. These materials will be screened with more isolates from both Andean and Mesoamerican groups to establish the extent of activity and durability of this resistance. Table 38 shows the distributions of resistance and susceptibility following inoculation with Andean and Mesoamerican isolates of *P. griseola*, while Figure 58 shows the response distribution of F_1 populations following inoculation with Mesoamerican races of *P. griseola*. Preliminary analysis of the results is indicative of two genes conferring resistance to *P. griseola* acting in a dominant manner to Andean races, and in a recessive manner to Mesoamerican races. However, more information will be obtained following QTL analysis and evaluation of backcrosses to DOR 364 and G 19833.

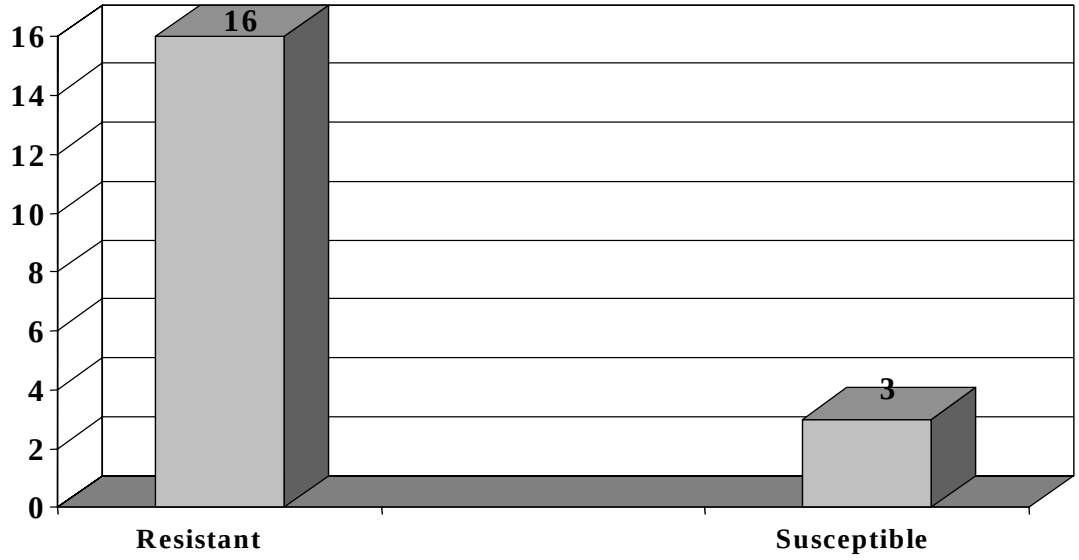


Figure 57. Proportion of DOR 364 x G 19833 recombinant inbred lines that were resistant and susceptible to Andean and Mesoamerican races of *Phaeoisariopsis griseola*.

Table 38. Response of DOR 364 x G 19833 recombinant inbred lines (RILs) and F_1 and F_2 families to inoculation with Andean and Mesoamerican races of *Phaeoisariopsis griseola*.

Isolate	Family	Materials evaluated	Resistant (R)	Susceptible (S)	Ratio	Expected ratio		
						1 gene	2 genes	3 genes
Andean	F_1	100	100	0	1R : 0S			
	F_2	100	87	13	6.7R : 1S	3R : 1S	15R : 1S	63R : 1S
	RILs	87	73	14	5.2R : 1S	1R : 1S	3R : 1S	7R : 1S
Mesoamerican	F_1	100	16	84	1R : 5.3S			
	F_2	100	14	86	1R : 6S	1R : 3S	1R : 15S	1R : 63S
	RILs	87	22	65	1R : 3S	1R : 1S	1R : 3S	1R : 7S

Similar results were observed in the evaluations of 100 plants each of the F_1 and F_2 populations. All F_1 materials were resistant to Andean races of *P. griseola*, while in the F_2 population, 13 were susceptible and 87 were resistant, thus indicating that resistance to this group of isolates might be controlled by two dominant genes (Table 38). F_1 plants that were inoculated with Mesoamerican isolates gave a 1:1 ratio of resistance to susceptible plants (Figure 58). However, in the F_2 population, 14 plants were resistant and 86 were susceptible (Table 38). These results show that recessive genes are conditioning resistance to this race.

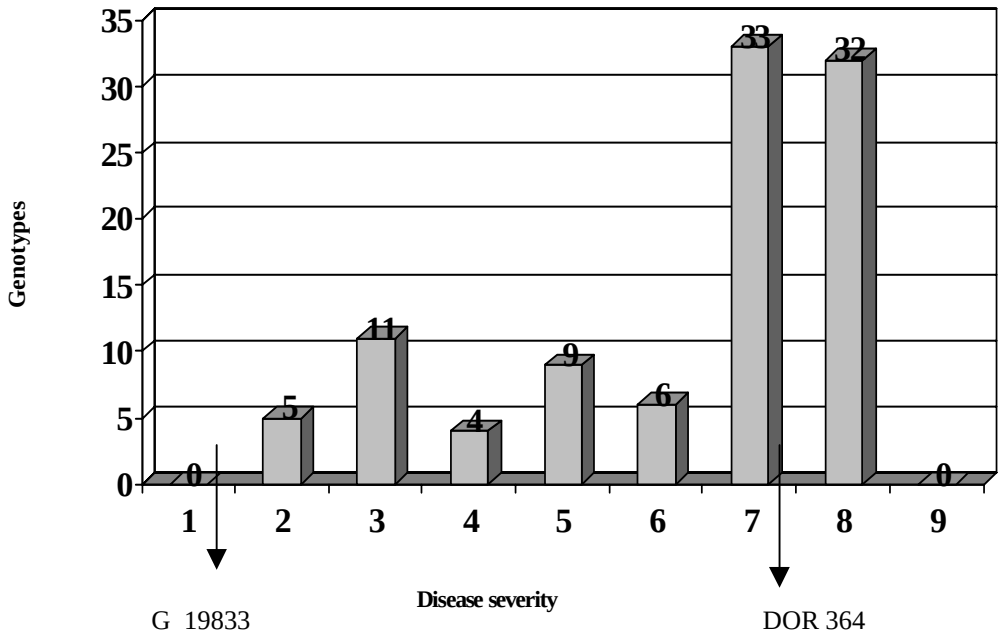


Figure 58. Response of DOR 364 x G 19833 F_1 populations inoculated with Mesoamerican races of *Phaeoisariopsis griseola*. Ratings of 1 to 3 were considered resistant and ratings >4 as susceptible.

Conclusions: The preliminary results reported here show the complex nature of inheritance of *P. griseola* resistance in the two genotypes under study. Resistance to the two major groups of *P. griseola* might be inherited differently, showing recessive genes for Mesoamerican isolates and dominant genes for Andean races. Preliminary analysis shows that at least two genes are conditioning resistance. In addition, there might be minor genes that are modifying the effect of the major genes in a race-specific manner. More information will be obtained following evaluations of the backcrosses to DOR 364 and G 19833, F_3 population, and QTL analysis of molecular profiles of the RILs.

Contributors: C Jara, G Mahuku, H Terán, S Beebe

3.2.4 Transfer of angular leaf spot resistance genes from Mesoamerican climbers to bush beans

Rationale: High levels of resistance to the most virulent and aggressive isolate of *P. griseola* (race 63-63) have been found in some materials from the highlands of Guatemala. Because of adaptation problems, this resistance has to be transferred to adaptable market-class type beans. In addition, the activity of these genes and the number and nature of this resistance has to be elucidated in order to take full advantage in using this resistance to manage ALS. This study is being conducted to transfer resistance genes from Mesoamerican climbers to Mesoamerican bush beans, to study the nature and inheritance of resistance in these climbers, and to develop populations for subsequent tagging of the genes with the broadest activity to facilitate transfer into other market-class type beans.

Materials and methods: Crosses were initiated to transfer ALS resistance in climbers (G 10474, G 10909, G 4691, G 18224, G 3991, and G 14301) into Mesoamerican bush types (G 4691, G 3991, and G 18224). In addition, crosses were made between these climbers and Sprite (a universal susceptible) to facilitate studying the nature and inheritance of ALS resistance in these materials. F_1 populations have been made and are currently being advanced to F_2 as well as backcrossed to resistant and susceptible (Sprite) parents.

Results and discussion: Results are pending evaluations and analysis of populations.

Conclusion: This study is in progress and results are pending evaluation of the generated populations.

Contributors: S Beebe, H Terán, C Jara, G Mahuku

3.2.5 Nature and inheritance of resistance in angular leaf spot differentials

Rationale: Because very little is known about the number of genes conditioning resistance in our differential materials, this has limited our ability to use virulence data to make inferences about our *P. griseola* populations. Knowledge of the resistance genes would facilitate precise definition of *P. griseola* races, their distribution, and relevance, and help in deploying resistant genes in ways that prolong their durability. Also, this would help in identifying the genes to combine for pyramiding in order to achieve long-lasting resistance.

Materials and methods: Crosses were made between PAN 72 and all the Mesoamerican differential varieties, and A 36 and all Andean differential varieties. F_1, F_2, and backcrosses to both the resistance and susceptible sources were recently completed. Host pathogen interactions are pending for this study.

Results and discussion: Results are pending evaluations of populations.

Contributors: G Mahuku, MW Blair, S Beebe

3.2.6 Inheritance of *Ascochyta* resistance

Rationale: Although *Ascochyta* blight is an important limitation in bean production in the moist highlands of the Andes and Africa, no good level of resistance has existed within the primary common bean gene pool until now, except for the variety ICTA Hunapú, which only recently was recognized as resistant. With the prospects of better sources of resistance, it is of interest to understand better the genetic control of this trait.

Materials and methods: Because the best resistance is found in the secondary gene pool, in *Phaseolus polyanthus* (PP) and in *P. coccineus* (PC), it was decided to study inheritance in resistant x susceptible crosses within and between these two species. A highly susceptible interspecific progeny with *P. coccineus* phenotype was identified and eventually coded as ASC 76. The F_1 generations of three crosses of ASC 7 x *P. coccineus* were evaluated in the field for their reaction to *Ascochyta* in seasons 1999B and 2000A, and the F_2 generation in the 2000A season (Table 39). Plants of the F_1 generation were replicated over time. This was possible because the plants were maintained alive between seasons by taking cuttings or by pruning old plants and taking data on regrowth. The F_2 generation was represented by the harvest of four F_1 plants of each cross, and the progeny of each F_1 plant was maintained as a separate subpopulation of 30 to 40 plants each. This permitted observations on possible segregation in the *P. coccineus* parent for resistance genes.

In the course of field evaluations, it was realized that a cross created for studying the resistance to BGYMV (G 35172 x G 35337, DRIN 13229) was also contrasting for resistance to *Ascochyta*. G 35172 is PC and G 35337 is PP. F_2 plants were being increased in the screenhouse to the F_3 generation, thus 144 F_2 plants were transplanted to

the field and inoculated with *Ascochyta* for evaluation in the 1999B season. For this population, no F_1 plants were available, but as in the case of the other populations, harvests from individual F_1 plants were managed as subpopulations to observe possible segregation from the parents.

Statistical analysis was carried out as per Mathers and Jinks (1977)[6] for estimating gene number controlling a quantitative trait, according to the formula:

$$k \text{ (gene number)} = ((P2 - P1)/2)^2 / \sigma^2$$

Results: All resistant parents of the three ASC 76 crosses rated about 3 on a 9-point scale, and the susceptible ASC 76 rated 6.1 (Table 39). Disease reaction in F_1 plants was intermediate between those of the resistant and susceptible parents in every case. In two crosses, the F_1 rating was very close to the mid-parent value (4.5) that would be expected in the case of additive gene action. The exception was the F_1 of ASC 76 x G 35509, which presented a reaction more similar to the resistant parent. In other words, resistance in this F_1 behaved as if it were slightly dominant.

Table 39. Means and ranges of *Ascochyta* reaction in F_1 hybrids evaluated in inoculated field trials in Popayán 1999B – 2000A, and F_2 populations in 1999B.

	F_1 1999B		F_1 2000A		1999B 2000A	F_2 1999B	
	Range	Mean	Range	Mean	Mean F_1	Range	Mean
ASC 76 x G 35509	3 - 7	3.8	2 – 7	3.7	3.7	2.8 - 6.8	4.7
ASC 76 x G 35358	2 - 7	4.4	2 – 7	4.0	4.2	2.3 - 7.5	5.1
ASC 76 x G 35369	2 - 6	4.9	2 – 8	4.3	4.6	2.0 – 7.0	4.4
Parents:							
ASC 76	4 - 8	6.1	4 – 8	6.0	6.1		
G 35509	2 - 4	3.1	1 – 6	2.8	3.0		
G 35358	2 - 5	3.5	1 – 5	2.7	3.1		
G 35369	2 - 5	3.2	2 – 5	2.7	3.0		
Checks:							
G 35182	2 - 3	2.0	1 – 2	1.7	2.0		
ICTA Hunapu	4 - 6	4.4	3 – 6	4.1	4.2		
G 12727	5 - 7	6.0	5 – 8	6.4	6.2		

In the F_2 of the three *P. coccineus* crosses (Table 39) resistance continued to behave as if it were generally additive; even in the population of ASC 76 x G 35509 where some evidence of dominance was observed in F_1. Thus, the general conclusion of additive gene action is maintained.

[6] Mather K, Jinks JL. 1977. Biometrical genetics: the study of continuous variation. Cornell University Press, Ithaca, NY. 382 p.

The population of G 35172 x G 35337, however, presented a slightly different pattern. Because G 35337 is a *polyanthus*, it has higher resistance (rating of 2.1) than the *coccineus* accessions used for the *Ascochyta* inheritance studies. G 35172 (*coccineus*) rated 6.3. The mid-parent of the two would be about 4.2, but the three F_2 subpopulations all presented symptoms of 5.3 to 5.4, or well above the mid-parent and at the upper extreme of the F_2 populations involving *coccineus*. It appears that the resistance of *polyanthus* may tend to be recessive.

Because all four populations consisted of subpopulations derived from individual F_1 plants, each subpopulation was evaluated for mean and variance. In the populations involving a *coccineus* source crossed to ASC 76, those with G 35509 and with G 35358 presented uniform variances, but means were distinct. Thus, one subpopulation was eliminated from the analysis of each population and the remainder was evaluated jointly. In two of the populations, the estimate suggested that two genes might be segregating, while in the third population, three or four genes might differentiate the parents. In the case of the population G 35172 x G 35337, the variances were distinct; thus two subpopulations were analyzed jointly and the third separately. Here also, the variances suggested that two genes might be segregating in the analysis of two subpopulations, and only one gene in the third subpopulation. It is in fact feasible that a single population gives different results, because the parents are possibly heterozygous and segregating, such that subpopulations are distinct.

These estimates are in any case approximate, because the analysis carries with it several assumptions that are of questionable validity in virtually any genetic analysis, especially that all gene effects are equal. Nonetheless, the results were fairly uniform in concluding that resistance is controlled by a limited number of genes, but in most cases more than one gene. For practical breeding purposes, this information is useful and is about as detailed as most genetic information often is.

Conclusions: Results of the genetic studies suggest that the expression of resistance genes in general tends to be additive, although with different parents, resistance could express as slightly dominant in some cases and slightly recessive in others, especially with the *polyanthus* parent that was used. Gene number is probably in the range of 2 to 4. Given the progress in selecting for resistance in the breeding populations and an apparently acceptable heritability, the conclusion of additive gene action would tend also to be confirmed through the breeding efforts.

Contributors: S Beebe, C Cajiao, G Mahuku (IP-1); MC Duque (SB-2)

3.2.7 Quantitative trait locus mapping and heritability of resistance to *Thrips palmi* in common bean

A better understanding of the genetics of insect and disease resistance will lead to improved management of these pests. Basic to this endeavor is a thorough analysis of the QTL that provide pest resistance and how they are organized at the genome level. An appreciation of gene organization will allow plant breeders to use MAS more effectively to pyramid multiple resistance factors into bean varieties with commercial seed types.

Rationale: *Thrips palmi* is a damaging insect pest of common bean and other dicotyledonous crops that was introduced from Asia (Java, Indonesia) into the Americas during the last decade. Starting in the Caribbean, (Cuba, Dominican Republic, Haiti, and Puerto Rico) the species spread rapidly into the United States and northern South America (Brazil, Colombia, Ecuador, and Venezuela). The greatest damage inflicted on common bean production in Colombia is seen in climbing bean varieties that are grown for the fresh market (including snap beans and Cargamanto dry beans). Sequential plantings, common in the production of snap beans, is highly conducive to heavy infestations of thrips and whiteflies, which are synergistic in the damage that they inflict. Misuse of insecticides also can lead to resurgence in thrips populations. The first studies in integrated pest management (IPM) of thrips were conducted recently at CIAT (see IP-1 Project Annual Report). BAT 881 has been a bean genotype with the best thrips resistance. The objective of this research was to study the inheritance and location of QTL controlling the resistance derived from this variety in a RIL population derived from BAT 881 x G 21212.

Materials and methods: The BAT 881 x G 21212 population, consisting of 139 RILs, was evaluated over two seasons at a field site in Pradera, Valle, Colombia. In the first season (semester 1999A-April), the population was planted as an unreplicated trial; while in the second season (semester 1999B-July) it was planted in an RCBD with three repetitions. The parents of the population were included in both seasons. The bean genotypes PVA 773 and RAZ 136 were used as check varieties in the first season, while PVA 773 was used alone in the second season. Infestation by *Thrips palmi* was increased with an initial planting adjacent to a heavily affected snap bean field. The lines were evaluated on a per row basis using a 1-9 scale according to the CIAT standard evaluation (1 = resistant. 9 = susceptible). DNA was extracted from 95 of the RILs by a standard miniprep procedure. One hundred and fifty one RAPD markers were run on these individuals and the segregation information was analyzed to construct a genetic map using the software program MAPMAKER. Quantitative trait loci were identified through single-point regression analysis of the phenotypic data onto the marker genotypes using the software program qGENE.

Results and discussion: The population of RILs was normally distributed for thrips resistance, suggesting that the inheritance of resistance was truly quantitative (Figure 59). Some of the RILs outperformed either parent suggesting transgressive segregation had occurred, however the best lines were different in each season. BAT 881 was the more resistant parent for both resistance scores (damage score = 6.0 in 1999A and 7.5 in

1995B; reproductive adaptation = 7.0 in 1999A and 7.1 in 1999B). However, G 21212 was similar for its reproductive adaptation (9.0 in 1999A and 7.6 in 1999B), but inferior for its susceptibility to thrips damage (8.0 in 1999A and 8.2 in 1999B). Overall the correlation between seasons was moderate (r = 0.277 for damage score and r = 0.371 for reproductive adaptation), while the correlation between the two resistance scores within a season were high (r = 0.873 for 1999A and r = 0.751 for 1999B).

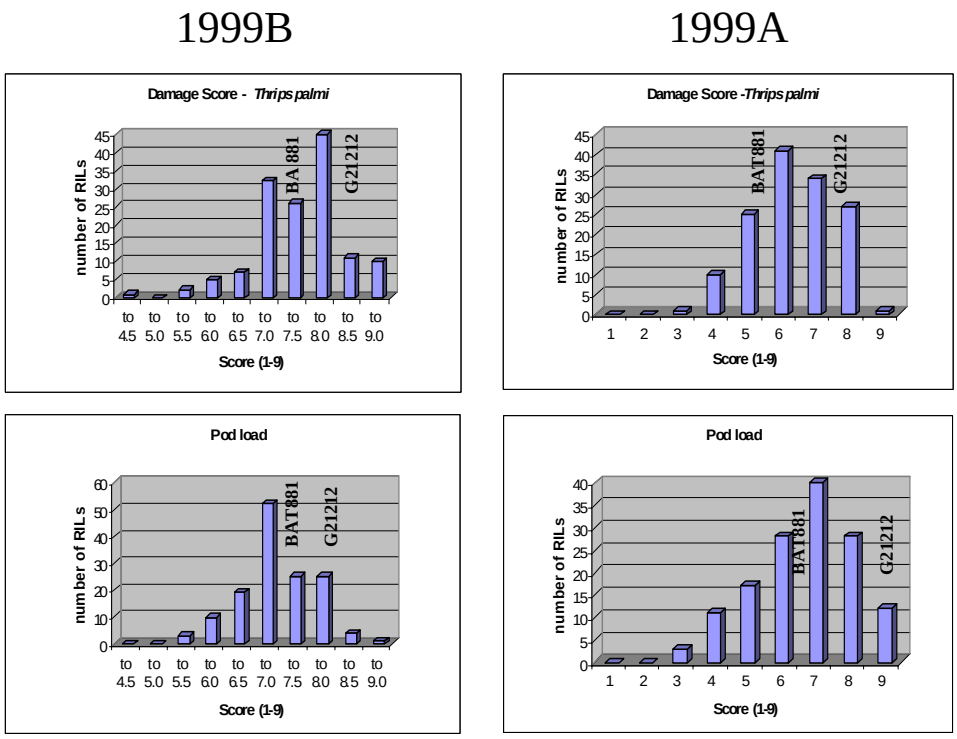


Figure 59. Population distribution for damage caused by *Thrips palmi* and pod load under heavy infestation in an unreplicated field trial conducted in Pradera, Valle, during two seasons (April - 1999A and July - 1999B).

Positive QTLs were found for thrips resistance in both seasons and were associated with both parents. However, it appeared that G 21212 was the principal source of QTLs for resistance to thrips damage, while both BAT 881 and G 21212 were the sources of QTLs for reproductive adaptation under thrips infestation. This varied from 1999A, when a mix of both genotypes provided QTLs for reproductive adaptation, to 1999B, when G 21212 provided all but one of the positive QTLs. An additional single QTL from BAT 881 provided resistance to damage in 1999B, but was unlinked to other markers. The existence of additive QTLs from both parents may explain why transgressive segregation was observed in some of the individual progeny.

Because the trial was replicated in 1999B, QTLs tended to be more highly significant for the 1999B data (up to F-value of 11.2) than for 1999A (maximum F-value of 6.5). In

addition, in 1999B the inheritance of resistance appeared to be fairly simple: only one QTL each could be identified for resistance to damage and reproductive adaptation. The QTL for resistance to damage consisted of six significant markers on a single linkage group and was associated with the G 21212 allele. The QTL for reproductive adaptation was found on a different linkage group and was associated with the BAT 881 allele. This QTL was also observed in the data from the first season, but at a slightly different location along the linkage group.

Broad-sense heritabilities of thrips resistance were estimated on an entry mean basis for the replicated data from the second season. Heritabilities were about 61% for the damage score and 44% for the reproductive adaptation score. The corresponding estimates of narrow sense heritabilities would be similar given that the genotypes were all F_6-derived RILs in which most of the genetic variance is caused by additive rather than dominant gene action.

Conclusions and future plans: The inheritance of thrips resistance was not as complex as might have been expected and the existence of major QTLs will allow thrips resistance to be selected in segregating populations. The heritabilities estimated were relatively high, indicating that the trait can be selected without extensive genotype replication. However, the variability in QTLs detected from one season to the next indicates that GxE interactions may be important. Another population derived from the cross BAT 477 x DOR 364 will be analyzed for thrips resistance to confirm the magnitude of the heritability estimates and to identify whether the QTLs are common across both the BAT 881 x G 21212 and BAT 477 x DOR 364 populations. The four genotypes used to produce these two populations are from the same race of Mesoamerican beans, therefore it will be interesting to see how well the genes for thrips resistance identified in these studies can be transferred into other gene pools. We will attempt to pinpoint the exact location of the QTLs identified so far and may develop new SCAR markers for the most important QTLs. In addition, we will test the validity of microsatellite or SCAR markers for selection of thrips resistance.

Contributors: MW Blair, C Cardona, S Beebe, JM Bueno

3.2.8 Quantitative trait locus mapping of angular leaf spot and anthracnose disease resistance in the common bean cross DOR 364 x G 19833

Rationale: All together, several dozen major resistance genes have been tagged in common bean, mostly through bulked segregant or simple genetic linkage analysis with either RAPDs or SCARs. Reliable markers have been developed for the well-known genes for resistance to BCMV (*I*, *bc-3*, *bc-12*), BGMV (*bgm-1*), rust (*Ur-3*, *Ur-5*, *Ur-11*), and anthracnose (*Co-4*^{2} and *Co-2*). Most of the resistance genes studied have been simply inherited. Relatively fewer studies have looked at the QTLs controlling partial resistance to diseases. The objective of this research was to identify QTLs for resistance to two pathogens of common beans, *Colletotrichum lindemuthianum*, the causal agent of anthracnose, and *Phaeoisariopsis griseola*, the causal agent of ALS, in a well-characterized, genetic mapping population based on the cross DOR 364 x G 19833. Recombinant inbred lines from this population have been a highly useful resource for field and greenhouse studies because they are a genetically stable set of advanced lines that can be tested in replicated trials with a series of disease isolates.

Materials and methods: The 87 RILs of the DOR 364 x G 19833 population were tested with six isolates each of ALS (PG3COL, PG260COL, PGCRI, PG3ELS, PG14HND, and PG12MEX), and anthracnose (CL5DOM, CL20COL, CL43COL, CL77CRI, CL235COL, and CL289COL) by artificial inoculation in the greenhouse. Disease evaluation was done qualitatively by treating disease reaction as a binary trait; and assigning the RILs into a susceptible or resistant category. For six of the disease isolates (PG260COL, PG3ELS, PG14HND, CL20COL, CL43COL, and CL77CRI) data were also taken quantitatively and the individual plants were scored for resistance on a 1-9 scale according to the CIAT standard evaluation (where 1 = resistant and 9 = susceptible). The genetic map for the population consisted of 417 markers (AFLPs, microsatellites, RAPDs, and RFLPs) and was constructed using the software program MAPMAKER. The microsatellite markers were placed at a minimum LOD score of 2.5, while the rest of the markers had a minimum LOD score of 2.0. The QTL were identified using the software program qGENE by: (1) single-marker contingency analysis using chi-square tests comparing the genotypic classes to the categorical data from the qualitative disease evaluation; and (2) single-point regression analysis of the quantitative disease score data onto the marker genotypes. A probability threshold of $P = 0.0001$ was used for the individual marker tests to reduce overall type I error rate to $P = 0.05$, based on the full set of genetic markers used in the experiment.

Results: For anthracnose, significant QTLs were found on chromosome 3, 4, 10, and 11 (Figure 60). Three isolates (CL77CRI, CL20COL, and CL5DOM) uncovered the same resistance QTL on chromosome 3. One of these isolates (CL5DOM) also uncovered a QTL on chromosome 4. Another isolate (CL235COL) uncovered a second QTL for resistance on chromosome 4 proximal to the previous QTL. A fifth isolate (CL43COL) uncovered two QTLs, one on chromosome 10 and another on chromosome 11. No significant QTLs were detected using the isolate CL289COL.

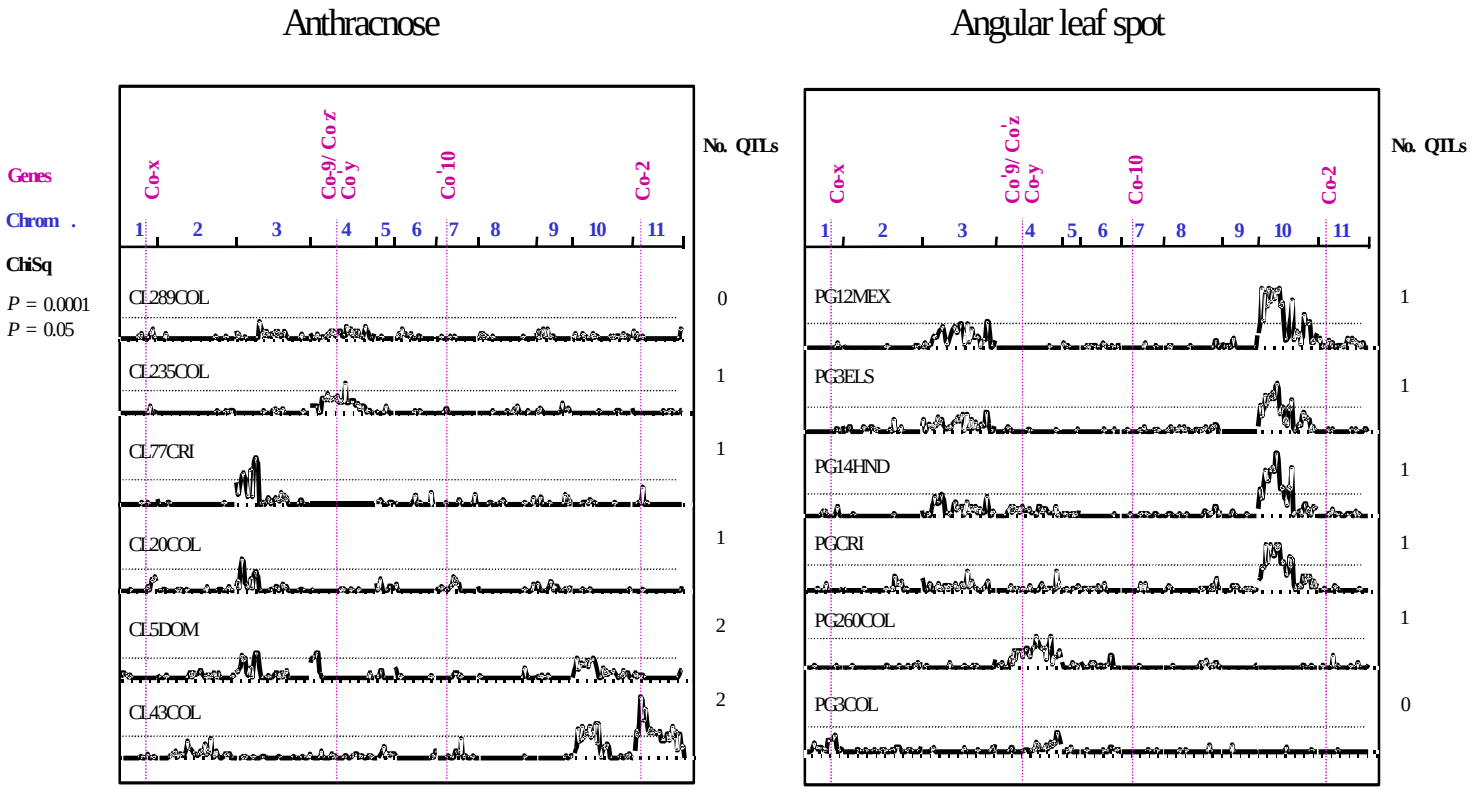


Figure 60. Location of quantitative trait loci (QTLs) for disease resistance identified in the population of DOR 364 x G 19833.

The QTLs on chromosome 4 coincided in location with known resistance factors for anthracnose (*Co-8*, *Co-y*, and *Co-z* cluster) and BGMV resistance. The QTL on chromosome 11 was probably a previously undescribed allele of the *Are* gene (renamed *Co-2*). The resistance QTLs on chromosomes 3 and 10 do not coincide with any previously mapped resistance genes in common bean, but may be allelic to the anthracnose genes that have been characterized and tagged, but not mapped (*Co-1, Co-4*, *Co-5*, and *Co-6*). Alternatively they may be allelic to the QTLs detected in the BAT 93 x JaloEEP 558 population studied by Geffroy et al.[7].

For ALS, significant QTLs were found on chromosomes 3, 4, and 10. Four isolates (PG12MEX, PG3ELS, PG14HND, and PGCRI) uncovered QTLs on chromosome 10 in the vicinity of the QTL for anthracnose described above. The isolate PG12MEX also detected a QTL at one end of chromosome 3. All along chromosome 3, minor genes appeared to be proportioning resistance against several isolates, but their effects were less significant. The isolate PG260COL uncovered a significant QTL on chromosome 4, while the isolate PG3COL uncovered no QTLs for resistance. The QTLs uncovered in this study may be alleles of the two ALS resistance genes, *Phg-1,* and *Phg-2*, which have been tagged with SCARs, but have not been localized on the genetic map of common bean.

Analysis of the quantitative data showed the same QTLs as those of the qualitative evaluation described above. For all the QTLs identified in this study, the positive effect was always from alleles of the resistant parent G 19833, not from those of the susceptible parent DOR 364. In contrast, a similar study showed that the DOR 364 provided resistance QTLs for BGMV.

Conclusions and future plans: The inheritance of resistance to both anthracnose and ALS appeared to be surprisingly simple in the DOR 364 x G 19833 population. A maximum of two chromosomes were involved in resistance to any single isolate and on the average only one significant QTL could be identified per inoculation. Quantum trait loci for disease resistance were found in five basic locations in the genome, both loci in areas that are already known to contain resistance genes and in areas where no resistance genes have been identified previously. A common QTL was found for resistance to both diseases. At resistance gene clusters, resistance genes are often found that function against multiple diseases and minor genes are found associated with major genes. Therefore, it is not surprising that the QTLs for resistance to the two diseases provided by G 19833 are clustered together with known resistance genes or with each other.

Contributors: MW Blair, S Beebe, C Jara, G Mahuku (IP-1); F Pedraza (SB-2)

[7] Geffroy V, Sévignac M, De Oliveira JCF, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M. 2000. Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantum trait loci with genes involved in specific resistance. Mol. Plant Microbe Interact 13: 287-296.

3.2.9 Populations developed for pyramiding and analyzing angular leaf spot resistance genes in common bean

Rationale: Few of the genes involved in resistance to the ALS pathogen, *Phaeoisariopsis griseola*, have been studied systematically. The objective of this work was to create F_2 populations and Andean nearly isogenic lines to analyze ALS resistance genes from the differential varieties and new sources of resistance discovered by the Bean Pathology section.

Materials and methods: The study was initiated with a search for a susceptible Andean bush variety to use in crosses for the genetic studies. The genotypes A 36, AND 277, AND 279, Araucaria 80, CAL 96, CAL 143, G 4494, G 5849, G 18370, G 18355, K 20, and PVA 800 A were inoculated with four strains - two from Colombia and one each from Malawi and Ecuador. The genotype A 36 was selected as the standard susceptible, because in addition to being susceptible to all four strains it has several other advantages including high yield, commercial seed type, and good architecture for a type II bush bean. Genotypes AND 277, AND 279, CAL 96, and CAL 143 were selected as additional donor parents because they were resistant to some strains.

For the inheritance study, the 10 differential resistant lines were crossed within gene pools to the susceptible varieties PAN 72 (Mesoamerican) and A 36 (Andean). For isoline development, the Mesoamerican differentials were crossed across gene pools to the Andean lines, CAL 96, CAL 143, and AND 277. In an effort to pyramid various Andean genes for resistance, four additional Andean sources of resistance from Rwanda (G 20523, G 20743, G 22255, and G 22267) were crossed to four semi-commercial Andean red-mottled bush beans with partial resistance (AFR 735, AND 277, AND 279, and CAL 143). Table 40 summarizes the crosses.

Results and discussion: are pending.

Conclusions and future plans: F_1 plants will be selfed to produce F_2 populations and backcrossed to produce isolines, which will be used for inheritance studies in the most pertinent crosses. It will be interesting to see whether the genes found in these studies are at the same locations of QTLs found in the DOR 364 x G 19833 population. Backcrossing will continue to incorporate the resistance genes from the Mesoamerican differentials into the Andean recipient parents. The pyramiding strategy of crossing between Andean differentials and Andean resistance sources with Andean commercial or semi-commercial genotypes will be tested for efficacy.

Contributors: MW Blair, G Mahuku

Table 40. Crosses made for pyramiding and analysis of angular leaf spot resistance genes in Andean beans.

Type of cross / donor parent	Seed type	Recipient parent	Seed type
PAN 72 x Meso differential			
G 2858	2M,P	PAN 72	1,P
Flor de Mayo	3J,P	PAN 72	1,P
MEX 54	2,M	PAN 72	1,P
BAT 332	2,P	PAN 72	1,P
Cornell 49242	8,P	PAN 72	1,P
A 36 x Andean differentials			
Don Timoteo	7,G	A 36	6M,G
Montcalm	6,G	A 36	6M,G
Amendoim	5M,M	A 36	6M,G
G 5686	4M,M	A 36	6M,G
AND 277	6M,G	A 36	6M,G
AND 279	6M,G	A 36	6M,G
PVA 800 A	6M,G	A 36	6M,G
CAL 143	6M,G	A 36	6M,G
Andean bush x Andean bush			
G 20523	6,G	AFR 735	6M,G
G 20743	6,G	AND 279	6M,G
G 22255	6,G	AND 277	6M,G
G 22267	6,G	CAL 143	6M,G
Andean bush x Meso differentials			
G 2858	2M,P	CAL 96	6M,G
Flor de Mayo	3J,P	CAL 143	6M,G
MEX 54	2,M	AND 277	6M,G
BAT 332	2,P		
Cornell 49242	8,P		

Progress towards achieving output milestones:

- Lines (16) that combine Andean and Mesoamerican ALS resistance genes were identified.

- Inheritance of resistance to Andean and Mesoamerican *P. griseola* races in G 19833 and DOR 364 was found to be complex and different for the two races, being recessive for one race and dominant for another, depending on the source of resistance.

- Lines with high levels of CBB resistance were identified. These will be screened with strains of a diverse origin to establish their suitability to other areas where CBB is a problem.

Activity 3.3 Developing integrated pest management components

Highlights:

- Developed reliable sampling methods for *Thrips palmi* on beans and snap beans.
- Established an action threshold for *T. palmi* on snap beans.
- Identified levels of resistance to insecticides in whitefly (*Trialeurodes vaporariorum*) and thrips populations.
- Developed components and successfully tested management strategies for combined populations of whiteflies and thrips affecting snap beans.

3.3.1 Development of a knowledge base on *Thrips palmi* as a pest of beans and snap beans

Rationale: Information on *T. palmi* as a pest of beans is very scarce. Development of appropriate management strategies for a major insect pest such as *T. palmi* requires basic knowledge of its phenology and distribution in the plant. This knowledge is essential for developing appropriate sampling methodologies, which will in turn facilitate understanding the various ways whereby the insect injures the crop and the response of the crop to injury. Sampling is also an essential tool for making decisions on when and how to intervene in order to bring populations below recommended action thresholds. Because the spatial pattern of individuals in the habitat has a tremendous influence on the sampling plan, we first studied the patterns of aggregation of adult populations on dry beans and on snap beans. We then estimated the optimum sample size and developed a sequential sampling method for adult thrips. With this information, and the yield response of snap beans to insect attack (estimated from replicated plots in the field), an action threshold was calculated.

Materials and methods: Commercial-sized fields of snap beans and dry beans were sampled every 3 days for the duration of two consecutive cropping seasons. Samples were examined for the presence of adults and larvae of thrips. The data thus recorded were analyzed for aggregation, sample size, and sequential sampling determination. Using an effective insecticide and preliminary information on mean adult populations, differential levels of insect attack were established and maintained. Yield responses at different levels of attack were measured. Regression analysis was used to obtain a loss function with which to calculate action thresholds.

Results and discussion:

Sampling methods for *Thrips palmi*. We found that, most frequently, samples of *T. palmi* adults per leaflet taken from natural populations have a variance that exceeds the mean. This was the first indication of aggregation. Further analysis using Taylor's power law showed that thrips populations fit a linear relationship between the base-10 log of mean and variance (Figure 61). Furthermore, the index of dispersion (*b* in the regression equation) was always higher than 1, meaning that populations are highly clumped.

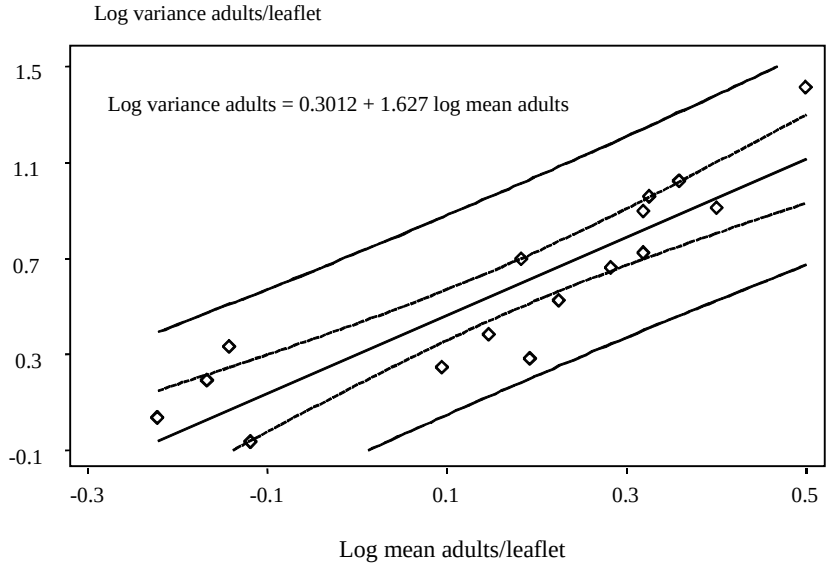


Figure 61. The relationship between mean and variance for adults of *Thrips palmi* per leaflet found on snap beans 49 days after planting.

Using values of *a* and *b* calculated from regression (Figure 61), we then calculated the optimum sample size at three levels of precision (Figure 62). The calculations showed that the required sample size at the 95% confidence level (the one that will assure us that our estimate is within 20% of the true value) is 40 leaflets taken at random in zigzag across the field.

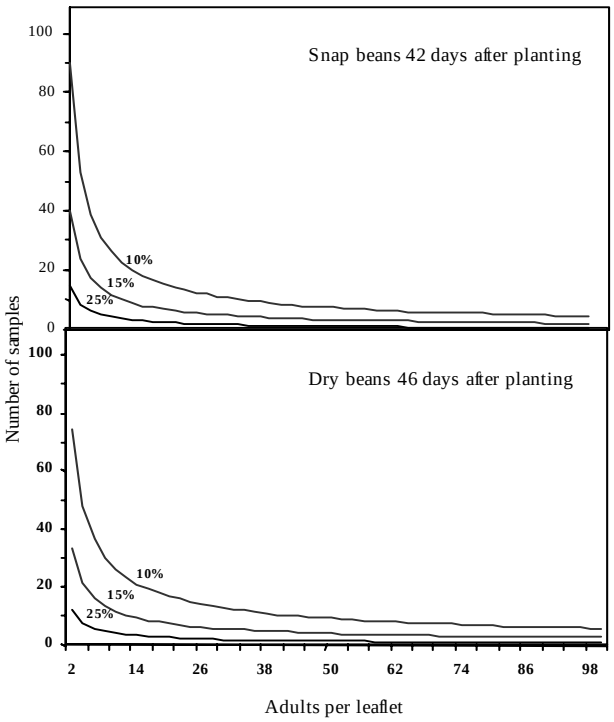


Figure 62. Estimates of optimum sample size for adults of *Thrips palmi* at three levels of precision.

The next step was to develop a sequential sampling method for *T. palm*i adult populations. This is a powerful tool used to categorize the pest population into density classes. If the population exceeds a pre-established action threshold, a curative treatment would be economically justified. Using an action threshold of seven adults per leaflet and values of *a* and *b* estimated from regression in Figure 61, we were able to develop decision lines (Figure 63). These lines are used to take decisions in the field. The number of samples taken is not fixed. Instead, after each sample, the cumulative number of insects is plotted on the graph. If the number falls above the upper line, treatment is necessary. If the number falls below the lower line, no treatment is necessary. In either case, sampling is stopped as soon as a line is crossed. If the cumulative number of insects sampled remains between the lines, sampling is continued.

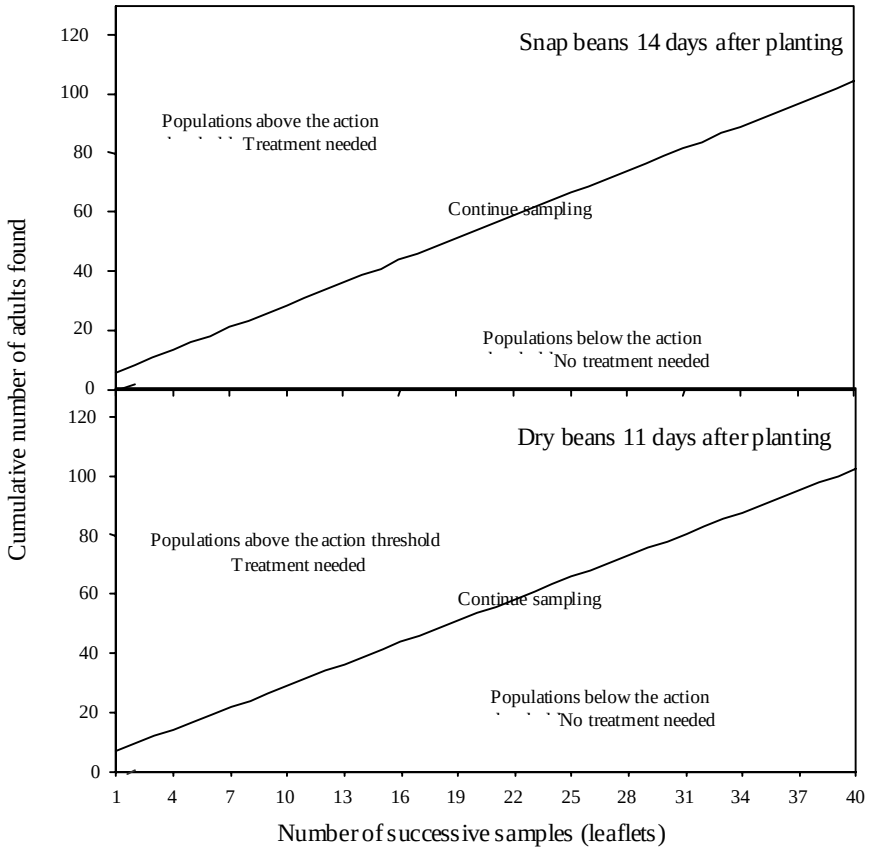


Figure 63. Sequential sampling plans for adults of *Thrips palmi* on dry beans and snap beans.

Contributors: J Osorio, C Cardona

Action thresholds for *T. palmi* on snap beans

Because insecticides are likely to remain the principal method of controlling *T. palmi*, decision aids for the rational and efficient use of insecticides can be important in the development of rational management strategies. One such tool is the action threshold, the level of population that warrants the economic use of a pesticide. In three consecutive trials, the response of snap beans to differential levels of attack by *T. palmi* adults was similar (Figure 64). The mean loss function (*b* in the regression) was 424 kg ha^{-1}. The action threshold calculated by means of the formula ranged from 7.1 to 8.0 adults per leaflet. Of course, the action threshold varies as the price of snap beans in the market changes (Figure 65).

$$AT = \frac{\text{Cost of control}}{\text{Price of produce X loss function X efficiency of control}}$$

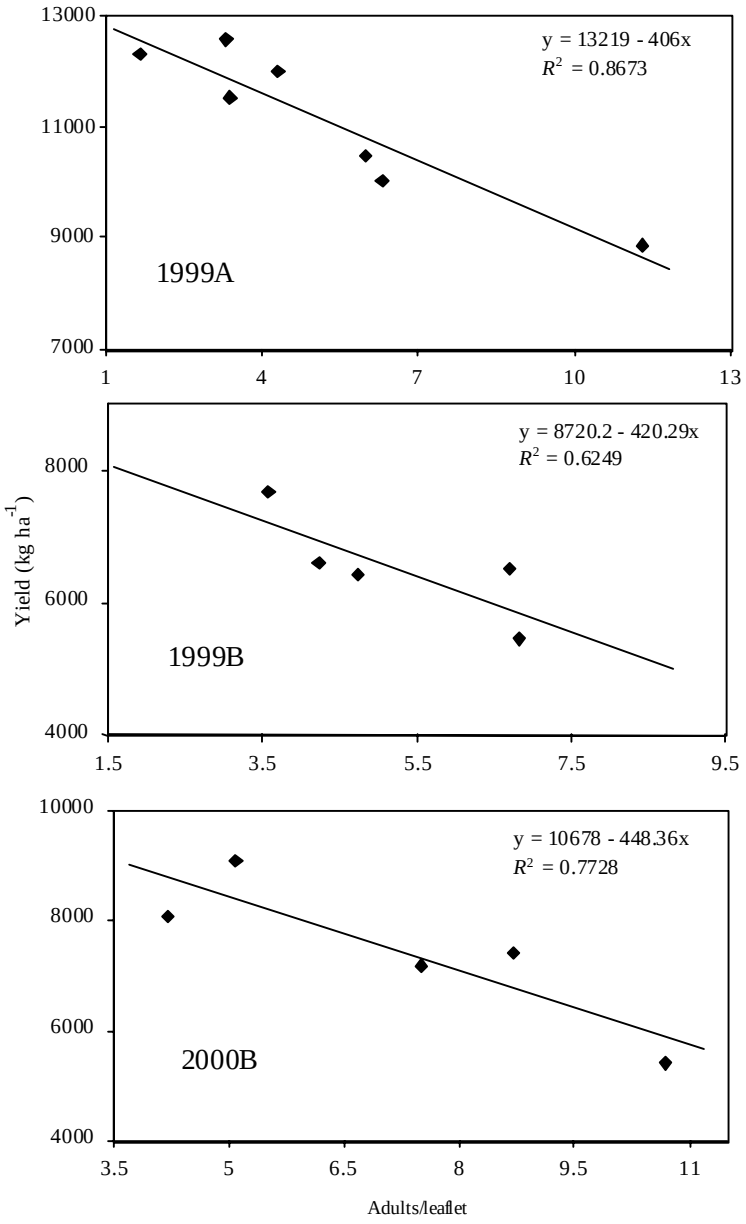


Figure 64. The relationship between adult *Thrips palmi* infestation and yields of snap beans.

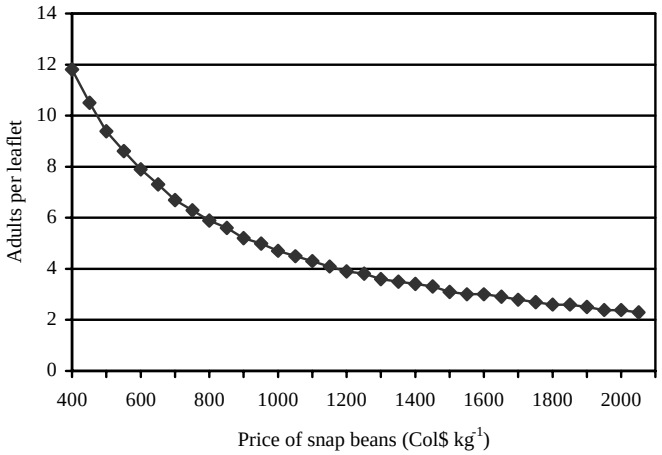


Figure 65. Action thresholds for *Thrips palmi* control as influenced by the market value of snap beans in a region of Colombia.

Losses in these trials were calculated. Results indicated that *T. palmi* is arguably one of the most important pests of snap beans. At high levels of population, the damage potential of thrips can be as high as 4.8 tons per hectare or almost 45% of the yield potential (Table 41).

Table 41. Yield losses caused by *Thrips palmi* on snap beans at varying levels of infestation.

Trial No./season	Level of infestation (adults/leaflet)	Losses	
		(t ha^{-1})	(%)
1/1999A	1.7	0.7	5.2
	3.3	1.3	10.1
	3.6	1.5	11.1
	4.1	1.7	12.6
	6.0	2.4	18.4
	6.3	2.6	19.3
	11.3 (Check)	4.6	34.7
2/1999B	3.6	1.6	18.0
	4.2	1.9	21.0
	4.8	2.1	24.0
	6.7	3.0	33.5
	6.8 (Check)	3.0	34.0
3/2000B	4.2	1.9	17.6
	5.1	2.3	21.4
	7.5	3.4	31.5
	8.7	3.9	36.5
	10.7 (Check)	4.8	44.9

Contributors: JM Bueno, J Díaz, C Cardona

Survey of thrips-affected areas in the Cauca Valley of Colombia

T. palmi has become a major pest of vegetables and beans in Colombia. A preliminary survey showed that snap beans, dry beans, pepper, melon, squash, and cucumber are the crops most affected by this pest in the Cauca Valley of Colombia. All farmers interviewed attempt chemical control. Conventional insecticides (organophosphates, carbamates, and pyrethroids) are ineffective. Some farmers are beginning to use new generation pesticides such as imidacloprid and compounds of biological origin such as abamectins and spinosad. Pesticides of biological origin can become powerful tools within an IPM system. Spinosad, for example, has shown a certain degree of selectivity for the control of thrips and there are no signs that *T. palmi* has become resistant to this new compound. Laboratory tests conducted at CIAT showed generalized susceptibility to spinosad in seven races tested, with a small increment of the LC_{50} (Figure 66) in Pradera, a hot spot for the insect where farmers usually abuse pesticides.

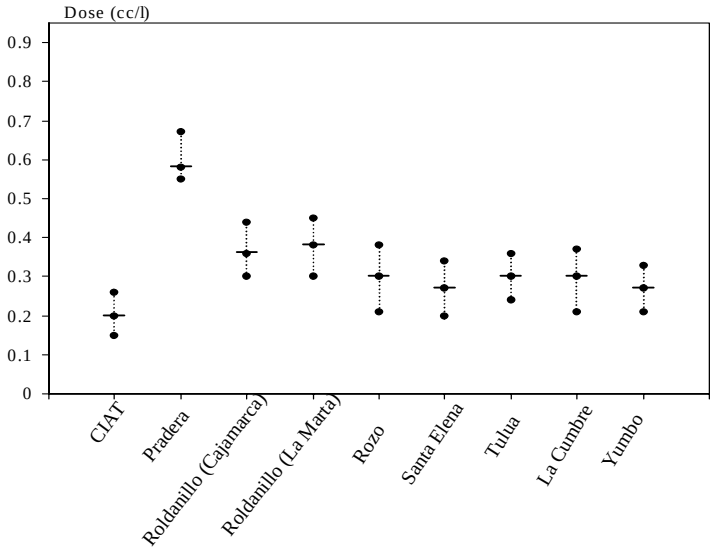


Figure 66. LC_{50} (—●—) and 95% fiducial limits (⁞) for nine races of *Thrips palmi* adults tested for toxicological response to spinosad. Two LC_{50} are considered significantly different if their 95% fiducial limits do not overlap.

Contributors: IC Durán, C Cardona

3.3.2 Monitoring of insecticide resistance levels in whitefly populations

Rationale: As indicated in the 1999 Annual Report, monitoring of insecticide resistance levels in adult populations of the greenhouse whitefly, *Trialeurodes vaporariorum*, is a major objective of the Systemwide Whitefly IPM Project. This work continued in 2000 with the calculation of baseline data for three additional insecticides and determination of diagnostic doses for five compounds. For the first time, we also calculated baseline data for nymphs of *T. vaporariorum* with three insecticides that are being used by farmers in Colombia and elsewhere.

Materials and methods: Baseline data and diagnostic dosages were calculated using mass rearings of susceptible strains of *T. vaporariorum* maintained at CIAT for several years. LC_{50} and LC_{90} values are calculated by exposing whitefly adults to increasing doses of a given insecticide in insecticide-coated vials and submitting the data to probit analysis. Once the baseline data are obtained, diagnostic doses (those causing mortalities of 95% or more in a susceptible strain) are tested. The diagnostic doses will be used to monitor resistance under field conditions by means of the insecticide-coated glass vials technique. Similar data are obtained for first instar nymphs using the dipping technique. Foliage is submerged for a few seconds in increasing concentrations of the insecticide tested, and mortality is recorded 72 h after treatment. Mortality data are used to calculate LC_{50} and LC_{90} values. Diagnostic doses are established as indicated above.

Results and discussion: Table 42 presents baseline data for monocrotophos, λ-cyhalothrin, and imidacloprid with the reference strain of *T. vaporariorum*. LC_{50} and LC_{90} values reflect toxicities to susceptible strains of whiteflies that have not been exposed to insecticides for over 10 years. Establishing baseline data for different insecticides is a fundamental step in resistance studies because the data thus obtained will serve for future comparisons in order to detect changes in insecticide resistance levels. Besides, calculation of baseline data permits the determination of diagnostic doses that can be used with less logistical difficulties in extensive monitoring of resistance such as the one carried out in the Andean zone.

Table 42. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* to five insecticides. Conventional insecticides were tested using insecticide-coated glass vials. Imidacloprid tests were conducted using the technique developed by Cahill et al[a].

Insecticide	N	LC_{50} (95% FL)[b]	LC_{90} (95% FL)[b]	Slope ± SEM	X^2
Monocrotophos	710	9.72 (6.77-13.37)	175.4 (115.5-299.8)	1.02 ± 0.08	4.80
λ cyhalothrin	714	9.38 (6.13-13.59)	264.9 (170.9-455.3)	0.88 ± 0.06	0.97
Imidacloprid	921	5.70 (4.6-6.9)	28.4 (20.9-44.9)	1.85 ± 0.22	7.80

a. Cahill M, Gorman K, Day S, Denholm I, Elbert A, Nauen R. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (*Homoptera*: *Aleyrodidae*) 1996. Bull. Entomol. Res. 86: 343-349.

b. Imidacloprid in ppm. All others in µg a.i./vial.

Data in Table 42 and data obtained in 1999 were used to determine diagnostic doses for five insecticides. The diagnostic doses (those causing at least 95% corrected mortality) in Table 43 will be used in 2001 to monitor the occurrence of resistance under field conditions. The baseline data for nymphs of *T. vaporariorum* in Table 44 will be used in 2001 to calculate corresponding diagnostic doses and to initiate field monitoring of resistance.

Table 43. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* adults to varying doses of five insecticides. Conventional insecticides were tested using insecticide-coated glass vials. Imidacloprid tests were conducted using the technique developed by Cahill et al[a].

Insecticide	Doses[b]	Percentage corrected mortality	
		Test No. 1	Test No. 2
Monocrotophos	300	100.0	96.8
	20	46.3	43.7
	1	18.9	20.6
	0.3	7.4	10.2
λ-cyhalothrin	500	100.0	96.9
	50	51.6	61.1
	1.25	21.1	25.8
	0.16	8.4	6.9
Bifenthrin	5	100.0	96.9
	1.25	50.5	54.6
	0.3125	26.3	28.9
	0.0781	5.3	10.9
Carbofuran	5	97.5	96.5
	1.25	65.0	64.0
	0.3125	31.0	28.0
	0.0781	15.0	17.0
Imidacloprid	40	97.9	93.9
	5	47.4	43.3
	0.625	12.4	16.8
	0.156	3.1	5.6

a. Cahill M, Gorman K, Day S, Denholm I, Elbert A, Nauen R. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (*Homoptera*: *Aleyrodidae*) 1996. Bull. Entomol. Res. 86: 343-349.

b. Imidacloprid in ppm. All others in µg a.i./vial.

Table 44. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* nymphs to three insecticides.

Insecticide	N	LC_{50} (95% FL) [a]	LC_{90} (95% FL) [a]	Slope ± SEM	X^2
Diafenthiuron	620	2.8 (0.8-6.3)	90-2 (40.0–346.8)	0.85 ± 0.1	11.30
Buprofezin 1st test	338	1.39 (0.98-1.87)	8.32 (5.7–14.0)	1.64 ± 0.18	3.90
Buprofezin 2nd test	600	0.5 (0.15-0.98)	7.68 (5.2-11.6)	1.01 ± 0.16	3.09
Imidacloprid	318	15.1 (9.5–22.2)	193.1 (118.2-382.0)	1.15 ± 0.12	5.30

a. Dosages in ppm of commercial formulation.

Contributors: I Rodríguez, C Cardona.

3.3.3 Management strategies for whiteflies and thrips

Rationale: Greenhouse whitefly and thrips have become the objects of excessive pesticide use by snap beans farmers. Insecticides will probably continue to be used until more biologically based management systems can be developed. However, with the information gathered so far on sampling methods, the action thresholds already developed for whitefly and thrips, and knowledge on the effectiveness of less toxic insecticides, systems can be developed that will at least contribute to reduce pesticide use. We report here on a series of field trials aimed at developing ways to reduce insecticide use in snap beans (see also the 1999 Annual Report).

Materials and methods: Different management tactics for whiteflies and thrips were compared using a Latin square design. There were five treatments:

(1) Seed treatment with imidacloprid;
(2) Imidacloprid applied as drench 15 days after planting;
(3) Timely application of imidacloprid or an equally effective insecticide when populations reach pre-established action thresholds;
(4) A simulation of farmers' current control practices; and
(5) Check (no insecticide use).

Whitefly and thrips populations were monitored twice a week. Yields and quality of the produce were recorded. Partial budget analysis was performed to compare benefit/cost ratios of the different treatments.

Results and discussion: As shown in Figures 67 and 68, significant differences occurred among treatments for levels of infestation, thus reflecting differential effectiveness of treatments in maintaining *T. vaporariorum* and *T. palmi* under control. Best results in two consecutive trials were obtained with the use of imidacloprid applied as drench 14-18 days after planting or as seed treatment at planting, followed by the timely application of effective foliar insecticides at pre-established action thresholds. Selective and timely use of insecticides resulted in a significant reduction in the number of applications per cropping season, from 7 in the "farmer's practices" treatment, to 2-3 in other treatments (Table 45). In terms of yields, the selective use of insecticides as seed treatment or as drench maintained adequate production levels. This is highly important. Our objective is to reduce pesticide use while maintaining yield levels. A management system that sacrifices yields for the sake of reducing insecticide use will not have impact in a farming community.

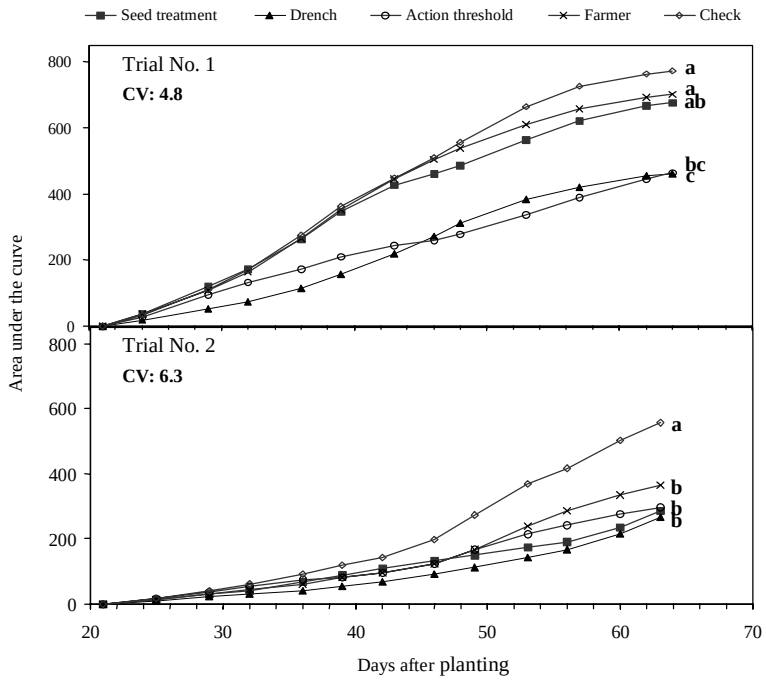


Figure 67. Areas under the curve for *Thrips palmi* larval populations on snap beans resulting from different management strategies. Letters show statistical differences at the 5% level (LSD Test). See text for explanation of treatments.

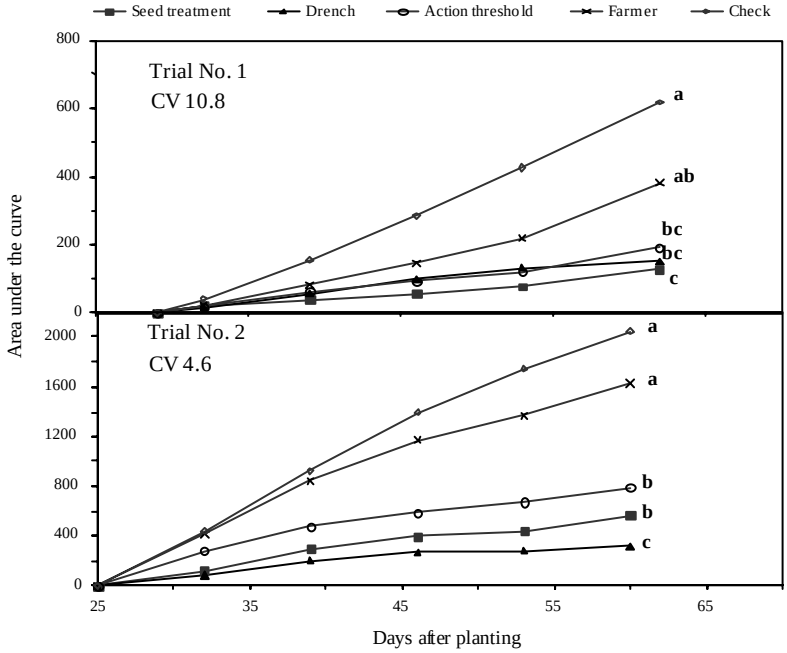


Figure 68. Areas under the curve for *Trialeurodes vaporariorum* nymphal populations on snap beans resulting from different management strategies. Letters show statistical differences at the 5% level (LSD Test). See text for explanation of treatments.

Table 45. Effect of different insect management strategies on damage levels and yields of snap beans; *Trialeurodes vaporariorum* and *Thrips palmi* acting together as key pests[a].

Treatment[b]	No. of applications		Farmers' appraisal[c]		Yield (t ha^{-1})		Percentage loss[d]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Seed treatment	3	2	3.6 ab	2.6 b	5.2 ab	10.3 a	25.7	-
Drench application	3	2	4.2 a	3.0 ab	7.0 a	10.0 a	-	2.9
Action threshold	4	3	3.2 bc	2.2 bc	4.0 b	8.4 a	42.8	18.4
Farmer's practices	7	7	2.6 c	4.2 a	4.0 b	9.6 a	4.3	6.8
Check	0	0	2.6 c	1.0 c	1.5 c	5.5 b	78.6	46.6

a. Means followed by the same letter in a column are not significantly different at $P = 0.05$.
b. See text for explanation.
c. On a scale of 1 (very poor) to 5 (excellent).
d. With respect to the best treatment.

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Progress towards achieving output milestones:

- We have greatly increased the body of knowledge on both *T. palmi* and *T. vaporariorum* as pests of beans and snap beans.

- This knowledge is being used to achieve output milestones: to develop and implement management systems aimed at reducing pesticide use.