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:

- Pathotype diversity in *Colletotrichum lindemuthianum* was shown to be not congruent to common bean gene pools, thus revealing a lack of co-evolution between this pathogen and common bean gene pools.

- Pathotype and genetic diversity for *C. lindemuthianum* was shown to be highest in the Middle American region, and the Middle American population was shown to contain all alleles found in the Andean population and more, suggesting that this pathogen originated and was disseminated from this region.

- It was shown that the loci specific markers (eight) that we developed could differentiate between *Phaseolus griseola* groups while revealing structuring geographically and according to host gene pool.

- Sequence information of the ribosomal genes was used to show that *P. griseola* is taxonomically close to *Mycosphaerella* spp. (anomorph *Cercospora* spp.) and *Cladosporium fulvum*.

- It was shown that 12 species of the genus *Pythium* can be found in association with common bean, although not all of them are pathogenic.

- A potential biocontrol agent (*Pythium oligandrum*) was identified among samples collected from bean roots.

- The sources of resistance to whitefly-transmitted geminiviruses selected so far have been effective to control at least four different geminivirus species known to attack beans in Latin America.

- Identification of the causal agent of the "amachamiento" disease of beans led to the implementation of effective management control practices directed towards the chrysomelid vectors of the causal virus.

3.1.1 Genetic diversity of *Colletotrichum lindemuthianum* and implications for anthracnose management

Justification: Knowledge of the genetic structure of plant pathogen populations is important because it reflects the evolutionary history and the capacity of the pathogen to evolve (Burdon 1993). This information has direct applications in diverse agricultural ecosystems (McDonald and McDermott, 1993). For example, the amount of genetic variation that is maintained in a

population reflects on the capacity of a pathogen to evolve, and this information is important in choosing (defining) a control strategy that is most effective. Pathogen populations with high levels of genetic variation will probably adapt more rapidly to new fungicides or introductions of new resistance gene(s) than will pathogens with very little variation. Thus, the information on the distribution of genetic variation can be used to select the most effective resistance gene(s) that are better suited to manage populations of the pathogen in a given locality. Anthracnose of common bean is one of the most devastating diseases of common bean, *Phaseolus vulgaris*. The disease is caused by the hemibiotrophic fungus, *Colletotrichum lindemuthianum*, and is highly variable. More than 200 pathotypes of this pathogen have been descried to date and this great pathotypic variability is an obstacle in the use of host resistance to manage the disease. Because the knowledge of the genetic structure of the pathogen has direct applications in disease management, this study was designed to use molecular markers and virulence to look at the genetic structure of *C. lindemuthianum* as a prerequisite to revealing other virulence aspects of this fungus. The information obtained was used to test the hypothesis that *C. lindemuthianum* coevolved with its common bean host. If this is true, then control strategies that seek to transfer and pyramid resistance genes from different *Phaseolus* gene pools may provide a more durable resistance to this disease. In addition, this information will help orient and make breeding strategies and other control methods more effective.

Materials and Methods: 200 isolates from different geographical zones were used in this study. Each of these isolates was classified as Andean or Mesoamerican, according to the gene pool of the variety from where they were collected. In order to establish if *C. lindemuthianum* isolates can be separated into Andean and Mesoamerican groups, according to the gene pools of the host, the isolates were characterized on 12 host differential varieties, three Andean and nine Mesoamerican, and classified as Andean or Mesoamerican based on their interaction with these genotypes (Table 39).

Molecular variation was assessed using eight random microsatellite primers (RAMS)—(CA)n, (AG)n, (GT)n, (TG)n, (CT)n, (CGA)n, (CCA)n, (ACA)n—and two primers derived from the DNA sequence of repetitive-elements - polymerase chain reaction (REP-PCR). For REP-PCR analysis, primers derived from the enterobacterial repetitive intergenic consensus (ERIC) sequence and the conserved repeated bacterial DNA element “BOX” were used. The banding profiles were analyzed using cluster and multiple correspondence analysis using statistical analysis system (SAS). Phenetic analysis was conducted using NTSYS and AMOVA programs.

Results and Discussion: 90 races were identified among the 200 *C. lindemuthianum* isolates that were studied, and all the races identified have been reported before (Table 39). Most of the races (54) were restricted to a single country, whereas only 36 were found in at least two countries. Race 9 was the most frequent and widely distributed, being recovered from both Andean and Mesoamerican genotypes and from eight countries (Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Mexico, and Peru) (Table 39). Most of the pathotype diversity was found in the Middle American region (Figure 41). Isolates belonging to race zero (nine isolates) only infected the universal susceptible variety, La Victorie. These isolates were recovered from the Andean as well as the Mesoamerican regions, and were collected either from wild beans or landraces, but never from advanced or bred lines. The isolates probably represent a group of simple isolates capable of infecting simple varieties that do not contain resistance genes effective against *C. lindemuthianum*.

Classification of isolates based on the host gene pool from which the isolates were collected identified 62 from Andean and 138 from Mesoamerican varieties. Virulence characterization identified only 19 isolates as true Andean, while 172 were Mesoamerican.

Table 39. Race, number of isolates, and origin of 200 *Colletotrichum lindemuthianum* isolates analyzed in this study.

Race	Number of isolates	Origin[a]
0	9	ARG, CRI, ECU, PER
1	9	ARG, BRA, COL, CRI, ECU, HND, PER
2	2	ARG, PER
3	10	AFR, ARG, COL, DOM, MEX, PER, USA
4	7	ARG, ECU, PER
5	6	ARG, COL, ECU, HND, MEX, PER
6	4	ARG, ECU, PER
7	8	ARG, COL, CRI, ECU, HND, NIC, PER
9	11	BRA, COL, CRI, ECU, GTM, HND, MEX, PER
11	2	BRA, COL
15	2	COL, ECU
17	1	BRA
23	2	BRA, EUR
31	1	BRA
36	1	ARG
38	5	CRI, DOM, ESP
39	1	DOM
47	1	DOM
64	1	ARG
65	3	ARG, BRA, ECU
73	8	CRI, GTM, MEX, PRI, USA
81	1	JAP
87	1	ARG
89	1	CRI
121	1	BRA
128	2	ECU, PER
129	5	COL, CRI, ECU, HND, MEX
132	2	ARG, PER
133	4	ARG, COL, ECU, PER
137	3	COL, CRI, HND
139	1	COL
192	1	MEX
256	4	ECU
257	1	MEX
261	2	ECU
320	1	MEX
385	3	COL, MEX
388	1	COL
393	2	CRI, MEX
448	1	MEX
449	1	MEX
453	1	MEX
457	1	CRI
513	1	BRA
515	2	COL

Continued.

Table 39. Continued.

Race	Number of isolates	Origin[a]
517	1	COL
521	4	COL, CRI
523	1	HND
525	1	COL
529	1	BRA
535	2	BOL, BRA
593	1	AFR
641	1	COL
647	1	COL
651	2	COL
653	1	COL
905	1	CRI
1025	2	CRI, GTM
1033	2	COL, CRI
1049	1	CRI
1088	1	MEX
1089	1	MEX
1093	1	MEX
1097	1	MEX
1153	2	CRI, ECU
1161	1	CRI
1217	1	HND
1417	1	HND
1433	1	CRI
1435	1	CRI
1473	2	HND, MEX
1481	1	CRI
1489	1	CRI
1497	1	CRI
1545	5	COL, CRI, GTM
1549	1	GTM
1561	1	CRI
1609	1	CRI
1645	1	GTM
1929	2	CRI
1945	1	CRI
1985	1	CRI
1993	1	CRI
2001	1	CRI
2009	1	CRI
2047	1	CRI
3481	5	CRI, ARG
3545	1	CRI
3977	1	CRI
3933	1	CRI, ARG

a. Country abbreviations: AFR = Africa, ARG = Argentina, BOL = Bolivia, BRA = Brazil, COL = Colombia, CRI = Costa Rica, DOM = Dominican Republic, ECU = Ecuador, ESP = Spain, EUR = Europe, GTM = Guatemala, HND = Honduras, JAP = Japan, MEX = Mexico, NIC = Nicaragua, PER = Peru, PRI = Puerto Rico, and USA = United States of America.

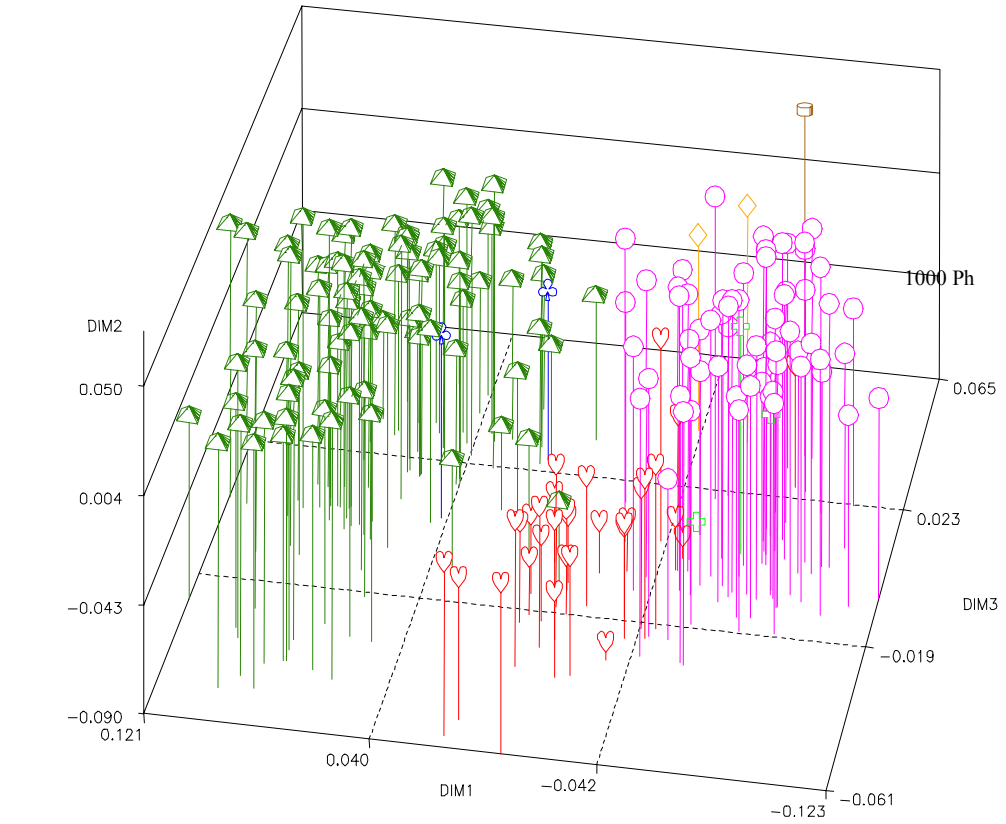

Figure 41. Three-dimensional representation of 200 *Colletotrichum lindemuthianum* isolates following multiple correspondence analysis of random amplified microsatellites (RAMS) data.

There was no correlation between the group designation of the pathotype and the gene pool of the host from where the isolate was collected. Four races (2, 6, and 36 from Argentina and race 4 from Ecuador) that were characterized as Andean based on their ability to infect only Andean differential varieties had been collected from Mesoamerican varieties (Table 40). An additional 42 isolates (32 races) collected from Andean varieties were classified as Mesoamerican showing that Andean and Mesoamerican isolates can infect both gene pools of the host. These results

show that the race diversity in *C. lindemuthianum* appears not to be structured according to gene pools that have been defined for common bean.

Table 40. Classification of 200 *Colletotrichum lindemuthianum* isolates according to common bean gene pools, Andean (A) and Mesoamerican (M).

Isolate origin	No. of isolates	No. of races	Cultivar gene pool	Isolate phenotype[a]
Africa	2	2	A	M
Argentina	4	4	M	A
	1	1	M	-
	11	11	M	M
Bolivia	1	1	M	M
Brazil	11	11	M	M
Colombia	13	12	A	M
	22	13	M	M
Costa Rica	1	1	A	A
	1	1	M	-
	1	1	A	M
	38	34	M	M
Dominican Republic	3	3	A	M
	1	1	A	A
Ecuador	5	2	A	A
	1	1	M	A
	13	11	A	M
	3	2	M	M
	5	1	A	-
Spain	3	1	A	A
Europe	1	1	A	M
Guatemala	6	6	M	M
Honduras	1	1	M	-
	10	9	M	M
Japan	1	1	M	M
Mexico	23	19	M	M
Nicaragua	1	1	M	M
Peru	4	3	A	A
	1	1	A	-
	9	8	A	M
Puerto Rico	1	1	M	M
USA	2	2	M	M

a. Virulence phenotype is based on the interaction of the isolate with a set of 12 common bean differential genotypes. The dash - represents isolates that were classified as pathotype zero, that did not infect any of the differential genotypes except the universal susceptible variety, La Victorie.

Analysis of RAMS data identified 199 haplotypes, while REP-PCR identified 174. High levels of genetic variation (97%) were observed within the entire *C. lindemuthianum* population. Cluster and multiple correspondence analysis did not separate isolates into Andean and Mesoamerican isolates. Isolates collected from Andean genotypes or characterized as Andean were distributed together with isolates classified or collected from Mesoamerican genotypes, showing the lack of

congruency between *C. lindemuthianum* and common bean groups (Figure 41). No correlations were observed between molecular markers and virulence ($P < 0.05$), but RAMS were highly correlated to REP-PCR ($r = 0.52$, $P < 0.0001$). In addition, AMOVA analysis revealed a lack of genetic differentiation (Gst = 0.08) within *C. lindemuthianum*. Taken together, the virulence and molecular results show that *C. lindemuthianum* might not reveal the common bean gene pool specialization that *P. griseola* has revealed.

Conclusions: Results from this study confirm that *C. lindemuthianum* is a highly variable pathogen, as shown by the number of races identified (90) and the high levels of genetic diversity (97%). The greatest levels of race diversity were observed in the Middle American regions. Neither molecular markers nor virulence analysis could differentiate isolates according to the gene pools that have been defined for common bean, showing that *C. lindemuthianum* is not a pathogen that is strongly structured according to host gene pools. Therefore, the strategy of combining Andean and Mesoamerican resistance genes to manage anthracnose might not be the best for this disease because transferring resistance genes between gene pools might not confer durable resistance.

References:

Burdon, J.K. 1993. Genetic variation in pathogen populations and its implications for adaptation to host resistance. *In*: Jacobs, T.; Parlevliet, J.E. (eds.). Durability of disease resistance. Kluwer, Dordrect, NL.

McDonald, B.A.; McDermott, J.M. 1993. Populations genetics of plant pathogenic fungi. Bioscience 43: 311-319.

Contributors: G. Mahuku, J.J. Riascos.

3.1.2 Development of locus specific microsatellite markers for identification and characterizing *P. griseola* groups and application to pathogen characterization

Rationale: Pathogen characterization continues to play a pivotal role in the quest to find a long-lasting solution to the management of the ALS pathogen. This information is important in (1) identifying suitable resistance genes for use in breeding programs or to deploy in a given area, and (2) monitoring the rate of change in the pathogen population, which is important in predicting the durability of a chosen control method. Previous analysis of *P. griseola* using molecular and virulence markers has divided isolates into groups that are structured along host gene pools as well as geographically. In the process, molecular fragments that can differentiate between these groups, both according to host and geographical specialization, were identified. The goal of this work was to develop locus-specific markers for use to differentiate isolates into identified groups, thereby avoiding the lengthy process of using virulence analysis. In addition to differentiating between groups, these markers would be used for assessing the genetic structure of *P. griseola* by tracing specific alleles within populations. In addition, this will provide a standardized tool for researchers working or interested in ALS research, thereby facilitating characterization and exchange of information. This will undoubtedly make research on ALS non-duplicative and more efficient on a global scale. Previous analyses have all shown that there are two major groups, therefore breeding for resistance should not focus individual races, but should focus specific groups.

Material and Methods: We took the approach of amplifying *P. griseola* genome using random microsatellite primers that are anchored at their 5' end. To make sure that the amplified region was a true microsatellite, we sequenced the fragments and found out that they contained microsatellite sequences. In a preliminary study, we amplified 131 isolates from different geographical regions and defined *P. griseola* groups using eight microsatellite primers. Based on the molecular profiles, eight fragments were selected that could distinguish between the different *P. griseola* groups (Andean isolates from Africa, Afro-Andean, Andean from Latin America, Mesoamerican from Africa, and Mesoamerican from Latin America), in addition to revealing the genetic diversity that exists within *P. griseola* (Figure 42 and Table 41). These markers were used to analyze DNA from 56 isolates and the data were analyzed using cluster and multiple correspondence analysis and isolates separated into groups depending on the molecular profiles. In addition, 37 isolates collected from nine countries were characterized using host differential varieties (Table 42).

Table 41. Random amplified microsatellites (RAMs) primer, band size, and the profiles expected for the different groups of *Phaeoisariopsis griseola*[a].

Primer- band size	Andean (LA)[b]	Andean (Africa)	Afro-Andean	Mesoamerican (Africa)	Mesoamerican (LA)
AG-650				+	+
AG-680	+	+	+		
CA-420	+			+	+
CA-500				+	+
CA-550	+	+	+		
CA-680	+	+	+		
GT-650				+	+
GT-820	+	+	+		+

a. + presence of band.
b. LA = Latin America.

Results and Discussion: 27 races were identified among 37 isolates characterized (Table 42). All the races identified in this study have been reported before. Of the 56 isolates characterized using molecular markers, 34 were identified as Andean, while 21 were Mesoamerican (Table 43). These markers can differentiate between Andean and Mesoamerican groups and also between Andean isolates from Africa and Latin America, as well as Mesoamerican form Africa and Latin America. Because "Afro-Andean" isolates have been found to be 98% similar to Andean isolates from Africa, these markers cannot differentiate between the two. However, since major groups have been identified, the breeding strategy that targets groups, rather than individual races is likely to be more efficient and produce good results. Apart from the ease with which these markers can be applied, they also reveal host and geographical differentiation that has been observed for this fungus.

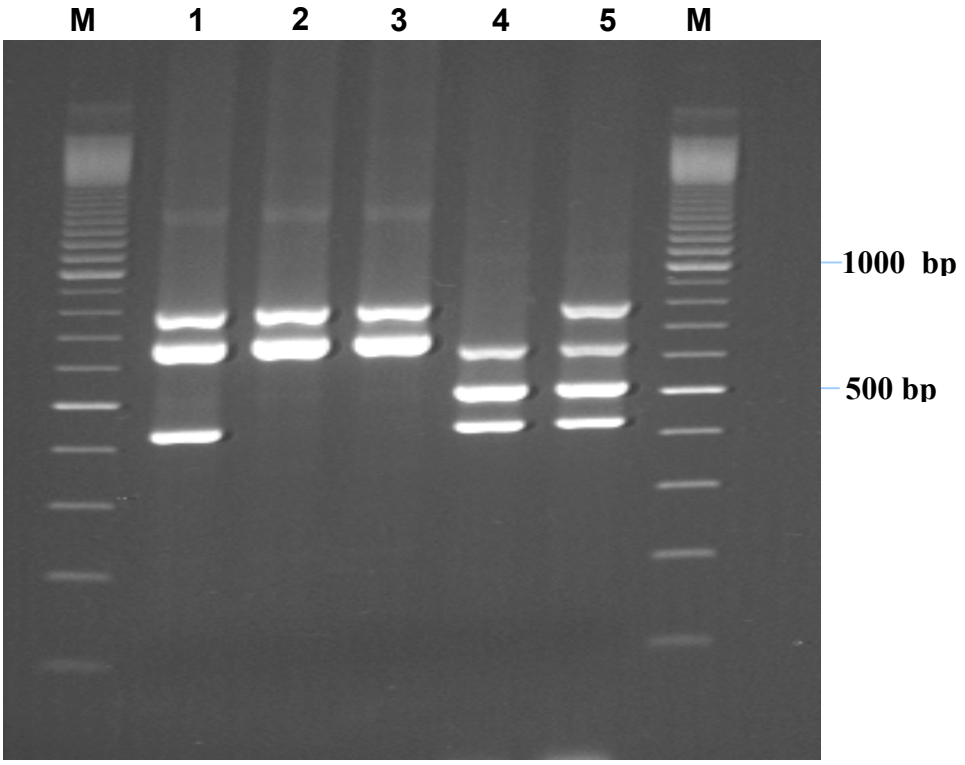

Figure 42. Banding patterns for differentiating groups of *Phaeoisariopsis griseola*. Lane 1 contains Andean isolates from Latin America, Lane 2 Andean isolates from Africa, Lane 3, Afro-Andean isolates, Lane 4 contains Mesoamerican isolates from Africa, and Lane 5 are Mesoamerican isolates from Latin America. Lane M is the 100 bp DNA stepladder.

Conclusions: Results from this study show that the molecular markers can be used to differentiate isolates into groups that have been defined for *P. griseola*. In addition, the markers can reveal host as well as geographical differentiation. This is ideal for breeding programs tasked with developing materials resistant to certain groups of pathogens, depending on the location being targeted. Final testings are being made to validate the specificity of these markers to *P. griseola*, after which they will be distributed to collaborating partners. Use of the standardized system will make characterization of *P. griseola* more efficient, will get rid of the need for the lengthy use of host differential genotypes, and will facilitate exchange of information between partners.

Table 42. Virulence phenotypes of 37 *Phaeoisariopsis griseola* isolates characterized during 2001.

Isolate	A	B	C	D	E	F	G	H	I	J	K	L	Race	Group	Location	Country
15 ARG	a	b	c	d	e	f	g	h	i	j	-	-	63-15	Meso		Argentina
9 ARG1	a	b	c	d	e	f	g	h	i	-	-	l	63-39	Meso		Argentina
312 COL 1	a	-	c	d	e	f	-	-	-	-	-	-	61-0	Andean	Tenerife	Colombia
50 CRI 1	a	-	c	-	e	-	g	h	i	j	k	l	21-63	Meso	Alajuela	Costa Rica
51 CRI 1	a	b	c	d	e	-	g	h	-	j	-	l	31-43	Meso	Puriscal	Costa Rica
52 CRI 1	a	b	c	d	e	-	g	h	-	j	-	l	31-43	Meso	Puriscal	Costa Rica
53 CRI 1	a	b	c	d	e	-	g	h	i	j	-	l	31-47	Meso	Puriscal	Costa Rica
54 CRI 1	a	b	c	d	e	-	g	h	i	j	-	l	31-47	Meso	Puriscal	Costa Rica
49 CRI 1	a	b	c	d	e	-	g	h	-	j	k	l	31-59	Meso	Alajuela	Costa Rica
55 CRI 1	a	-	c	-	-	-	g	h	-	-	-	l	5-35	Meso	Puriscal	Costa Rica
48 CRI 1	a	b	c	d	e	f	g	h	i	j	-	l	63-47	Meso	Puriscal	Costa Rica
14 HTI	a	b	c	d	-	-	g	h	i	-	k		15-23	Meso	Damien	Haiti
18 HTI	a	b	c	d	-	-	g	h	i	-	-	l	15-39	Meso	Deron	Haiti
13 HTI	a	b	c	d	-	-	g	h	i	-	k	l	15-55	Meso	Camperrin	Haiti
9 HTI	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	San Mathurine	Haiti
10 HTI	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	Rempe	Haiti
11 HTI	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	Rempe	Haiti
17 HTI	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	Deron	Haiti
12 HTI	a	b	c	d	e	-	g	h	i	-	k	l	31-55	Meso	Camperrin	Haiti
15 HTI	a	b	c	d	e	-	g	h	i	-	k	l	31-55	Meso	Landrace	Haiti
8 HTI	a	b	c	d	e	-	g	h	i	-	-	-	31-7	Meso	San Mathurine	Haiti
16 HTI	a	b	c	-	-	f	g	h	i	-	-	l	39-39	Meso	Deron	Haiti
19 HTI	a	b	c	-	-	-	g	h	i	-	-	l	7-39	Meso	Deron	Haiti
33 HND1	a	b	c	d	e	-	g	h	i	j	k	-	31-31	Meso	Danli	Honduras
30 HND1	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	Danli	Honduras
32 HND1	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	El Obrero	Honduras
36 MWI	a	-	-	-	-	-	g	h	i	-	-	-	1-7	Meso	Bembeke	Malawi
37 MWI	a	b	c	-	e	-	g	h	i	j	-	l	23-47	Meso	Bembeke	Malawi
24 NIC1	a	b	c	-	e	-	g	h	i	j	k	-	23-27	Meso	Carazo	Nicaragua
23 NIC1	a	b	c	d	e	-	g	h	i	j	k	-	31-27	Meso	Matagalpa	Nicaragua
25 NIC1	a	b	c	d	e	-	g	h	i	j	k	l	31-63	Meso	San Marcos	Nicaragua
6 DRC	a	b	c	d	e	-	-	-	-	-	-	-	31-0	Andean	Kabare	RD Congo
8 DRC	a	b	c	d	e	-	-	-	-	-	-	-	31-0	Andean	Kabare	RD Congo
10 DRC	a	b	c	d	e	f	-	-	i	-	-	l	63-36	Meso	S. Kivu	RD Congo
12 DRC	a	b	c	d	e	f	g	h	i	-	-	l	63-39	Meso	Mwenge	RD Congo
11 DRC	a	b	c	-	-	-	g	h	i	-	-	-	7-7	Meso	Kalehe	RD Congo
11 RWA	a	b	c	d	e	-	g	h	i	-	-	l	31-39	Meso	Rubona	Rwanda

Table 43. Classification of 56 *Phaeoisariopsis griseola* isolates[a] into groups based on molecular profiles from eight markers.

Isolates	Evaluated bands								PG-Group
	Primer AG		Primer CA				Primer GT		
	650	680	420	500	550	680	650	820	
15TZA		X			X	X		X	Andean from Africa
16TZA		X			X	X		X	Andean from Africa
18TZA		X			X	X		X	Andean from Africa
19TZA		X			X	X		X	Andean from Africa
22TZA		X			X	X		X	Andean from Africa
24TZA		X			X	X		X	Andean from Africa
25UGD		X			X	X		X	Andean from Africa
26TZA		X			X	X		X	Andean from Africa
27TZA		X			X	X		X	Andean from Africa
28TZA		X			X	X		X	Andean from Africa
30TZA		X			X	X		X	Andean from Africa
31TZA		X			X	X		X	Andean from Africa
33TZA		X			X	X		X	Andean from Africa
34MWI		X			X	X		X	Andean from Africa
35TZA		X			X	X		X	Andean from Africa
36TZA		X			X	X		X	Andean from Africa
39TZA		X			X	X		X	Andean from Africa
45TZA		X			X	X		X	Andean from Africa
46TZA		X			X	X		X	Andean from Africa
50TZA		X			X	X		X	Andean from Africa
52TZA		X			X	X		X	Andean from Africa
53TZA		X			X	X		X	Andean from Africa
55TZA		X			X	X		X	Andean from Africa
57TZA		X			X	X		X	Andean from Africa
5COG		X			X	X		X	Andean from Africa
60TZA		X			X	X		X	Andean from Africa
61TZA		X			X	X		X	Andean from Africa
62TZA		X			X	X		X	Andean from Africa
6COG		X			X	X		X	Andean from Africa
6TZA		X			X	X		X	Andean from Africa
7COG		X			X	X		X	Andean from Africa
8COG		X			X	X		X	Andean from Africa
8TZA		X			X	X		X	Andean from Africa
9TZA		X			X	X		X	Andean from Africa
10TZA	X		X	X			X		Mesoamerican from Africa
12TZA	X		X	X			X		Mesoamerican from Africa
17TZA	X		X	X			X		Mesoamerican from Africa
1MDG	X		X	X			X		Mesoamerican from Africa
27UGD	X		X	X			X		Mesoamerican from Africa
28UGD	X		X	X			X		Mesoamerican from Africa
2MDG	X		X	X			X		Mesoamerican from Africa
32TZA	X		X	X			X		Mesoamerican from Africa
33MWI	X		X	X			X		Mesoamerican from Africa
36MWI	X		X	X			X		Mesoamerican from Africa
37MWI	X		X	X			X		Mesoamerican from Africa
43TZA	X		X	X			X		Mesoamerican from Africa
44TZA	X		X	X			X		Mesoamerican from Africa
48TZA	X		X	X			X		Mesoamerican from Africa
49TZA	X		X	X			X		Mesoamerican from Africa
4MDG	X		X	X			X		Mesoamerican from Africa
58TZA	X		X	X			X		Mesoamerican from Africa
59TZA	X		X	X			X		Mesoamerican from Africa
5MDG	X		X	X			X		Mesoamerican from Africa
7MDG	X		X	X			X		Mesoamerican from Africa
7TZA	X		X	X			X		Mesoamerican from Africa

a. Origin of isolates: COG = Congo, MDG = Madagascar, MWI = Malawi, TZA = Tanzania, and UGD = Uganda.

Contributors: G. Mahuku, M.A. Henríquez.

3.1.3 The taxonomic position of *Phaeoisariopsis griseola* and relationship between defined ALS groups

Rationale: Characterization of *P. griseola* using microsatellites and intergenic spacer region (IGS)- DNA restriction fragment length polymorphisms (RFLP) has revealed two groups, Andean and Mesoamerican, that correspond to common bean gene pools (CIAT 2001, p. 106-110). In addition, significant genetic differentiation was shown between Andean isolates from Africa and Latin America (Gst = 0.68) as well as Mesoamerican isolates from the two continents (Gst = 0.25). To further understand the relationship between these two groups and elucidate the taxonomic classification of *P. griseola*, we sequenced the internally transcribed spacer region (ITS1 and ITS2) and 5.8 S ribosomal gene, and compared them between the *P. griseola* representative of each group as well as sequences retrieved from GenBank. The intergenic transcribed spacer (ITS) region of the rDNA is a fast evolving region of the genome and can be used to look at the taxonomic relationship within and between species. The objective of this study was to use the sequences of the ITS regions of rDNA to determine the relationship between *P. griseola* groups (Andean, Mesoamerican, and "Afro-Andean", as well as find or establish the taxonomic position of *P. griseola* by comparing these sequences to known sequences of other species. In addition, precise definition of the taxonomic position of *P. griseola* will allow us to adopt methods or strategies that have been used for other, but closely related, pathogens to identify and isolate avirulence genes. This of course, will depend on how closely *P. griseola* is related to pathogens for which work in this area has progressed.

Materials and Methods: 10 *P. griseola* isolates were selected for this study. Two were Mesoamerican isolates from Africa, two Mesoamerican isolates from Latin America, two Andean isolates from Africa, two Afro-Andean isolates, and two Andean isolates from Latin America. The ITS region of each isolate was amplified using conserved primers (ITS4, which anneals to the 18S ribosomal gene, and ITS5, which anneals to the 28S). The fragment was digested with six (Taq 1, Cfo 1, Tsa II, Mbo 1, Alu III, and Alu 1) restriction endonucleases (4-base cutters) to determine differences between groups. The PCR products were purified using the Wizard PCR purification kit and direct sequencing of the fragment was done using the ABI PRISM 377 DNA sequencer. Sequences were analyzed using the SeqMan program and aligned using the MegAlign program in the software package, DNASTAR. A blast search was done; sequences with some homology to *P. griseola* were retrieved from Genbank and used to determine the taxonomic position of *P. griseola*.

Results and Discussion: The fragment was 580 bp for all the isolates tested in this study (Figure 43). No differences were found in the size of the ITS fragment (580 bp) between the different *P. griseola* groups, or isolates, thus revealing no apparent polymorphisms in the size of the amplified fragment. Restriction analysis of the amplified ITS region revealed no polymorphisms in the restriction site for all *P. griseola* isolates analyzed (Figure 44). Sequence comparison showed no differences between ITS sequences of *P. griseola* groups. Blast results showed that *P. griseola* was closely related to *Mycosphaerella* spp., *Pseudocersospora, Cercospora, Cladosporium fulvum*, and *Cladosporium* spp. (Figure 45). A lot of work has been done on *Cladosporium fulvum* and several avirulence genes have been cloned.

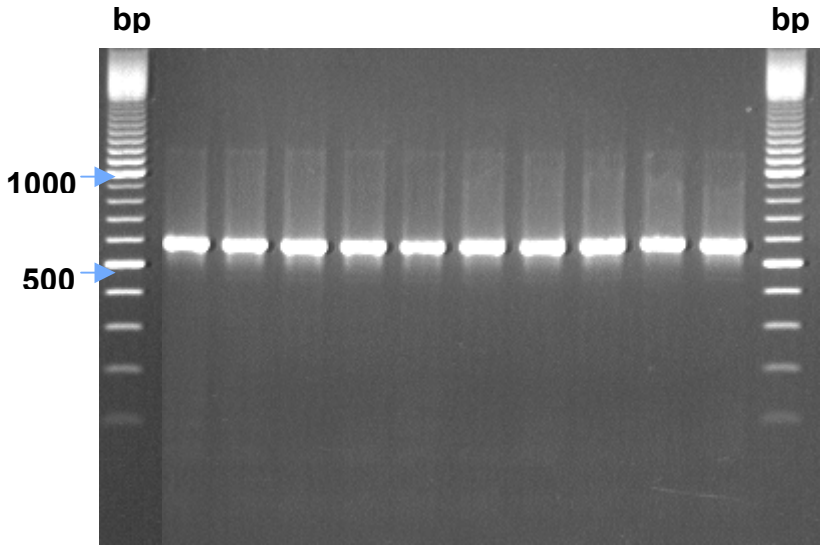

Figure 43. Polymerase chain reaction (PCR)-amplified products of the ITS region of different *Phaeoisariopsis griseola* isolates.

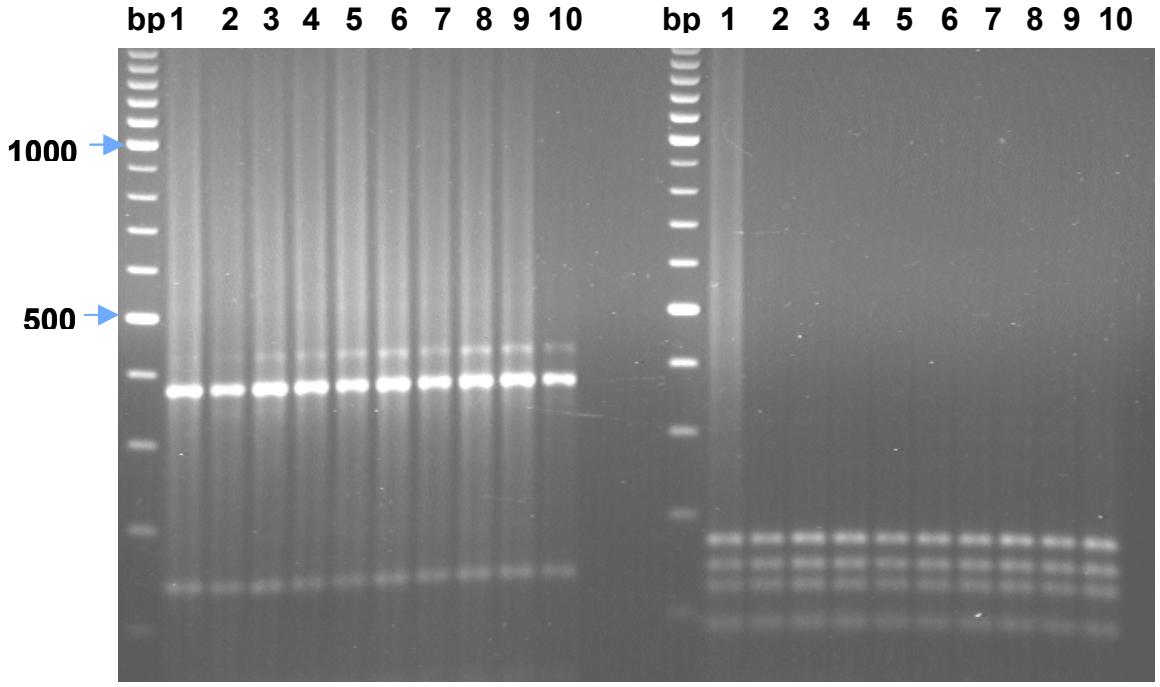

Figure 44. Banding patterns from digestion of amplified ITS region of ribosomal genes using the restriction endonucleases (A) *Alu* I and (B) *Cfo* I. Lanes 1 – 2 (Andean isolates from Africa); 3-4 "Afro-Andean"; lanes 5-6 (Mesoamerican isolates from Africa); lanes 7-8 (Andean isolates from Latin America), 9 – 10 (Mesoamerican isolates from Latin America). Lanes marked with bp represent the 100 bp DNA stepladder.

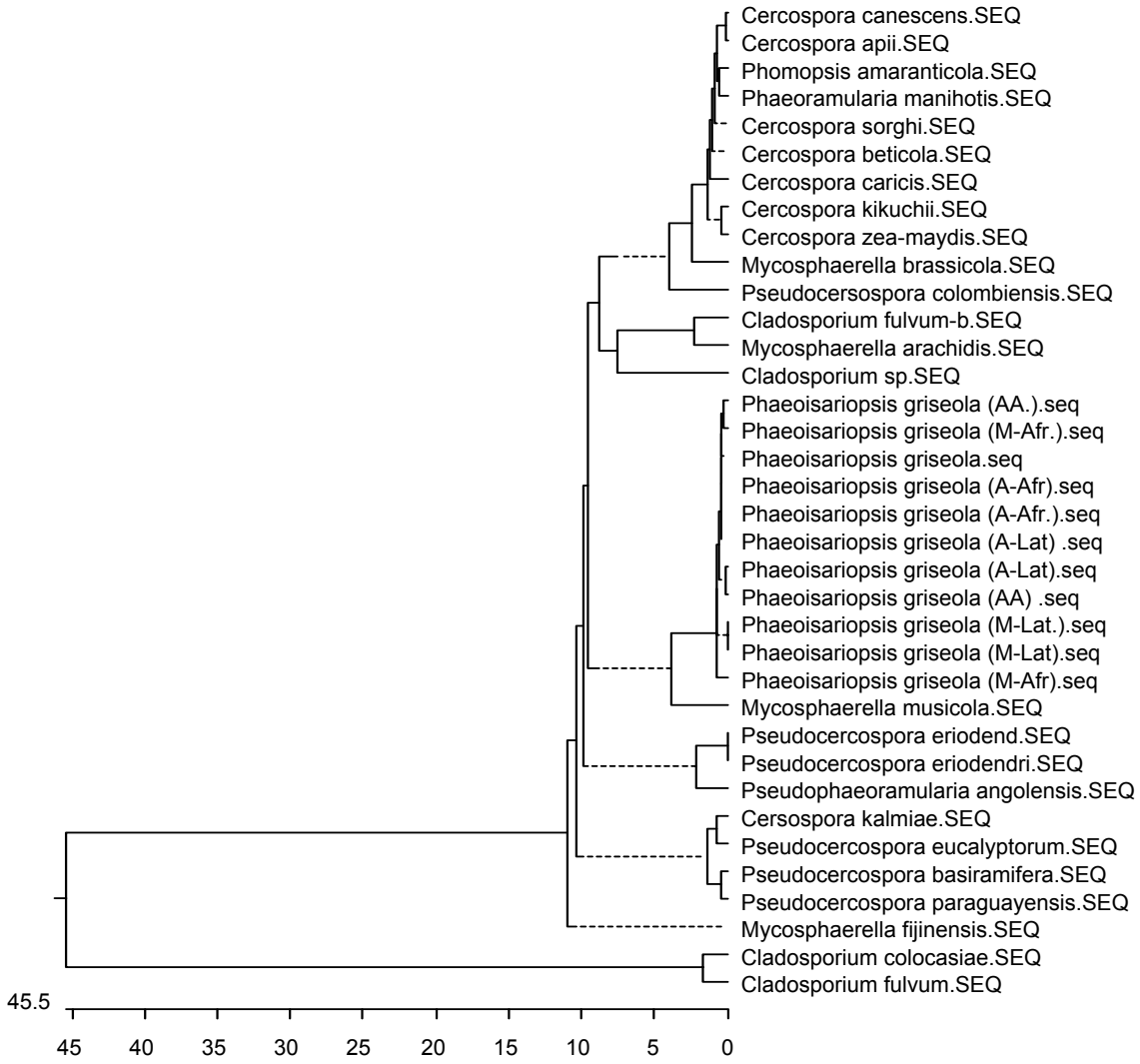

Figure 45. Taxonomic classification of *Phaeoisariopsis griseola* showing the fungi that are closely related to it based on sequences of the ITS regions of the ribosomal genes.

Conclusions: Although the ITS region is known to be highly variable, no differences were found between the different *P. griseola* isolates, showing that this region is fairly conserved within this species. The differences that have been observed between *P. griseola* groups are from other regions, especially those associated with pathogenicity, thus revealing the importance of host specialization in influencing the structure of *P. griseola*. Taxonomically, *P. griseola* was close to *Mycosphaerella* spp. (anamorph *Cercospora* spp.) and *Cladosporium fulvum*. *C. fulvum* is one of the most studied fungi and some avirulence genes have been cloned. It will be interesting to see if there is homology between the *C. fulvum* avirulence genes and avirulence genes in *P. griseola* or if the elicitor approach that was used to clone the avirulence gene in *C. fulvum* will work for *P. griseola*.

Reference:

CIAT. 2001. Annual Report 2000 Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: G. Mahuku, M.A. Henríquez.

3.1.4 Identification of *Pythium* species prevalent in bean growing areas from Uganda, Rwanda, and Kenya

Rationale: Several species of *Pythium* are important pathogens, with variable host ranges and spatial distribution, while others are saprophytes or have potential for biological control. *Pythium* root rot is the most destructive soil-borne disease of beans in East and Central Africa and development of effective management strategies against the disease requires accurate, reliable, and rapid detection assays. A diagnostic test has been developed for the detection of most known species (particularly in the temperate) of *Pythium* using reverse dot blot hybridization (RDBH) (Levesque, 1998). It is based on species-specific oligonucleotides that have been designed and blotted on to a membrane array. There are about 100 species in the genus *Pythium*. Some *Pythium* species are highly pathogenic, some are almost exclusively saprophytes, and some species are biological control agents. Given the wide genetic variation within the *Pythium* genus, some strains (pathogenic or beneficial), particularly in the tropics, could be almost certainly novel. This study was initiated to adopt the RDBH technique to rapidly identify the different species commonly associated with bean root rots in East and Central Africa. Because the sequences used to develop the probes were obtained exclusively from *Pythium* spp. found in temperate zones, the first step was to test the suitability of this assay for tropical zones. The hypothesis was that some *Pythium* populations are only found in tropical zones.

Materials and Methods: This work was done in Canada. DNA from 100 *Pythium* isolates collected from Uganda, Kenya, and Rwanda were carried to Canada for analysis. All isolates were amplified with primers targeting the ITS regions of the ribosomal genes and specific to *Pythium* spp. This first step allowed the differentiation of *Pythium* and non-*Pythium* species. Direct sequencing of the PCR fragment was done using primers that annealed inside of the first fragment and the sequencing products were run on an ABI prism-automated sequencer. After editing, the sequences were compared to sequences of known *Pythium* species from the *Pythium* database managed by Dr A. Levesque.

Results and Discussion: Amplification with *Pythium* specific primers identified 17 isolates as *Mortierella* spp. The rest of the isolates were identified as *Pythium* spp. *Mortierella* is a common saprophyte that can be isolated on *Pythium* specific media (Figures 46 and 47). Morphologically, it cannot be differentiated from *Pythium*. This molecular method (using *Pythium*-specific primers) is a highly useful tool that can be used to eliminate *Mortierella* spp. from the collection of *Pythium*. Sequence analysis of the isolates identified 12 different species. Of the four species reported to infect beans, only *Pythium ultimum* and *P. irregulare* were identified; and *P. ultimum* var. *ultimum* had the highest incidence (25), showing that this species is the most prevalent in the areas under study (Table 44). Other species identified included *P. acanthicum, P. dissotocum, P. indigoferae, P. oligandrum, P. salpingophorum, P. spinosum, P. torulosum,* and *P. vexans*. Of interest were new putative species found in association with beans, and whose sequences were significantly different from their closest match (*P. torulosum)* among the neo (type) strains implying that they could be novel. Additional characterization studies are underway to look at the morphology and pathogenicity of these isolates, as well as other parts of the genome to establish if indeed these isolates represent new species within the genus *Pythium*. Also of interest was the occurrence of *P. oligandrum,* a known potent biocontrol agent, effective against a number of soil-borne pathogens including *Pythium* species. We are currently verifying its effectiveness to manage soil-borne pathogens. Other species identified, *P. vexans* and *P. indigoferae,* have been implicated as possible biocontrol agents.

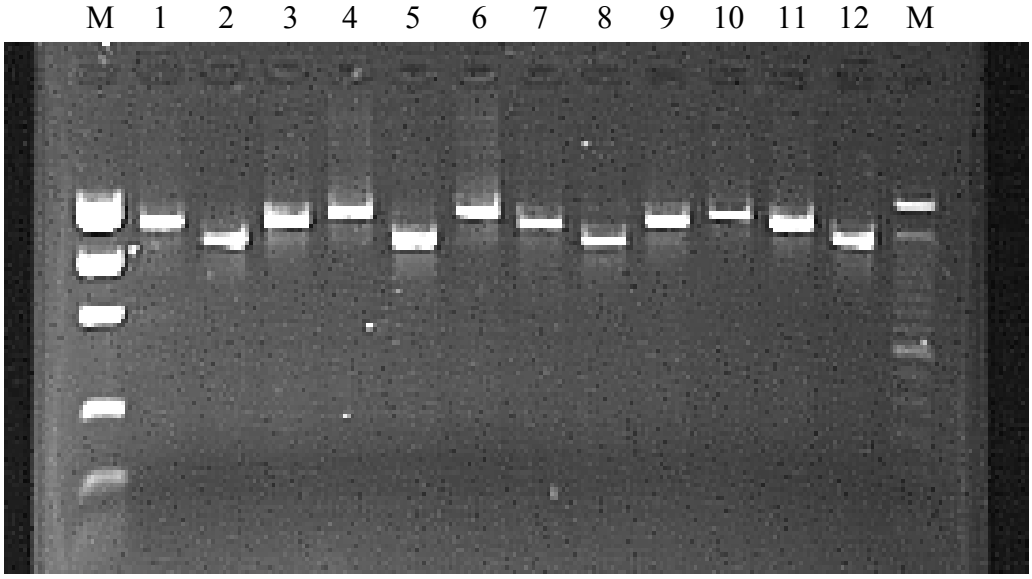

Figure 46. Banding patterns from amplifying ribosomal DNA spacer region using Oomycete specific primers and used to distinguish between *Mortierella* spp. and *Pythium* spp. Lanes 1, 3, 4, 6, 7, and 9 –11 represent DNA from *Pythium* spp., while DNA in lanes 2, 5, 8, and 12 contains DNA from *Mortierella* spp. Lane M is a DNA molecular ladder.

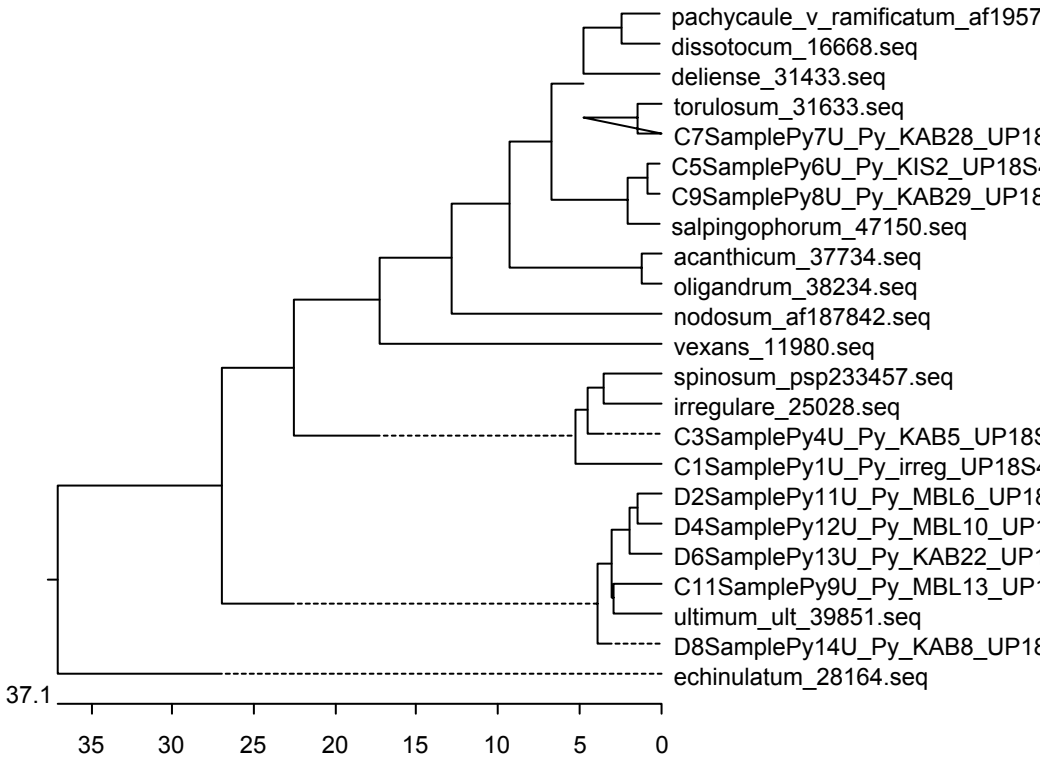

Figure 47. Dendogram derived from sequence data of some *Pythium* isolates and classification into different *Pythium* species.

Table 44. Identification and classification of *Pythium* isolates collected from bean growing areas in southwest Uganda, western Kenya, and Rwanda.

Species	Number of isolates	Comment
Pythium ultimum	25	Pathogenic
P. dissoticum	1	
P. deliense	1	
P. irregulare	2	Known to be pathogenic
P. nodosum	2	
P. oligandrum	2	Known biocontrol agent
P. salpingophorum	2	
P. echinulatum	1	
P. spinosum	4	
P. torulosum	9	Possibly new species in its group
P. vexans	7	Saprophyte/possibly biocontrol
P. pachycuale	11	
P. indigoferae	1	Possibly biocontrol agent

Conclusion: This study demonstrated the complex nature of the genus *Pythium*. Twelve *Pythium* species were identified from bean fields in Uganda, Rwanda, and western Kenya. Of these, *P. ultimum* var. *ultimum* was the most prevalent. This species has been reported as the most important incitant of bean root rots. Two isolates that are potential biological control agents were identified. Studies have been initiated to test the effectiveness of these isolates as biocontrol agents against *P. ultimum* var. *ultimum* and other pathogenic soil-borne species. More samples are being collected from these areas to have an extensive coverage of the bean-growing areas experiencing root rot problems. In addition, DNA microarrays are being adopted for fast detection and identification of *Pythium* species in order to accelerate the diagnosis process.

Reference:

Lévesque, C.A.; Harlton, C.E.; de Cock, A.W.A.M. 1998. Identification of some comycetes by reverse dot blot hybridization. Phytopath 88:213-222.

Contributors: G. Mahuku (IP-1); R. Buruchara (IP-2); A. Lévesque (Agriculture and Agri-Food Canada [AAFC]-Ottawa)

3.1.5 Whitefly-transmitted geminiviruses infecting beans

Bean virology is constantly monitoring the different geminiviruses transmitted by the whitefly *Bemisia tabaci* to beans in Latin America and elsewhere in the world. In the Americas, we have BGMV in Brazil, Argentina, and Bolivia. This year we received bean samples (var. INTA-Cerrillos) from the Bolivian National Program, collected in bean plantings in the locality of Comarapa. These samples were tested for the presence of geminiviruses, but the assay yielded negative results. Most of these samples, however, were shown to be infected by a potyvirus, probably BCMV.

Guatemala and El Salvador were surveyed this year to study pathogenic variability in the whitefly-transmitted geminiviruses that attack beans in those countries. In both countries, subtle changes have taken place in the molecular composition of the BGYMV isolate present there, probably in response to the arrival of Biotype B of the whitefly *B. tabaci*. However, these molecular changes do not seem to affect the overall pathogenicity or transmissibility of the viruses in bean plantings. Integrated pest management methods are being implemented in prime bean production regions of El Salvador and Guatemala to control whitefly-transmitted geminiviruses, in collaboration with the National Program of El Salvador (CENTA), the Instituto de Ciencia y Tecnología Agrícolas (ICTA), and PROFRIJOL. Bean virology has linked the IPM Systemwide Whitefly Projects in Central America, to boost the production of common bean in regions affected by whitefly-transmitted geminiviruses. This year, a project was started with CENTA to recover bean production in prime agricultural regions and particular seasons that were abandoned to bean production because of the high incidence of the whitefly *Bemisia tabaci* and the viruses it transmits.

3.1.6 Controlling the "amachamiento" disease of beans in Central America

The "amachamiento" problem (failure to produce pods) of beans was studied at CIAT in past years, and diagnosed as a disease caused by cowpea chlorotic mottle virus (CCMV). In the past, this disease was known as "bean yellow stipple", particularly in the lowlands of Central America and the Caribbean island of Cuba. This virus, however, has been attacking beans at higher altitudes, particularly in the Brunca region of Costa Rica, causing yield losses in excess of 50%. Because beetles (chrysomelids) transmit this virus, vector control is a must to reduce yield losses. Costa Rican scientists showed this year that chemical control of the vector (mainly *Diabrotica* spp.) resulted in yields in excess of 1 ton per hectare versus 728 kg per hectare for the untreated control, even under a low incidence (15%) of the "amachamiento" problem in the control.

Yield loss analyses conducted under controlled conditions, using the Costa Rican bean landrace "Sacapobres", showed a reduction in the number of pods produced by bean plants affected by "amachamiento" of up to 54.8%, with respect to the virus-free control. In the original study on the etiology of "amachamiento", it was pointed out that the local landraces Sacapobres and "Generalito" were also doubly infected by BCMV. In this yield loss study, Sacapobres yielded 20% less pods than the virus-free control, when infected singly by BCMV. However, when the test plants were doubly infected by BCMV and CCMV (the causal agent of the "amachamiento" disease) pod yield losses increased to almost 84%, hence the importance of breeding for resistance to BCMV.

Rationale: Proper diagnosis of biotic problems affecting bean production is critical to implement effective disease management practices.

Materials and Methods: The Virology Research Unit has in stock antisera and molecular diagnostic tools needed to identify bean pathogens known to affect bean production throughout the world. The enzyme-linked immunabsorbent assay (ELISA) and PCR tests were used in these studies.

Results and Discussion: Results are presented above. These findings have contributed to the understanding of pathogen interaction in complex cropping systems.

Contributors: M. Castaño (IP-1); A.C. Velasco, R. Sedano (Virology Research Unit)

Progress towards achieving output milestones:

Characterizing and monitoring pathogen diversity / stable strategies for managing pathogens developed

- It was shown that *C. lindemuthianum* exhibits incredible diversity that is not structured along host gene pools. Therefore the strategy of combining Andean and Mesoamerican genes might not lead to stable anthracnose resistance

- It was shown that the loci specific markers we developed can be used to characterize *P. griseola* and separate isolates into different groups based on geographic origin and host gene pool. These markers will be shared with collaborators so as to have a standardized system for characterizing this pathogen.

- *P. griseola* was shown to be closely related to *C. fulvum* and *Mycosphaerella* spp. The strategies used to clone avirulence genes from *C. fulvum* might be applicable to *P. griseola*.

- Twelve *Pythium* species were identified in association with bean roots and one species (*P. oligandrum*) is a known biocontrol agent. Studies are underway to test the effectiveness of this species to manage *Pythium* and other soil-borne pathogens.

- Yields are being increased through proper pathogen identification and implementation of effective IPM control methods.

Activity 3.2 Characterizing disease and insect resistance genes

Highlights:

- A single dominant gene was shown to condition resistance to ALS in the bred line MAR 1.

- A marker was identified that segregated with the resistance gene in MAR 1.

- It was shown that resistance to common bacterial blight (CBB) in VAX 6 is highly stable and that the *fuscans* strain of *Xanthomonas campestris* pv. *phaseoli* is highly pathogenic.

- Both dominant and recessive genes were shown to condition resistance to ALS. G 10909 and G 5686 have two dominant epistatic genes, Don Timoteo has one dominant gene, while Montcalm and Amendoim have two recessive genes.

3.2.1 Nature and inheritance of angular leaf spot resistance genes in MAR 1

Rationale: Previous studies have shown that the variety MAR 1 is resistant to several ALS races, but is highly susceptible to CBB. VAX 6 is susceptible to ALS, but is highly resistant to both strains of CBB. MAR 1 has been shown to do moderately well under low soil fertility, especially low phosphorus, and does reasonably well under drought conditions, although it has low levels of

tolerance to *Macrophomina phaseolina* (CIAT 1999, p 84-6; CIAT 2000, p 58-61). This variety has been reported to be especially good in managing ALS in Brazil and some parts of Africa. 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 ALS race 31-55 under field conditions, and races 63-23 and 31-39 under greenhouse conditions. The goal of this study was to elucidate the nature and inheritance of ALS resistance in MAR 1 as well as to identify and develop markers linked to the resistance gene(s).

Materials and Methods:

Evaluation of MAR 1 x VAX 6 RILs under field conditions: The parents, VAX 6 and MAR 1, and RILs (233) were evaluated for their reaction to ALS under field conditions at Santander de Quilichao, about 900 m in altitude. The RILs were planted in 4-m rows replicated three times. Each entry had four rows. Parental materials (MAR 1 and VAX 6) and local checks (susceptible and resistant) were sown after every 10 lines. Plants were inoculated three times using the local ALS race, 31-55. The first inoculation was done 25 days after planting and at weekly intervals thereafter to a maximum of four inoculations. Disease severity was evaluated starting at time of inoculation, and thereafter at weekly intervals up to a maximum of six evaluations. Evaluations were done using a CIAT 1 – 9 scale, where 1 represents no visible symptoms and 9 = severe symptoms and disease expression. Plants that had a rating of 3 or less were considered resistant, 4-6 as intermediate, and a rating greater than 6 as susceptible.

Greenhouse evaluation: The same RILs (223) and their parents (MAR 1 and VAX 6) were evaluated in the greenhouse using two races, 31-55, 63-23 and 31-39. In addition, the ALS differential varieties were included to verify the race designation of the isolates. Plants were planted three per pot with three pots for each entry. Inoculations were done 21 days after planting and plants were put in a humid chamber for 4 days, after which they were moved to a bench. Evaluations were started 8 days after inoculation and were done every 2 days until a total of six evaluations were collected.

DNA isolation: Leaf samples (young trifoliate) were collected from each RIL, and DNA extracted using standard procedures and stored at –20 ºC until required. After obtaining the phenotypic data, a susceptible and resistant bulk were created in addition to the parents and used to screen 123 RAPD primers, eight microsatellite primers, six resistant gene analog (RGA) primers, and 10 SCARS for polymorphism. A marker that exhibited polymorphism was later used to screen all 223 RILs.

Data analysis: For both field and greenhouse experiments, the area under disease progress curve was calculated and this was used to divide RILs into resistant and susceptible. For the purposes of this study, all intermediate reacting lines were considered as susceptible. Some RILs had combinations of both highly resistant and highly susceptible, showing that they were still segregating and not stable. The chi-square test was used to test the goodness of fit of the data to different models depending on how many resistance genes are expected in the population. Simple regression analysis was used to measure the association between the polymorphic marker and the phenotypic data.

Results and Discussion: The observed segregation of resistance to races 31-55, 63-23, and 31-39s was 1:1, signifying that a single dominant gene conditions ALS resistance in MAR 1 (Table 45). No significant differences were observed between repetitions both for field and greenhouse conditions. An advantage of using RILs is that phenotypic data can be collected many times, something that cannot be done using segregating populations, such as F_2s.

Table 45. Response of 233 recombinant inbred lines (RILS) derived from crossing MAR 1 (angular leaf spot [ALS] resistant) by VAX 6 (ALS susceptible) to inoculation with three races of *Phaseolus griseola*.

Race	Resistant	Susceptible	Observed ratio	Expected ratio for one gene
31-55	106	115	1:1.08	1:1
63-23	109	114	1:1.05	
31-39	111	112	1:1	

Of the 146 primers tested, only three were polymorphic. Further testing revealed that only the microsatellite primer gave reliable and reproducible results. A DNA band (230 bp) was amplified from most RILs that had a resistant phenotype (Figure 48). Of all RILs with resistant phenotypes, only 11 did not have the 230 bp band, while 12 of the susceptible RILs had the fragment. These RILs were observed to be still segregating, with some plants showing a resistant phenotype, while others had a susceptible phenotype. Because the DNA used came from a single plant, the DNA was possibly from the plant that was contrary to the final phenotype assigned to these entries. We are in the process of verifying the reaction of the 23 RILs by inoculating plants in the greenhouse and collecting another sample for analysis.

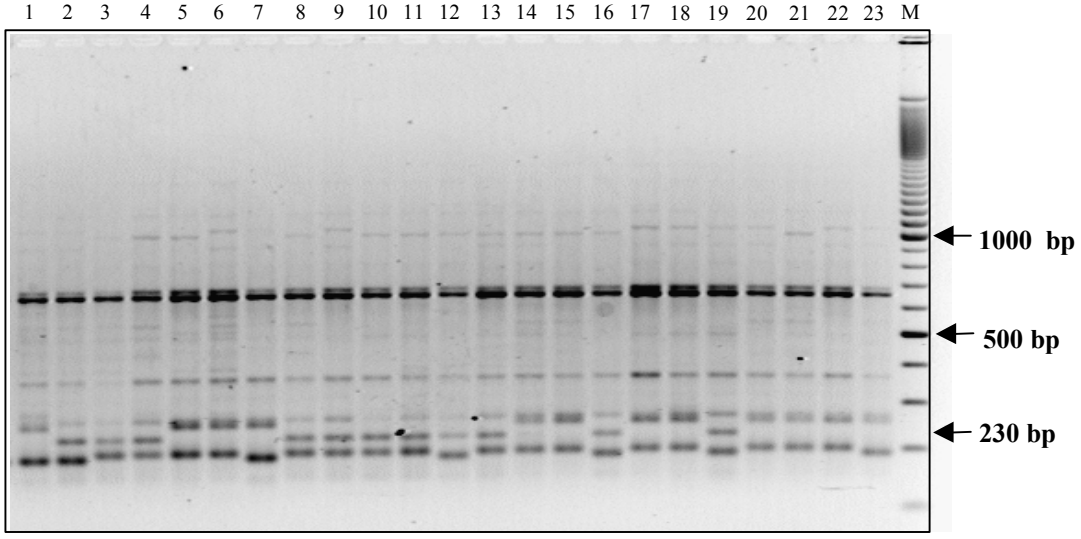

Figure 48. Banding patterns generated by a microsatellite primer for resistant and susceptible recombinant inbred lines (RILs) derived from crosses of MAR 1 (angular leaf spot [ALS] resistant) and VAX 6 (ALS susceptible). The polymorphic band is 230 bp. Lanes 1, 5-7, 14-15, 17-18, and 20-23 correspond to susceptible RILS, while lanes 2-4, 8-13, 16, and 19 represent resistant genotypes. Lane M is the 100 bp DNA stepladder.

Although some RILs had conflicting results, simple regression analysis using SAS showed that this marker explained between 86% and 91% of the total phenotypic variation observed in the RILs. Analysis to establish the distance of the marker from the resistance gene is pending. This will determine how useful this marker will be for MAS breeding.

Conclusions: ALS resistance in the variety MAR 1 is conditioned by a single dominant gene. A marker that is associated with resistance has been identified. However, the distance of this marker form the resistance gene will be determined following linkage analysis. Meanwhile, the fragment (230 bp) has been cloned and will be sequenced after verifying ambiguous results. Additional work will determine and verify the usefulness of this marker as a candidate for MAS breeding.

References:

CIAT. 1999. Annual Report 1999 Project IP-1. CIAT, Cali, CO. 134 p. (Working doc. no. 180)
CIAT. 2000. Annual Report 2000 Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: G. Mahuku, J.J. Riascos, C. Jara

3.2.2 Nature and inheritance of common bacterial blight resistance in VAX 6

Rationale: CBB is the most widely distributed disease of common bean and 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. VAX 6 was developed by pyramiding resistance genes from several sources, and to achieve and maintain the same level of resistance as found in VAX 6, all the resistance genes in this line must be transferred to the new commercial type variety. A more practical way is to tag and develop markers for all the QTLs that are found in VAX 6 to ensure that the genes in this variety are transferred to commercial type beans. 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 developing markers for all the QTLs involved in CBB resistance found in VAX 6. Last year, we reported the results of screening 223 RILs under field conditions. This year, we report the results of screening the same RILs under field and greenhouse conditions, relating yield to incidence of the bacteria.

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 the local CBB isolate, XCP 123, previously isolated from Santander de Quilichao. Each RIL was planted in four rows of 3 m, with parental materials and local control BAT 41 (susceptible check) planted every 20 rows. 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. In addition, yield and yield components (weight of

100 seeds, etc.) were taken at harvest to determine the contribution of increased resistance to CBB coming from VAX 6.

Similarly, the same RILs were screened in the greenhouse with the same XCP strain (XCP 123), and the *fuscans* variant (XCPF 2). Plants were inoculated using the razor blade method on a fully expanded trifoliate and the experiment was repeated. Evaluations were done on individual plants, starting 10 days after inoculation to a total of six evaluations using the scale described above.

Results and Discussion: Most of the MAR 1 x VAX 6 RILS (81%) were resistant, 15.6% intermediate, and 2.2% susceptible (Figure 49). The results were highly similar to the results reported for the first semester of evaluation with the same isolate in the same location, showing the stability of these materials. As expected, MAR 1 had a susceptible reaction (rating 8) while VAX 6 had a resistant reaction (rating 2).

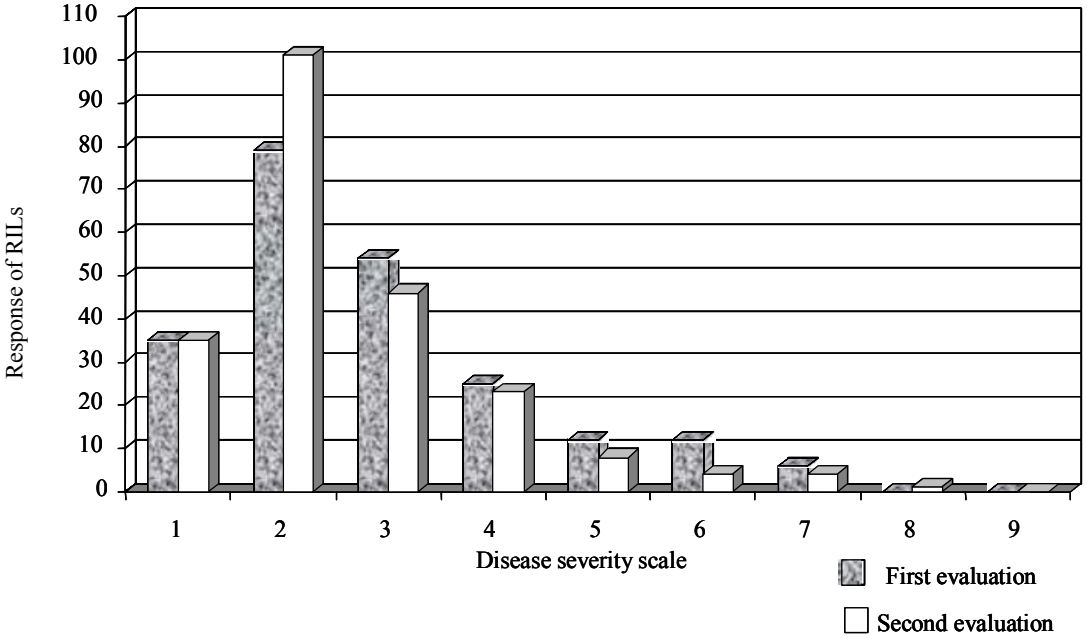

Figure 49. Response of MAR 1 x VAX 6 recombinant inbred lines (RILs) to inoculation with *Xanthomonas campestris* under field conditions on a scale of 1-9, where 1 represents no symptoms and 9 represents severe symptoms.

Under greenhouse conditions, 80% of the RILS were resistant, 18% intermediate, and 2% susceptible (Figure 50). For the isolate XCP 123, field and greenhouse results were highly correlated (r = 0.94) showing the high levels of resistance that were introgressed into VAX 6. Evaluations with the *fuscans* variant of XCP showed that this strain is more aggressive than the XCP strain. Most of the plants were resistant (110), while 96 were intermediate, and the rest susceptible (17). The proportion of susceptible plants was 7%. These results show and support previous reports that the *fuscans* variant is more aggressive than the XCP normal strain. DNA has been extracted from these RILs and is being screened with the SCARS that have been developed for XCP, to verify if the tagged QTLs are also present in VAX 6. In addition, other markers,

random amplified polymorphic DNAs (RAPDs), microsatellites, and RGAs are being tested for polymorphism to identify potential markers for tagging CBB resistance QTLs in VAX 6.

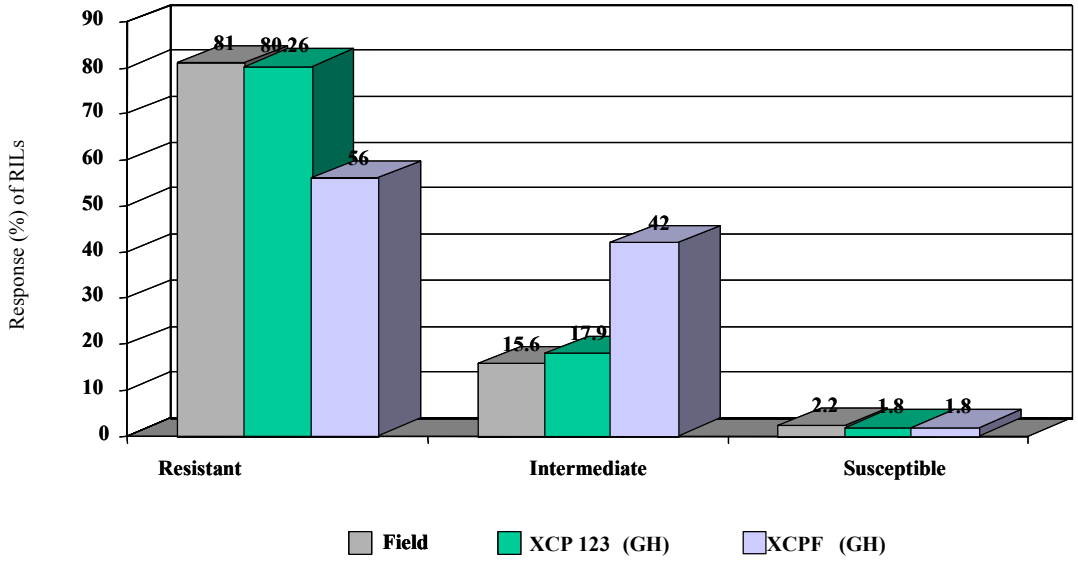

Figure 50. Reaction of MAR 1 x VAX 6 recombinant inbred lines (RILs) to inoculation with different strains of *Xanthomonas campestris* pv. *phaseoli* (XCP) and its *fuscans* (XCPF) variant under greenhouse conditions.

Conclusion: Results from field and greenhouse evaluations have shown the high levels and stability of CBB resistance in VAX 6. Studies to find QTLs for all the genes contributing to the high levels of resistance in this variety are continuing and a broader picture will be obtained once this work has been completed.

Contributors: G. Mahuku, C. Jara

3.2.3 Nature and inheritance of angular leaf spot resistance in ALS differential genotypes and identified resistance sources

Rationale: It has been established that all *P. griseola* races can be separated into two major groups that correspond to the common bean gene pools. Therefore, combining Andean and Mesoamerican genes is expected to give broad and durable resistance to this pathogen. A clear understanding of the nature and inheritance of ALS resistance in identified sources of resistance and differential varieties is necessary to fully take advantage of this host pathogen co-evolution to manage ALS disease. Very little is known about the number of genes conditioning resistance in our differential materials, and this has limited our ability to use virulence data to precisely define the distribution of *P. griseola* races, which would facilitate deploying resistant genes in ways that prolong their durability. In addition, defining *P. griseola* populations in terms of resistance genes will help identify genes to combine or pyramid to achieve durable resistance. 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 the transfer of resistance to well-adapted, market class type bean.

Materials and Methods: The snap bean variety, Sprite, has been identified as universally susceptible to *P. griseola*. All *P. griseola* races (~200) tested to date have a compatible interaction with this variety. We therefore chose Sprite as a susceptible parent in all crosses to elucidate the nature and inheritance of ALS resistance. Populations (F_1, F_2 backcrosses to resistant and susceptible parents) were made for 10 ALS common bean differential genotypes (four Andean and six Mesoamerican) and identified sources of resistance (G 10474, G 10909, G 4691, G 18224, G 3991, and G 14301). To date, five genotypes (G 10909, G 5686, Amendoin, Montcalm, and Don Timoteo) have been screened under greenhouse conditions. Andean materials have been screened with Andean races in order to identify Andean genes, while Mesoamerican varieties have or will be screened using Mesoamerican races. The source of resistance (G 10909) was screened with race 63-63, the most virulent race that knocks down all the resistance genes in our differential genotypes. Evaluations for disease severity were assessed using a CIAT 1 – 9 scale, where 1 represents no visible symptoms and 9 represents severe symptoms and disease expression. Ratings of 1 to 3 were considered resistant, and ratings > 4 susceptible. Area under disease progress curves was calculated to assign genotypes to resistance and susceptibility classes. Several different genetic hypotheses were tested for each population using a chi-squared test.

Results and Discussion: Results obtained reveal that inheritance of ALS resistance is complex, and both recessive and resistance genes are involved in conditioning resistance to the ALS pathogen. The observed segregation ratios from F_2, BC1-R and BC1-S, reveal that a single dominant gene conditions ALS resistance in Don Timoteo, while two dominant genes probably condition resistance in G 5686 and G 10909. The resistance in Montcalm and Amendoin may be conditioned by two recessive genes (Table 46).

Conclusions: The results reported show the complex nature of inheritance of resistance to *P. griseola*. Major genes (whether recessive or dominant) are involved in conferring resistance to ALS, and the complex segregation patterns observed in Montcalm and Amendoin reveal a possibility that minor genes might be involved as well. However, more F_2 populations of the genotypes Montcalm and Amendoin need to be screened again before drawing firm conclusions about minor genes.

Table 46. Segregation of common bean populations following inoculation with different races of *Phaeoisariopsis griseola*.

Cross	Generation	Resistant	Susceptible	Expected ratio	Ratio	Likely inheritance
Montcalm x Sprite	F_1	6	49	0:1	0.12:1	Poss. two recessive genes
	F_2	6	128	1:15	1:21	
	BC_1-R	5	48	1:15	1:9.6	
	BC_1-S	3	19	0:1	0.16:1	
Don Timoteo x Sprite	F_1	59	3	1:0	1:0.05	One dominant gene
	F_2	90	39	3:1	2.3:1	
	BC_1-R	28	3	1:0	1:0.1	
	BC_1-S	18	17	1:1	1:1	
Amendoin x Sprite	F_1	0	136	0:1	0:1	Poss. two recessive genes
	F_2	5	136	1:15	1:25	
	BC_1-R	4	88	1:15	1:22	
	BC_1-S	0	58	0:1	0:1	
G 5686 x Sprite	F_1	64	16	1:0	1:0.25	Two dominant epistatic genes
	F_2	81	58	9:7	9:6.4	
	BC_1-R	18	4	1:0	1:0.22	
	BC_1-S	1	21		0:1	
G 10909 x Sprite	F_1					
	F_2	100	63	9:7	9:5.6	Two dominant epistatic genes
	BC_1-R					
	BC_1-S	7	35	1:3	1:5	

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

Progress towards achieving output milestones:

Strategy developed for stable angular leaf spot resistance / markers for marker assisted selection developed for various biotic constraints

- Several resistance genes, both dominant and recessive, were identified. A marker that segregates with resistance gene in MAR 1 was identified and cloned and its suitability for MAS is currently being evaluated.

- Angular leaf spot resistance genes in some differential lines were identified and lines will be developed that contain only single genes.

- Several lines that combined CBB and ALS resistance were identified among RILs developed from MAR 1 x VAX 6 crosses. These lines are currently being evaluated using other isolates and races.

Activity 3.3 Developing integrated pest management components

Highlights:

- It was shown that use of resistant varieties can increase yields by as much as 64%, while tolerant varieties can increase yields by 56%.
- An action threshold for *Thrips palmi* was validated on snap beans.
- Levels of resistance to insecticides in whitefly (*Trialeurodes vaporariorum*) and *T. palmi* populations were identified.
- Integrated pest management (IPM) components were developed and management strategies were successfully tested for combined populations of whiteflies and thrips on snap beans.

3.3.1 Contribution of varietal resistance to yield stability

Rationale: ALS has emerged as the most important and widely distributed disease of common bean. In recent years, this disease is increasing in importance and is reaching epidemic proportions in many countries. For example, in 2000 and 2001, ALS reached epidemic proportions in Argentina, especially in regions cultivating the small, black-seeded variety, DOR 590. The increase in the incidence of ALS appears to be structured with decreased fertility (low fertility) and use of susceptible genotypes. Several resistant sources have been found and are being used. It has been argued that using resistant genotypes will increase yield. However, studies to quantify the contribution to yield by resistance genes have not been done for ALS and no studies have looked at the cost associated with using resistance genes. This study was initiated to quantify the contribution to yield of full or partial resistance to ALS disease, and the risks associated with the use of susceptible varieties.

Materials and Methods: The trial was carried out during 2001A at Darién and Santander de Quilichao, in Colombia. Darién is at about 1300 m altitude, and Santander de Quilichao is at about 900 m. The population structure of *P. griseola* in these two locations is very different and distinct. The varieties AFR 703 (resistant), AFR and AND 829 (intermediate), and Montcalm (susceptible) belonged to the Andean gene pool. Mesoamerican varieties were G 9462 (susceptible), RIZ 97 (intermediate), and A 223 (resistant). The experiment was arranged in completely randomized block designs, with natural infection inoculated and protected as the main blocks, and the different varieties as the treatments. Plants were protected by a combination of fungicides, Chlorothalinil, Benlate, and deoxycarboxin, which were inoculated starting 20 days after inoculation to a total of four sprays. The first inoculations using mixtures of ALS races from that site (Darién: 63-0; 31-47; 5-47; 63-15 and Santander de Quilichao: 63-0; 31-55; 1-55; 13-63; 31-47) were started about 25 days after planting, and thereafter at weekly intervals to a maximum of four inoculations. At each inoculation time, plants in the protected block were sprayed with a mixture of fungicides. Rating for disease incidence and severity was started just before the first inoculation and was done at weekly intervals thereafter. All yield parameters were recorded at harvest time and included date of maturation, number of pods, seed number, and yield in 2.4 m^2 area.

Results and Discussion: The data for Santander de Quilichao were unreliable and were therefore excluded. There was severe manganese toxicity in our plots and some varieties were affected, more so for the Andean genotypes. Disease takeoff and expression was severely affected. Figure 51 shows disease progression for the different varieties at Darién.

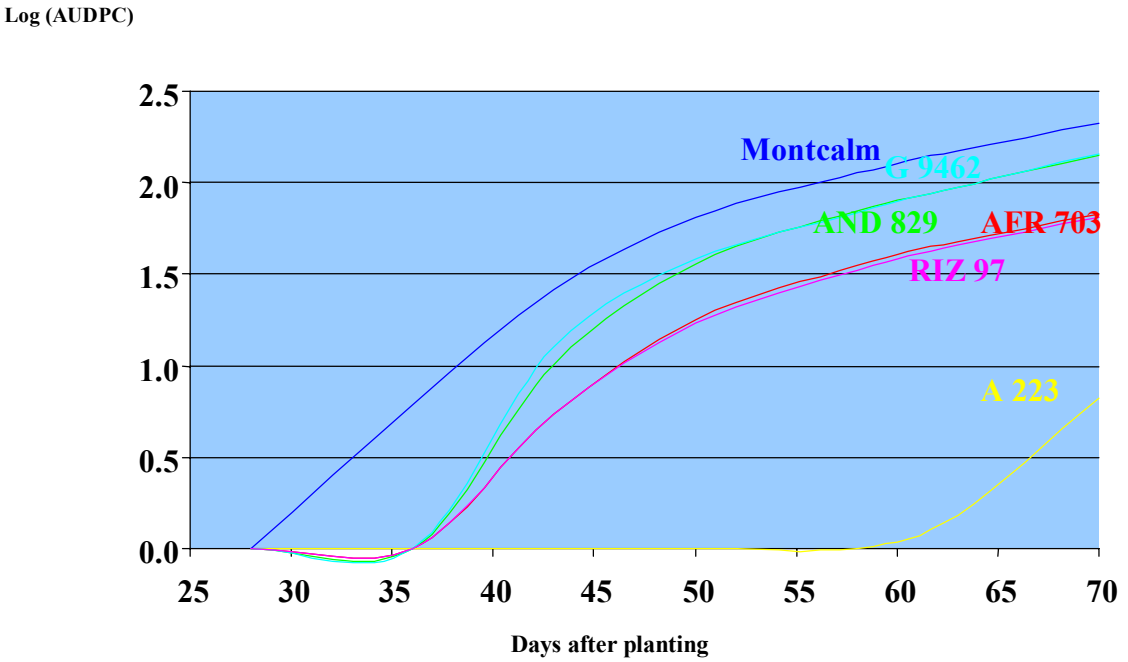

Figure 51. Disease progress (expressed as area under disease progress curve [AUDPC] of six varieties differing in their response to Andean and Mesoamerican races of *Phaeoisariopsis griseola* under field conditions.

Montcalm was the most susceptible of all varieties and was completely defoliated 15 days before the protected trials were defoliated. Yield reduction was highest for the susceptible varieties Montcalm (64%) and G 9462 (56%). For the tolerant variety RIZ 97 (Mesoamerican) yield was reduced by 30% and for tolerant AFR 703 (Andean) by 43%, whereas for the resistant varieties, yield was reduced by 13% (A223) and 36% (AND 829) (Table 47 and Figure 52).

Table 47. Yield response (kg ha^{-1}) of six varieties differing in their resistance to *Phaeoisariopsis griseola.*

Treatment[a]	Mesoamerican varieties			Andean varieties		
	A 223	RIZ 97	G 9462	AFR 703	AND 829	Montcalm
Inoculated	4785.1	3239.6	1410.6	3378.2	2924.5	1210.9
% yield gain	70.5	56.5	-	64.0	58.5	-
Natural infection	4208.6	3935.4	2759.2	3713.1	4328.9	1735.8
% yield gain	34.4	29.9	-	53.2	59.9	-
Protected	5475.6	4619.7	3217.3	5883.8	4595.0	3356.1
F	3.19	3.59	17.98	5.95	2.56	29.08
Pr > *F*	0.1136	0.0943	0.0029	0.0376	0.1569	0.0008

a. Percentage of yield gain is calculated relative to the yield obtained in the susceptible variety, G 9462 (Mesoamerican), and Montcalm (Andean).

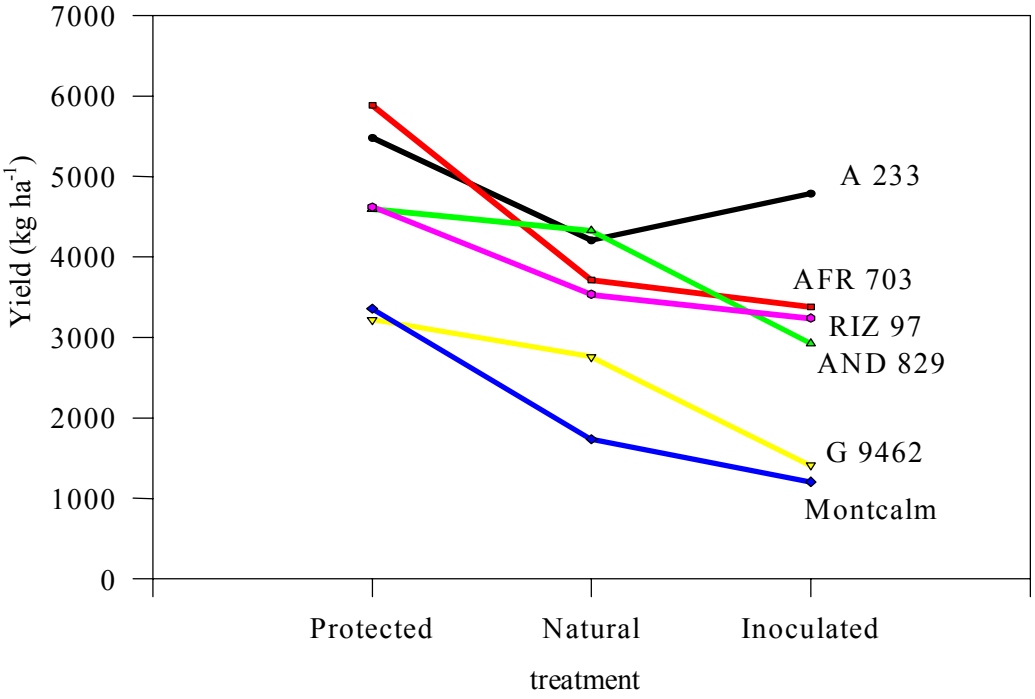

Figure 52. Yield distribution in response to inoculation with Mesoamerican and Andean races of *Phaeoisariopsis griseola* under field conditions.

Strong correlations were observed between the amount of disease expressed and yield loss, and this relationship was stronger for susceptible genotypes than tolerant or resistant genotypes (Figure 53). However, there were no significant differences in yield for the tolerant and resistant varieties (Table 47 and Figure 52).

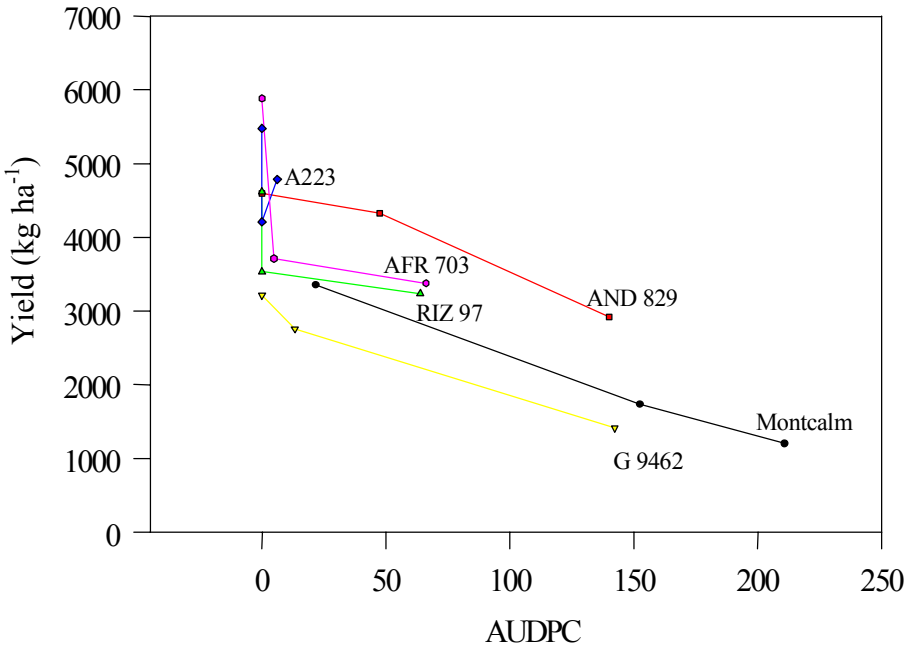

Figure 53. Relationship between yield and disease incidence (expressed as area under disease progress curve [AUDPC]) for *Phaeoisariopsis griseola* under field conditions.

Interestingly, the inoculated plot of the resistant variety A 233 had a higher yield than the non-inoculated plots. Although these data are preliminary, we can speculate that cross protection or induced resistance is protecting these plants from other pathogens that may impact negatively on yield. The reduction in yield between the protected and unprotected plots observed for A 223 might be a result of expression of resistance genes. No other diseases were observed for this variety. When comparing within gene pool and under inoculation (high disease pressure), use of resistant varieties increased yield by 64%, while use of tolerant varieties increased yields by 58.5%. A similar situation was observed for Mesoamerican varieties, where yield was increased by 70.5% by using resistant varieties, and by 56.5% by using varieties tolerant to ALS. Under natural conditions, using resistant varieties increased yields by 34.4% for Mesoamerican varieties and 53.2% for Andean varieties, whereas using tolerant varieties increased yields by almost 30% for Mesoamerican and almost 60% for Andean varieties.

Conclusion: These are preliminary results, but nevertheless demonstrate the importance of using resistance genes. Under heavy disease pressure, use of resistance can increase yield by as much as 64%, while tolerant varieties can result in 56% yield increases. This study is being repeated at both Santander de Quilichao and Darién and a clear picture will be obtained with data from several seasons.

Contributors: G. Mahuku, C. Jara.

3.3.2 Validation of an action threshold for *Thrips palmi* control

Rationale: It has been stated before that insecticides are likely to remain as the main method of control of *T. palmi*. The development of an action threshold (AT) may serve as a decision tool in the design and implementation of rational management strategies. The AT for *T. palmi* as a pest of snap beans was established as 7-8 adults per leaflet (CIAT 2001, p. 134-36). We now have moved into the validation of this AT in order to ensure that future management recommendations have a sound and scientific basis.

Materials and Methods: To validate the AT for *T. palmi* we used a 4 x 4 Latin square design. The following treatments were compared (1) Foliar applications of an effective insecticide (imidacloprid) at AT populations; (2) Seed treatment followed by foliar applications of an effective insecticide at AT populations; (3) Drench application of an effective insecticide followed by foliar applications of an effective insecticide at AT populations; and (4) Check (no insect control). Insect populations and damage levels, yields, and costs were recorded and analyzed.

Results and Discussion: All treatments protected the crop in such a manner that differences with the absolute check were high and significant in terms of damage scores, farmers' appraisal of the quality of the crop, and yields (Table 48). Yield losses in the absolute check were very high (40.7%). All possible uses of the AT can be categorized as appropriate and viable in order to rationalize the use of chemicals against *T. palmi.* Further research will attempt to simplify management strategies in order to facilitate adoption by participating farmers. Existing ATs for

whitefly control will be incorporated in our research to develop a strategy for both insect pests in those regions in which they coincide.

Table 48. Effect of four management strategies[a] on damage and yield losses caused by *Thrips palmi* on snap beans.

Treatment	Damage[b]	Farmer's appraisal[c]	Yield (kg ha^{-1})	Percentage yield losses[d]
Foliar applications at AT[e] populations	2.7 b	3.7 a	15246 a	10.9
Seed treatment + foliar applications at AT	1.5 c	3.5 a	16073 a	7.2
Drench + foliar applications at AT	2.0 bc	3.5 a	17314 a	-
Check	7.2 a	1.7 b	10272 b	40.7

a. Means within a column followed by the same letter are not significantly different at $P = 0.05$.
b. On a 1 - 9 score scale, where 1 = no damage and 9 = severe damage.
c. On a 1 - 5 score scale, where 1 = very poor and 5 = excellent appearance of the crop.
d. With respect to the best treatment.
e. Action threshold.

Reference:
CIAT. 2001. Annual Report Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: C. Cardona, J. M. Bueno, I. Rodríguez.

3.3.3 Monitoring of insecticide resistance levels in whitefly and thrips populations

Rationale: Monitoring of insecticide resistance levels in whitefly populations is a major objective of the Systemwide Whitefly Project. It is also an important step in the design of appropriate insect pest management strategies given the fact that for several major insect pests of beans (such as whiteflies and thrips), insecticides will continue to be an essential method of control. In 2001, we established new baseline data for two insecticides with whitefly adults, for three insecticides with whitefly nymphs, and for three insecticides with thrips adults. This is essential information needed to monitor the development of resistance in insect populations. For the first time, we initiated periodic monitoring of insecticide resistance levels in whitefly and thrips adult populations in the areas in which IPM activities of the Project are being conducted.

Materials and Methods: Baseline data and diagnostic dosages were calculated using mass rearings of susceptible strains of *T. vaporariorum* and *T. palmi* maintained at CIAT for several years. LC_{50} and LC_{90} values are calculated by exposing whitefly or thrips 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 mortality of 95% or more in a susceptible strain) are tested. The diagnostic doses are then used to monitor resistance under field conditions by means of the insecticide-coated glass vials technique. Similar data are obtained for first instar whitefly 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 49 presents baseline data for carbosulfan and thiamethoxam 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 49. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* adults to two insecticides tested using insecticide-coated glass vials.

Insecticide	N	LC_{50} (95% FL)[a]	LC_{90} (95% FL)[a]	Slope ± SEM	X^2
Carbosulfan	712	1.8 (1.5 – 2.1)	19.9 (16.3 – 24.9)	1.2 ± 0.05	3.3
Thiamethoxam	670	8.6 (6.0 – 11.7)	101.0 (68.8 - 170.4)	1.2 ± 0.12	5.9

a. Carbosulfan in µg a.i./vial; thiamethoxam in ppm commercial formulation.

Data in Table 49 were then used to calculate diagnostic doses for carbosulfan and thiamethoxam (Table 50). These doses (causing at least 95% corrected mortality) were then used for field monitoring of resistance.

Table 50. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* adults to varying doses of two insecticides.

Insecticide	Dose[a]	Percentage corrected mortality	
		Test 1	Test 2
Carbosulfan	100	99.0	97.1
	2.5	46.4	46.5
	0.3	27.3	28.3
	0.1	8.8	11.9
Thiamethoxam	200	95.8	-
	7	52.1	-
	0.8	15.8	-
	0.2	5.3	-

a. Carbosulfan in µg a.i./vial tested using insecticide-coated vials; thiamethoxam in ppm of commercial formulation tested using the technique developed by Cahill et al. (1996).

Monitoring of resistance in whitefly adult populations was conducted in five critical areas of the Cauca Valley of Colombia where pesticide use is very high. Results (Figure 54) indicated widespread resistance (less than 50% corrected mortality) to organophosphates, susceptibility (more than 80% mortality) to carbamates, and intermediate resistance (50%-80% mortality) to pyrethroids. This information is being used in the implementation of IPM strategies that are being researched at present.

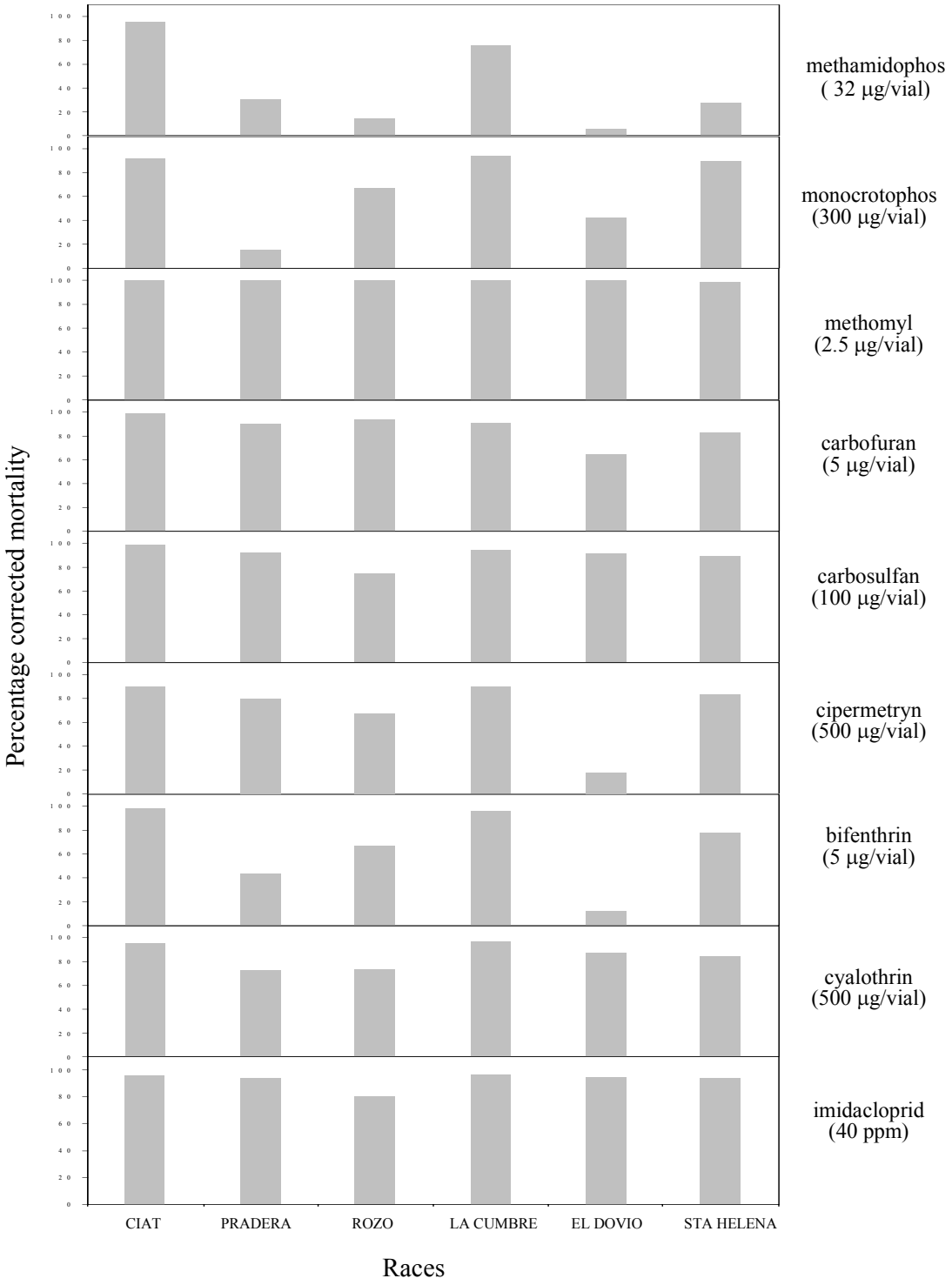

Figure 54. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* adults to nine insecticides in the Cauca Valley region of Colombia. Diagnostic dosages were tested under field conditions using insecticide-coated glass vials (except in the case of imidacloprid). Imidacloprid was tested using the petri dish technique of Cahill et al. (1996).

Similar work was conducted with nymphs of *T. vaporariorum.* Table 51 presents baseline data for three commonly used insecticides. With this information we calculated the diagnostic doses (Table 52). Field monitoring of resistance is in progress.

Table 51. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* first instar nymphs to three insecticides.

Insecticide	N	LC_{50} (95% FL)[a]	LC_{90} (95% FL)[a]	Slope ± SEM	X^2
Buprofezin	907	0.8 (0.6 – 1.1)	9.2 (6.9 – 13.0)	1.2 ± 0.09	7.3
Diafenthiuron	904	3.2 (2.3 – 4.4)	60.1 (41.8 – 92.8)	1.0 ± 0.06	10.5
Imidacloprid	483	16.5 (10.7 - 23.4)	171.5 (115.6 – 290.9)	1.2 ± 0.10	3.6

a. In ppm of commercial formulation.

Table 52. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* nymphs to varying doses (in ppm of commercial formulation) of three insecticides.

Insecticide	Dose	Percentage corrected mortality	
		Test 1	Test 2
Buprofezin	16.6	97.6	98.2
	1.5	68.6	52.3
	0.3	15.5	16.5
Diafenthiuron	300	94.6	97.6
	3	52.7	50.5
	0.3	15.8	16.8
Imidacloprid	300	94.9	97.2
	30	51.5	56.9
	3	15.1	12.2

Table 53 presents baseline data for adult *T palmi*. These figures represent the toxicological responses of a thrips population that has not been exposed to insecticides for years. With these data, the diagnostic doses shown in Table 54 were estimated.

Table 53. Toxicological responses of laboratory strains of *Thrips palmi* adults to three insecticides.

Insecticide	N	LC_{50} (95% FL)[a]	LC_{90} (95% FL)[a]	Slope ± SEM	X^2
Carbosulfan	719	160.6 (130.5 – 193.0)	837.0 (669.4 – 1103.0)	1.7 ± 013	2.5
Spinosad	764	210.0 (150.0 – 260.0)	1450.0 (1080.0 – 2120.0)	1.5 ± 0.10	7.5
Imidacloprid	827	26.6 (19.3 – 35.6)	465.6 (312.2 – 768.7)	1.0 ± 0.07	5.2
Fipronil 1	718	2.5 (1.6 – 3.7)	64.1 (39.1 – 123.8)	0.9 ± 0.08	4.4
Fipronil 2	705	1.7 (0.8 – 3.1)	45.1 (20.7 – 147.9)	0.9 ± 0.09	9.5

a. In ppm of commercial formulation.

Table 54. Toxicological responses of laboratory strains of *Thrips palmi* adults to varying doses (in ppm of commercial formulation) of three insecticides.

Insecticide	Dose	Percentage corrected mortality	
		Test 1	Test 2
Carbosulfan	2000	98.0	97.0
	250	52.6	51.1
	62.5	27.6	29.0
	31.2	14.2	10.2
Spinosad	2000	99.0	98.0
	300	77.2	61.0
	100	48.5	36.2
	31	16.7	15.1
Imidacloprid	1000	96.1	95.8
	30	44.9	47.5
	3	20.2	25.3
	1	7.3	6.2

The following step was to conduct field monitoring of resistance in five critical areas of heavy pesticide use. Results (Figure 55) indicated that *T. palmi* populations in the five areas surveyed are still susceptible to the three novel insecticides currently in use to control this insect.

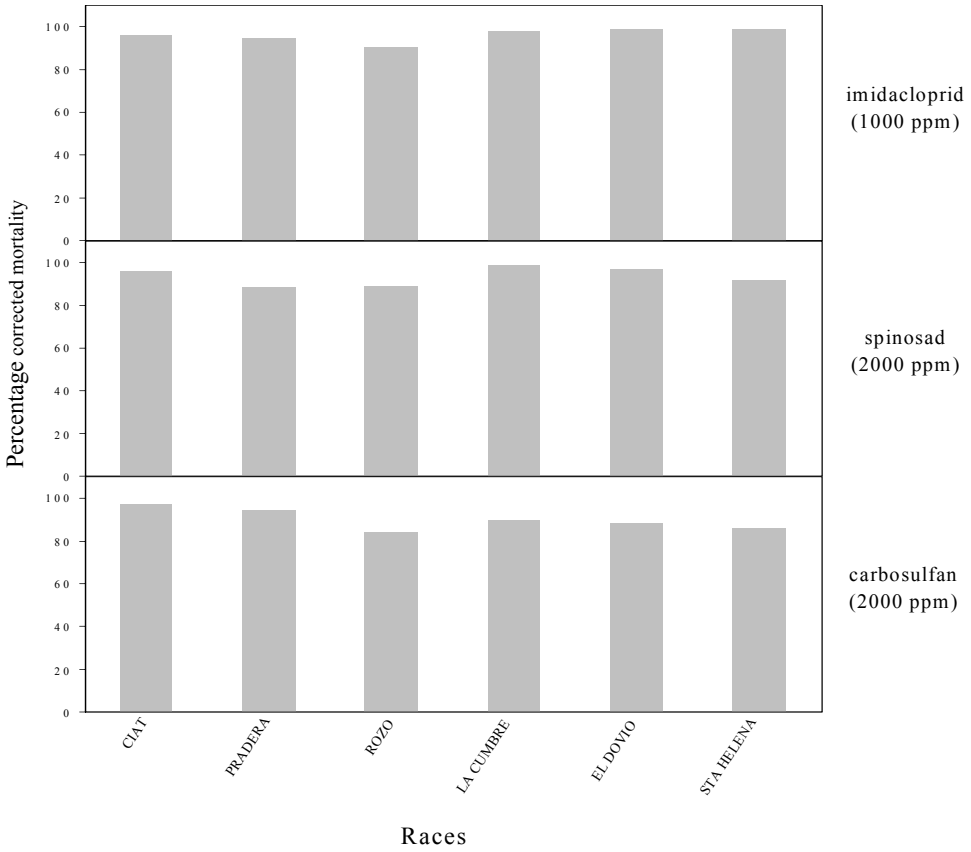

Figure 55. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* adults to nine insecticides in the Cauca Valley region of Colombia. Diagnostic dosages were tested under field conditions.

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

Contributors: C. Cardona, I. Rodríguez

3.3.4 Management strategies for whiteflies and thrips

Rationale: Whiteflies and thrips have become the object of excessive pesticide use by snap bean farmers. With the body of knowledge acquired in previous years (see CIAT 2001), systems can be developed that will at least contribute to reduce pesticide use. We now report on trials conducted to develop ways to reduce pesticide use in snap beans.

Materials and Methods: Two trials were conducted. In the first, we compared three approaches to thrips and whitefly management: (1) Drench application of an effective insecticide (imidacloprid) followed by foliar applications of an effective novel insecticide; (2) Seed treatment followed by foliar applications of an effective novel insecticide; (3) Farmers' practices. In the second trial we used a 5 X 5 Latin square design. Treatments were: (1) Drench application of an effective insecticide (imidacloprid) followed by foliar applications of an effective novel insecticide; (2) Drench application of an effective insecticide (imidacloprid) followed by foliar applications of less costly conventional insecticides; (3) Foliar applications of effective novel insecticides at pre-established action thresholds; (4) Foliar applications of conventional insecticides at pre-established action thresholds; (5) Farmers' practices.

Results and Discussion: In both trials, alternative management strategies resulted in the same levels of damage as those sustained by the farmer following his traditional approach to insect control (Table 55).

Table 55. Effect of different management strategies on damage caused by thrips (*Thrips palmi*) and whitefly (*Trialeurodes vaporariorum*) on snap beans.

Trial 1[a]			Trial 2[a]	
Treatment	Damage scores		Treatment	Damage by thrips (58 DAP)
	By thrips (58 DAP)	By whitefly (35 DAP)		
Drench + novel insecticides	5.0 a	5.6 a	Drench + novel insecticides	2.4 a
Seed treatment + novel	5.0 a	5.3 a	Drench + conventional	2.4 a
Farmers' practices	6.0 a	6.6 a	Novel at action threshold	2.6 a
			Conventional at AT[b]	2.2 a
			Farmers' practices	2.4 a

a. Damage scores on a scale of 1-9, where 1 = no damage and 9 = very severe damage. DAP = days after planting. Means within a column followed by the same letter are not significantly different at $P = 0.05$.
b. AT = action threshold.

In both trials, yields obtained with alternative insect control approaches were statistically the same as those obtained by the farmer (Table 56). The only real difference was in terms of the number of applications necessary to maintain a viable production. Although new approaches to whitefly and thrips control resulted in 2-3 applications per season, the farmers' practice treatment demanded 6-7 applications. This means that it is technically feasible to reduce the number of applications in snap beans. Further research should concentrate on elucidating the economic viability of the new approaches proposed and in the refinement of action thresholds so that farmers adopt them.

Table 56. Effect of different management strategies for thrips (*Thrips palmi*) and whitefly (*Trialeurodes vaporariorum*) on yields of snap beans.

Treatment	Number of insecticide applications	Yields [a] (kg ha^{-1})	Percentage loss[b]	Benefit/cost ratio
Trial 1:				
Drench + novel insecticides	2	9447 a	-	1.8
Seed treatment + novel	2	8366 a	11.4	1.6
Farmers' practices	6	8833 a	6.4	1.8
Trial 2:				
Drench + novel insecticides	2	12703 a	0.7	2.4
Drench + conventional	2	12156 a	4.9	2.5
Novel at action threshold	3	11737 a	8.2	2.2
Conventional at action threshold	3	12205 a	4.5	2.9
Farmers' practices	7	12789 a	-	2.4

a. For each trial, means within a column are not significantly different at $P = 0.05$.
b. With respect to the best treatment.

Reference:

CIAT. 2001. Annual Report 2000 Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: C. Cardona, I. Rodríguez, J.M. Bueno

Progress towards achieving output milestones:

Strategy developed for stable ALS resistance

- Resistant and tolerant varieties can substantially increase yields. This study is continuing at different locations.

- Knowledge on thrips and whitefly responses to alternative approaches will result in the development of management systems aimed at reducing pesticide use.