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DEVELOPING IMPROVED BUSH BEAN VARIETIES IN ECUADORIAN MARKET CLASSES USING FARMER PARTICIPATORY CROP IMPROVEMENT METHODS AND MARKER ASSISTED SELECTION OF AN ANTHRACNOSE RESISTANCE GENE

presented by

Emmalea Garver Ernest

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Plant Breeding and Genetics

James D. Kelly
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ABSTRACT

DEVELOPING IMPROVED BUSH BEAN VARIETIES IN ECUADORIAN MARKET CLASSES USING FARMER PARTICIPATORY CROP IMPROVEMENT METHODS AND MARKER ASSISTED SELECTION OF AN ANTHRACNOSE RESISTANCE GENE

By

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Bush beans are an important cash and subsistence crop in the Mira and Chota River Valleys of northern Ecuador. This project was undertaken to introduce genetic resistance to anthracnose into bean market classes grown in this region, and to determine the bean production practices, problems and variety preferences of bean producers in the Mira and Chota Valleys.

Co-4^{2}, a gene conferring broad-based resistance to anthracnose (*Colletotrichum lindemuthianum*) was introduced into bush bean varieties from three Ecuadorian commercial classes using marker assisted backcrossing. Anthracnose resistant lines were developed in two of the market classes but the *Co-4*^{2} gene failed to function in the genetic background of two of the Ecuadorian varieties used as recurrent parents. Verification of disease reaction through direct inoculation is essential to support the use of marker assisted selection in resistance breeding.

Farmer surveys revealed that bean producers in northern Ecuador are switching to bean types with a domestic market after losing the Colombian bean export market. Major production constraints are bean rust, whitefly, bean weevil, anthracnose, and root attacking pests and diseases. Few bean growers maintain their own seed but rather rely on the local grain markets as a source of bean seed. This system of seed management may be incompatible with the introduction of improved bean varieties in Ecuador.

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KEY TO ABBREVIATIONS

CRSP Collaborative Research Support Program

CIAL Comité de Investigación Agrícola Local, Local Agricultural Research Committee

CIAT International Center for Tropical Agriculture

INIAP Instituto Nacional Autonomo de Investigaciones Agropecuarias

PCI Participatory Crop Improvement

PCR Polymerase Chain Reaction

PRONALEG-GA Programa Nacional de Leguminosas y Granos Andinos

PVS Participatory Varietal Selection

QTL Quantitative Trait Loci

RAPD Random Amplified Polymorphic DNA

RIL Recombinant Inbred Line

SCAR Sequence Characterized Amplified Region

WHO World Health Organization

CHAPTER 1

IMPROVING ANTHRACNOSE RESISTANCE OF ECUADORIAN BEANS

Introduction

Beans in Ecuador

Production and Consumption

Common bean (*Phaseolus vulgaris*) is the most cultivated and most consumed legume crop in Ecuador. Ecuadorians consume not only dry beans but also the immature seed (*tierno*) and pods (snap bean). Although the Ecuador's national average per capita bean consumption rate of 6 kg/year is one of the lowest in Latin America (Broughton *et al,* 2003), consumption rates are believed to be much higher in certain regions of the country. According to Ecuador's Third National Agricultural Census, about 122,000 ha of beans for consumption dry or *in tierno* are planted every year (INEC-MAG-SICA, 2002). Climbing beans intercropped with maize constitute the majority of this area. Monocropped beans, principally bush beans with determinant or upright indeterminate growth habits, occupy 30% of the area planted to beans consumed *in tierno* and only 18% of the area planted to dry bean. However, bush beans account for 63% of the *tierno* production and 47% of the dry bean production. Additionally, the majority of the beans sold in Ecuador are bush beans (INEC-MAG-SICA, 2002).

The bulk of Ecuador's bean production area is in the highlands (80%), with smaller areas on the coast (18%), and in the eastern lowlands (2%) (Peralta et al., 1997). In the highlands, climbing beans are typically grown at 2400 to 2800 m.a.s.l., while the bush types are grown at lower elevations between 1500 and 2400 m.a.s.l. (Voysest, 2000). The focus of this project is the highland bush bean production area in the Northern Ecuadorian provinces of Imbabura and Carchi.

Bean Classes Grown in the Ecuadorian Highlands

Morphological (Singh et al., 1991) and genetic differences (Nodari et al., 1992) between domesticated beans from Central America and Mexico and those from South America indicate that there are two distinct bean gene pools and sites of domestication in *Phaseolus vulgaris*. Andean beans are typically larger-seeded and some possess a determinant growth habit, which is infrequent in beans of Middle American origin. Despite possessing a wide variation in traits such as seed color and protein type, Andean beans have been shown to be less genetically diverse than their Middle American counterparts. This narrow genetic base may account for the difficulty breeders have encountered in improving traits, such as yield, in Andean beans (Beebe et al., 2001). Most of the bean classes cultivated in Ecuador are from the Andean bean gene pool. However, beans of Middle American origin have been grown in South America since Pre-Colombian times, and some landraces grown in Colombia and Northern Ecuador show evidence of gene introgression from Middle American beans (Beebe et al., 2000; Beebe et al., 2001). Such landraces are an important genetic resource and potential source of unique variability within the Andean gene pool (Beebe et al., 2001)

According to Voysest (2000) the predominant commercial classes in the highland regions of Ecuador are large-seeded red mottle types (45-55 g/100 seeds), cranberry (45-55 g/100 seeds), and the round-seeded *bola* types (60-70 g/100 seeds) in a range of colors from yellow and red to white. Red mottle seed types have historically been grown for export to Colombia, while the other classes are marketed domestically. Both bush (type I and II growth habits) and climbing varieties (type IV growth habits) of these commercial classes are cultivated (Singh, 1982). Other Ecuadorian bean classes reported by Voysest (2000) include *panamito*, a small white bean, which may be Middle American in origin; *amarillo matahambre*, a yellow seeded bean; and *vaquita* which includes a variety of different kinds of spotted beans that are grown primarily for home consumption.

Bean Production Problems in Ecuador

Anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*) are the two bean diseases which have been most researched and characterized in Ecuador. Rust can result in severe yield losses if plants are infected before or during flowering. The disease is transmitted by wind-blown spores but not through seed. Rust can be controlled with fungicide applications made early in plant development, or through the use of resistant varieties (Stavely and Pastor-Corrales, 1989). A survey of rust diversity in Ecuador detected 17 different populations of the pathogen, and found that all of the Ecuadorian bean varieties tested were susceptible to at least one of the collected rust populations (Ochoa et al., 2002). Work to develop rust resistant bean varieties in Ecuadorian market classes is underway at INIAP (Murrillo, 2002)

Other bean diseases which have been reported in Ecuador include: ascochyta blight (*Ascochyta* sp.), halo blight (*Pseudomonas syringae* pv *phaseolicola*), powdery mildew (*Erysiphe polygoni*), angular leaf spot (*Phaeoisariopsis griseola*), root rot (*Fusarium solani, Rhizoctonia solani*) and bean common mosaic virus (BCMV) (Peralta et al., 1997; van Schoonhoven and Voysest, 1989).

Insect pests are more problematic in bush beans than in climbing beans. Bean pests that have been reported in Ecuador include: greenhouse whitefly (*Trialeurodes vaporariorum*), spider mite (*Tetranychus* sp. and *Polyphagotasonemus latus*), leafhopper (*Empoasca kraemeri*), pod borer (*Epinotia aporema* and *Laspeyresia leguminis*), and bean weevil (*Acanthosalides obtectus*) (Peralta *et al*, 1997). Whiteflies and spider mites, which also affect other crops, such as tomato, feed on the leaves of bean plants. Large populations of the pests can cause yield losses by weakening the plant. However, the whitefly species reported, *Traleurodes vaporariorum*, does not transmit Bean Common Mosaic Virus. Leafhoppers can be especially damaging and may cause severe yield losses. Their feeding stunts the plant and causes leaf curling. Pod borers can have a direct effect on yield by damaging developing seeds and allowing secondary rot pathogens infect the pod. Bean weevil larvae feed on stored grain and can cause substantial post-harvest losses (Schwartz et al., 1978).

The Bean/Cowpea Collaborative Research Support Program (CRSP) has funded an initiative to improve bean yield and yield stability in Ecuador. The project involves the bean breeding programs at Michigan State University and at Ecuador's National Agricultural Research Institution (INIAP). One goal of this project is to develop bean varieties possessing improved genetic resistance to anthracnose as well as the agronomic and seed quality traits that Ecuadorian farmers desire.

Colletotrichum lindemuthianum

Anthracnose of bean is caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav. The disease has a world-wide distribution, although it tends to be more problematic in temperate and subtropical climates since high humidity and moderate temperatures of between 13 and 26 C favor disease development (Pastor-Corrales and Tu, 1989).

Symptoms of the disease in bean include dark brown or black lesions on the leaves, pods, stems and hypocotyl. Veins of infected leaves often become darkened and necrotic. Conidia are produced in larger lesions and serve as a source of secondary inoculum that may be spread by wind and rain (Tu, 1992). Mycelia of the fungus can also infect seeds causing discoloration and cankers. Moreover, anthracnose is transmitted efficiently by pathogen infected seed, which is a source of inoculum if planted the following year (Pastor-Corrales and Tu, 1989).

The infection process of *C. lindemuthianum* begins with the germination of conidia in free water on the plant surface. The fungus then penetrates the cuticle and epidermal cells via appressoria. The infection peg enlarges between the plant cell wall and the plasma membrane and primary hyphae from the infection peg ramify intracellularly through the plant tissue. Within a few days, lateral branches of the primary hyphae begin to grow into the host's cells, marking the beginning of the destructive, necrotrophic phase (O'Connell and Bailey, 1991). Eventually, acervulli bearing conidia form in the water-soaked lesion infected by the fungus (Pastor-Corrales and Tu, 1989).

C. lindemuthianum reproduces asexually through conidia -- the sexual phase of the fungus has very rarely been observed (Pastor-Corrales and Tu, 1989). Despite its infrequent sexual reproduction, *C. lindemuthianum* exhibits a wide range of pathogenic variability. One means of assessing the variability of the pathogen is inoculation of a set of twelve differential cultivars (Table 1), which possess different anthracnose resistance genes or alleles. The differential series includes representative cultivars from the Andean and Middle American bean gene pools. Each differential cultivar is assigned a binary number. An isolate of *C. lindemuthianum* is assigned a race number, which is the sum of the binary numbers of the differential cultivars on which the isolate is pathogenic (Drijfhout and Davis, 1989; Pastor-Corrales, 1991).

Table 1. Anthracnose differential cultivars, their gene pool, binary number and resistance genes

	Differential Cultivar	Gene Pool*	Binary Number	Resistance Genes
A.	Michelite	MA	1	
B.	Michigan Dark Red Kidney	A	2	*Co-1*
C.	Perry Marrow	A	4	$Co\text{-}1^3$
D.	Cornell 49242	MA	8	*Co-2*
E.	Widusa	MA	16	*Co-9?*
F.	Kaboon	A	32	$Co\text{-}1^2$
G.	Mexico 222	MA	64	*Co-3*
H.	PI 207262	MA	128	$Co\text{-}4^3$, *Co-9*
I.	TO	MA	256	*Co-4*
J.	TU	MA	512	*Co-5*
K.	AB 136	MA	1024	*Co-6, co-8*
L.	G2333	MA	2048	$Co\text{-}4^2$, *Co-5, Co-7*

* MA- Middle American, A-Andean, ?- allele of *Co-9,* yet to be determined

However, the differential series does not detect all pathogenic variability, as there have been reported differences in pathogenicity between isolates with the same race number. In a study of *C. lindemuthianum* diversity in Ecuador, thirteen isolates which were not pathogenic on any of the differential cultivars (race 0) had six distinct patterns of pathenogenicity when inoculated on a set of nine "local differential cultivars" (Falconí et al., 2003). Molecular genetic studies of *C. lindemuthianum* have detected additional variability both within and between races of the pathogen (Fabre, et al., 1995; Balardin, et al., 1997).

Control of Bean Anthracnose

Cultural practices, fungicides and genetic resistance have all been used to control anthracnose with varying degrees of success. Pathogen-free seed has been used successfully in some countries, such as the United States, that have semiarid regions where disease-free seed can be produced. In developing countries, however, farmers are unlikely to have access to disease-free seed, and are unable to avoid possible introduction of the disease into their fields through infected seed. Seed treatments and foliar fungicide applications are not sufficiently effective to prevent crop losses under conditions favorable for disease development (Pastor-Corrales and Tu, 1989). Overuse of chemical controls could lead to the emergence of fungicide resistant biotypes of *C. lindemuthianum* (Tu and McNaughton, 1980) resulting in loss of disease control. Additionally, chemical controls can be prohibitively expensive to small-scale farmers in developing countries (Pastor-Corrales and Tu, 1989).

Generally, genetic resistance is considered the best control for bean anthracnose. Historically, the introduction of major anthracnose resistance genes into commercial cultivars has reduced the economic importance of this disease in Europe, the United States and Canada, but dependence on single resistance genes has led to the breakdown of cultivar resistance when new races of the pathogen are inadvertently introduced or arise through mutation (Fouilloux 1976, Kelly et al., 1994).

Genetic Resistance to Bean Anthracnose

Both quantitatively and qualitatively inherited genetic resistance to *C. lindemuthianum* are present in bean, but research on quantitative resistance has been limited. Geffroy et al. (2000) located ten quantitative trait loci (QTL) involved in partial resistance to *C. lindemuthianum* races 7 and 45 on the BAT93 x JaloEEP558 RIL map. Two of the QTL identified were located near genes involved in plant defense and three were adjacent to genes conferring specific disease resistance. This finding lends support to the theory that defeated resistance genes are involved in conferring partial resistance (Nass et al., 1981; Li et al., 1999) and may represent members of a cluster of resistance genes as proposed by Michelmore and Meyers (1998).

One recessive and nine dominant anthracnose resistance genes are reported in the literature. Some genes, namely *Co-1, Co-3* and *Co-4*, have multiple resistance alleles that have been characterized (Kelly and Vallejo, 2004). The names of the known resistance genes, their gene pools of origin, sources, and linked molecular markers are shown in Table 2. The Andean sources of resistance that have been characterized all possess alleles at the same locus, *Co-1*. The Middle American sources of resistance are more diverse and represent eight different loci.

A number of studies have demonstrated the gene pool specificity of *C. lindemuthianum* (Sicard et al., 1997; Balardin and Kelly, 1998; Geffroy et al., 1999). Generally, Andean races of *C. lindemuthianum* are more virulent on bean lines carrying the Andean resistance alleles at the *Co-1* locus than on lines with resistance genes from the Middle American gene pool. Yet *Co-1* alleles confer resistance to several highly virulent races of Middle American origin (Balardin and Kelly, 1998). Similarly, the Middle American resistance genes confer resistance to Andean races of the pathogen. Consequently, the best approach to anthracnose resistance breeding is to employ genes based on a knowledge of the *C. lindemuthianum* races present in the region. In regions where Andean races predominate, the Middle American genes should be used and vice versa. In the U.S., where both Andean and Middle American races of the pathogen are present, resistance is best achieved by pyramiding both Andean and Middle American anthracnose resistance genes into a cultivar (Balardin and Kelly, 1998). The most complete and durable resistance to anthracnose will be achieved through gene pyramiding, as evidenced by the outstanding resistance of a three gene pyramid in G 2333, one of the anthracnose differential cultivars (Table 1).

Table 2. Anthracnose resistance genes and alleles, their previous names, differential cultivars containing them, their gene pool of origin, other sources and linked markers.

Gene/ Allele	Previous Name	Differential Cultivar[a] [binary number]	Gene Pool[b]	Other Sources	Markers	References
Co-1	*A*	Michigan Dark Red Kidney [2]	AND		$OF10_{530}$	Young and Kelly, 1997
Co-1^2		Kaboon [32]	AND		SE_{ACT}/M_{CCA}	Melotto and Kelly, 2000 Vallejo and Kelly, 2002
Co-1^3		Perry Marrow [4]	AND			Melotto and Kelly, 2000
Co-1^4			AND	AND 277		Alzate-Marin et al.,2003a
Co-2	*Are*	Cornell 49242 [8]	MA		$OQ4_{1440}$ $B355_{1000}$, SCH20	Young and Kelly, 1996 Adam-Blondon et al., 1994
Co-3	*Mexique 1*	Mexico 222 [64]	MA			
Co-3^2			MA	Mexico 227		
Co-4	*Mexique 2*	TO [256]	MA		SAS13	Young et al., 1998 Awale and Kelly, 2001
Co-4^2		G2333 [2048]	MA	SEL 1308	SH18, SBB14	Awale and Kelly, 2001
Co-4^3		PI 207262 [128]	MA			
Co-5	*Mexique 3*	TU [512]	MA	SEL 1360	SAB3	Vallejo and Kelly, 2001
Co-6		AB 136 [1024]	MA	Catrachita	$OAH1_{780}$, $OAK20_{890}$	Young and Kelly, 1997
Co-7		G2333 [2048]	MA	SEL 111		
co-8		AB 136 [1024]	MA		OPAZ20	Alzate-Marin et al., 2001
Co-9		PI 207262 [128]	MA	BAT 93	SB12	Geffroy et al., 1999
Co-10			MA	Ouro Negro	F10	Alzate-Marin et al., 2003b

a Binary number indicated in brackets   b AND, Andean ; MA, Middle American

CIAT accession G 2333 is a landrace known as Colorado de Teopisca that was collected in Chiapas, Mexico. G 2333 belongs to a genetically unique group within the Middle American gene pool, termed race Guatemala, which includes several highly disease resistant landraces (Beebe et al., 2004; Beebe et. al., 2000). Schwartz et al. (1982) found that G 2333 was resistant to *C. lindemuthianum* isolates from Colombia, Brazil, Guatemala and Europe. Subsequent testing revealed that G 2333 was resistant to 380 isolates of *C. lindemuthianum* (representing 15 different races) from 11 Central and South American countries (Pastor-Corrales et al., 1994). Pastor-Corrales et al. (1994) and Young et al. (1998) determined that G 2333 carries a pyramid of three anthracnose resistance genes: *Co-4*^{2}, *Co-5,* and *Co-7*. The most effective gene in this pyramid is *Co-4*^{2} which conferred resistance to 33 out of 34 different races of *C. lindemuthianum* collected from 9 different countries in the Americas (Balardin et al., 1997).

Pyramiding of resistance genes presents a challenge for breeders, as the effects of two or more resistance genes may confound one another, making their detection through disease screening difficult or impossible. Molecular markers, which allow breeders to indirectly test for the presence or absence of a gene without disease inoculation, facilitate gene pyramiding (Kelly, 1995). RAPD and SCAR markers have proven useful in such applications because they are relatively inexpensive and efficient to run. Both RAPD and SCAR markers make use of in vitro DNA synthesis, or PCR. For RAPDs short sequences of DNA, called primers, anneal to particular sequences in the genomic DNA and initiate DNA replication at the site. Since RAPD primers are short, they initiate replication at several sites in the genome and produce DNA fragments different lengths which can be separated by agarose gel electrophoresis, and visualized as discrete bands.

Slight differences in the genomic DNA sequences of the individual plants being tested affect primer annealing and thereby result in different patterns of DNA fragments amplified. A RAPD marker for a disease resistance gene amplifies a fragment of the genomic DNA in the resistant plant that is located near the gene of interest but is absent in the susceptible plant. SCAR markers are developed by sequencing the fragment that is polymorphic between the resistant and susceptible plant and designing longer primers (of ~24 base pairs) which are specific to the fragment of interest. SCAR markers then amplify only a specific region of the genome, which is linked to the resistance gene, resulting in only one band. The advantages of SCAR markers over RAPDs are that SCARs are more reproducible and do require as long for electrophoresis (Staub et al., 1996).

Molecular markers have been developed for many of the anthracnose resistance genes that have been characterized, including *Co-4*^{2}, the most effective gene from G 2333. SCAR markers linked to *Co-4*^{2} include: SAS13 at 0.39 cM from the *Co-4*^{2}gene (Young *et al.*, 1998; Melotto and Kelly, 2001), SH18 at 4.27±2.37 cM from the gene and SBB14 at 5.87±1.93 cM from the gene (Awale and Kelly, 2001). SAS13 and SH18 are dominant markers coupled to the *Co-4*^{2} gene. SBB14 is codominant, which means it amplifies two different sized bands with the larger band associated with the resistance gene. SH18 and SBB14 are specific to the *Co-4*^{2} allele while SAS13 is present in lines carrying other alleles at the *Co-4* locus. Even though it is not as specific as the other two markers SAS13 has the desirable characteristic of being tightly linked to the *Co-4* locus. In a population of 1,018 plants segregating for *Co-4*^{2}, only four recombinations between the gene and SAS13 were observed. SAS13 has successfully been used to introduce *Co-*

4^2 into highly susceptible pinto bean through backcrossing without pathogen screening (Miklas and Kelly, 2002).

Anthracnose in Ecuador

Anthracnose is one of the most important bean diseases in Ecuador, particularly in cool, moist, highland regions. A 1994 survey of *C. lindemuthianum* diversity and virulence in the country identified 14 races from 94 different isolates of the fungus (INIAP, 1995). Twenty- one percent of the isolates were not pathogenic on any of the differential cultivars and were designated race 0. The most frequently identified races, 133, 128, 129, 5, and 4, collectively overcome the resistance genes in the differentials Perry Marrow (*Co-1*^{3}), and PI 207262 (*Co-4*^{3} and *Co-9*). Other races identified in the survey were, 2, 15, 65, 131, 1153, and 1409, which overcome resistance genes in Michigan Dark Red Kidney (*Co-1*), Cornell 49242 (*Co-2*), Mexico 222 (*Co-3*), TO (*Co-4*), and AB136 (*Co-6* and *co-8*). Twenty-nine isolates collected from wild beans in Ecuador were mostly race 0; only one isolate was pathogenic on the differentials and was characterized as race 6 (Sicard, 1997). A more recent survey of 31 *C. lindemuthianum* isolates identified races 0, 3, 4, 256, 260, and 1346. Again, most of the isolates were race 0, which suggests a low level of virulence among isolates from Ecuador. Collectively, the six races identified in the most recent study overcame *Co-1*, *Co-1*^{3}, *Co-3*, *Co-4*, *Co-6*, and *co-8* (Falconí et al., 2003). None of the races identified thus far in the country are pathogenic on G 2333 (*Co-4*^{2}, *Co-5* and *Co-7*) or Kaboon (*Co-1*^{2}). These two differential cultivars are potential sources of genes which would confer resistance to all known races of *C. lindemuthianum* in Ecuador.

This project was undertaken with the objective of developing Ecuadorian bean varieties with enhanced anthracnose resistance. To reach this objective, *Co-4*^{2} was introduced into six Ecuadorian bush bean varieties, which represent three different commercial classes. Use of molecular markers allowed for rapid, indirect selection for the resistance gene so that three backcrosses could be made in quick succession. A backcross breeding approach was used in order to maintain the required adaptation and seed quality traits important to Ecuadorian bean farmers.

Materials and Methods

Use of Molecular Markers

Indirect selection was conducted based on RAPD (AS13) and SCAR (SAS13, SBB14, SH18) markers linked to the *Co-4²* gene. DNA was extracted from bean leaf tissue according to the mini-prep procedure of Afanador et al. (1993). The DNA was quantified using a fluorometer (Hoefer DyNA Quant 200, Amersham Biosciences) or by comparison to Low DNA Mass Ladder (Invitrogen) in a 0.8% agarose gel. DNA samples were diluted to a standard concentration of 10 ng/μl. RAPD marker reactions were prepared in a 25 μl reaction volume with the following component concentrations: template DNA, 1.2 ng/μl; primer, 1.2 ng/μl; $MgCl_2$, 5 mM; PCR buffer, 1x; dNTP, 0.2 mM each base; Taq polymerase, 0.05 u/μl. SCAR marker reactions were prepared in a 20 μl reaction volume with the following component concentrations: template DNA, 1 ng/μl; primer (Integrated DNA Technologies, Coralville, IA), 0.5 ng/μl each primer; $MgCl_2$, 3.75 mM; PCR buffer, 1x; dNTP, 0.2 mM each base; Taq polymerase, 0.05 u/μl. Amplification by Polymerase Chain Reaction (PCR) in a MJ Research PTC-100 thermal cycler (MJ Research, Inc., San Francisco) followed the thermal profiles recommended by the markers' developers (Young et al, 1998; Awale and Kelly, 2001). PCR products were loaded into a 1.4% agarose gel containing 0.02 μg/ml ethidium bromide, 40 mM Tris-acetate, and 1 mM EDTA. After electrophoresis the DNA bands were visualized under ultraviolet light and photographed.

Introduction of Co-4^2 into Ecuadorian Varieties Through Marker Assisted Backcrossing

Crossing between Ecuadorian varieties, Je.Ma. and Cocacho, and Red Hawk*2/SEL 1308, a line carrying *Co-*4^2, began in November of 2001. A description and pedigree for the parental materials appears in Table 3, and a summary of the crossing and selection activities appears in Table 4. All crosses were made in the Michigan State University Greenhouses, East Lansing, MI.

RH*2/SEL1308 is a single backcross of Red Hawk, an improved dark red kidney variety of Andean origin, to SEL 1308 a type II small black which carries *Co-*4^2 derived from G2333 (Young et al, 1998). Rather than using G2333 or SEL 1308 as the donor parent, RH*2/SEL1308 was chosen as the source of *Co-*4^2, since this line produced seed nearer in size to the large-seeded Ecuadorian recurrent parents. The RH*2/SEL1308 plants were segregating for *Co-*4^2, so the RAPD marker AS13 was used to select those plants likely to be carrying the resistance gene. The seed for Je.Ma. and Cocacho was supplied by Dr. James Beaver, University of Puerto Rico, Mayaquez. Je.Ma is a large seeded Calima type of Colombian origin and Cocacho is a medium seeded yellow Canario, which may have Peruvian origins (Voysest, 2000). The donor parent was also crossed with Genuine, a McCaslan type climbing snap bean which carries two resistance genes for bean rust, *Ur-3* and *Ur-4* and the Bean Golden Mosaic Virus resistance gene *bgm-1*. Snap beans are grown in Ecuador and we speculated that this line could be useful in Ecuador because of its multiple disease resistance genes.

Table 3. Parents used in crossing

Line	Class (g/100 seeds)	Growth Habit[a]	Resistance Genes	Pedigree	Source[b]
INIAP-414 -- Yunguilla	Calima (50 g)	I	--	G13922//G2172/G6474	CIAT
ARME-2	Medium Red Mottle (45 g)	IIa	--	AND 1005/Paragachi	CIAT
Paragachi	Medium Red Mottle (45 g)	IIa	--	Possibly a selection from PVA 1441[c]	possibly CIAT
INIAP-418 -- Je.Ma	Calima (55 g)	IIb	--	G12722/G21720	CIAT/ICA
Cocacho	Canario (50 g)	I	--	possibly of Peruvian origin[c]	Landrace
ACE-1	Canario (50 g)	I	I gene	CAP9/Canario Bola	CIAT
Genuine	McCaslan type, indeterminate snap	IV	*Ur-3, Ur-4, bgm-1*	McCaslan42*3/5/Goldrush *2/3Slenderette *2/Mexico 235	Florida Ag. Exp. Sta., Puerto Rico Ag. Exp. Station, USDA-ARS
Red Hawk	Dark Red Kidney (50 g)	I	*I gene, Co-1, Co-2*	Charlevoix/2*Montcalm[d]	Michigan Ag. Exp. Sta., USDA-ARS
SEL 1308	Small Black (20 g)	II	*Co-4*^{2}	G2333/2*Talamanca[e]	CIAT

[a] I – Determinate
IIa – Indeterminate with upright stem and branches, lacks climbing ability
IIb – Indeterminate with upright stem and branches, possesses some climbing ability
IV - Indeterminate climbing
(Singh, 1982)

[b] CIAT – Centro Internacional de Agricultura Tropical
ICA - Instituto Colombiano Agropecuario
USDA-ARS - United States Department of Agriculture Agricultural Research Service

[c] (Voysest, 2000)
[d] (Kelly et al., 1998)
[e] (Young et al., 1998)

Table 4: Planting dates for lines grown as the pollen parent for crosses, number of seeds planted, number of plants selected for crossing, molecular marker used for selection, and varieties with which the selected plants were crossed.

Date	Pollen Parent	# Planted	# Selected	Marker	Selected Plants Crossed With:
11/01	RH*2/SEL 1308	15	10	AS13	Cocacho, Je.Ma., Genuine
Backcross 1					
2/02	Cocacho//RH*2/SEL 1308	12	9	AS13	Cocacho, ACE-1
2/02	Genuine//RH*2/SEL 1308	10	9	AS13	Genuine
3/02	Je.Ma.//RH*2/SEL 1308	16	8	AS13	Je.Ma., Yunguilla, Paragachi, ARME-2
Backcross 2					
5/02	Cocacho*2//RH*2/SEL 1308	6	5	SAS13	Cocacho
5/02	Genuine*2//RH*2/SEL 1308	6	6	SAS13	Genuine
6/02	ARME-2/3/Je.Ma. //RH*2/SEL 1308	6	3	SAS13	ARME-2
6/02	ACE-1/3/Cocacho//RH*2/SEL 1308	6	2	SAS13	ACE-1
6/02	Yunguilla/3/Je.Ma.//RH*2/SEL 1308	6	3	SAS13	Yunguilla
6/02	Paragachi/3/Je.Ma.//RH*2/SEL 1308	6	3	SAS13	Paragachi
6/02	Je.Ma.*2//RH*2/SEL 1308	9	6	SAS13	Je.Ma.
Backcross 3					
8/02	Cocacho*3//RH*2/SEL 1308	12	4	SAS13	Cocacho
8/02	ACE-1*2/3/Cocacho//RH*2/SEL 1308	23	11	SAS13	ACE-1
9/02	ARME-2*2/3/Je.Ma.//RH*2/SEL 1308	10	9	SAS13	ARME-2
9/02	Genuine*3//RH*2/SEL 1308	18	4	SBB14, SH18	Genuine
9/02	Paragachi*2/3/Je.Ma.//RH*/SEL 1308	12	6	SAS13, SBB14	Paragachi
9/02	Yunguilla*2/3/Je.Ma.//RH*/SEL 1308	11	6	SAS13, SBB14	Yunguilla

Recurrent parent followed by *2 indicates one backcross; recurrent parent followed by *3 indicates two backcrosses

In February of 2002, seed of four additional Ecuadorian varieties was obtained from the National Legume and Andean Grains Program (PRONALEG-GA) at INIAP: Yunguilla, an improved Calima type; ARME-2 and Paragachi, both medium seeded red mottle types; and ACE-1, an improved Canario. These four varieties were introduced into the crossing program as recurrent parents during the first backcross in February and March of 2002. Je.Ma.// RH*2/SEL1308 plants carrying the AS13 marker were crossed with Yunguilla, ARME-2, Paragachi and Je.Ma. Je.Ma., Paragachi, ARME-2 and Yunguilla are sensitive to photoperiod and required the use of blackcloth to shorten the daylight period to induce flowering in Michigan. Cocacho//RH*2/SEL1308 plants carrying AS13 were crossed with ACE-1 and Cocacho. Genuine// RH*2/SEL1308 plants carrying AS13 were crossed with Genuine. In all cases this was considered the first backcross even though it was the first cross involving Yunguilla, ARME-2, Paragachi and ACE-1, since the first cross had been to plants of similar seed type.

Seed for the second backcross was sown in May and June of 2002. The Je.Ma., Yunguilla, ARME-2 and Paragachi crosses and parents were sown in a growth chamber (22C, 10 hour daylength) and maintained there until they began flowering. At flowering, they were moved to the greenhouse for crossing. The BC_1F_1 plants were screened with the SCAR marker SAS13 and those carrying the marker were crossed with their respective recurrent parent.

Seed of the BC_2F_1 generations for Cocacho, ACE-1, Yunguilla, ARME-2, Paragachi and Genuine was sown in August and September of 2002 in order to generate the third backcross. Je.Ma. was not included since its long generation time did not allow for harvest of seed in time to make a third backcross. SAS13 was used to select plants from the Cocacho, ACE-1, and ARME-2 populations. Both SBB14 and SAS13 were used to select plants from the Yunguilla and Paragachi populations. SBB14 and SH18 were used to select plants from the Genuine population since it was discovered that that Genuine tested positive for the SAS13 marker. The BC_2F_1 plants which were positive for these markers were crossed with their respective recurrent parent.

Marker Assisted Selection in Ecuador

In February of 2003 a sample of the BC_2 and BC_3 generations was planted in pots in a greenhouse on INIAP's Santa Rosa substation near Quito, Ecuador. The greenhouse is located at 2750 m.a.s.l. and consists of a fiberglass roof and screened walls. The potting media was pasteurized soil and pumice. Fifteen seeds from the BC_2F_2 generations and ten from the BC_3F_1 generations of the Cocacho, ACE-1, Yunguilla, Paragachi, ARME-2 and Genuine populations were planted in the greenhouse in addition to 10 seeds from the Je.Ma BC_2F_1 generation. Two seeds of each recurrent parent were also sown.

Leaf tissue was collected from each plant and DNA extracted as in previous selections. SAS13 primers failed to amplify a band after repeated attempts. Consequently, the SBB14 and SH18 SCAR markers were used to select plants believed to be carrying the *Co-4*^{2} anthracnose resistance gene. Selfed seed of the selected plants was harvested and was retained in Ecuador for further testing.

Field Inoculation of Backcross Populations

The majority of the Cocacho, ACE-1, Yunguilla, Paragachi, ARME-2 and Genuine BC_2F_2 and BC_3F_1 generation seed was planted in a research plot on the Santa Rosa Substation. The plot was at an elevation of 2740 m.a.s.l. - higher than bush beans are typically grown in Ecuador. However, the cool, wet conditions at this elevation were ideal for anthracnose infection. The field was planted on February 11, 2003 according to the layout in Figure 1. Paragachi, which is highly susceptible to anthracnose, was planted in the guard rows. Additionally, three seeds of Paragachi were planted at the end of each experimental row to serve as a disease spreader.

The plot was cultivated at 28, 55, 88 and 118 days after planting in order to control weeds. A foliar fertilizer was applied at 34 days after planting and a sidedressing of urea (approximately 20 kg/ha) at 55 days. Dimetholate was applied at the recommended rate at 88 days to control leafhoppers (*Empoasca krameri*).

The field was inoculated three times with a race 0 isolate of *Colletotrichum lindemuthianum* collected from the Santa Rosa Substation. The fungus used for inoculation was grown in Erlenmeyer flasks on chopped and sterilized snap beans pods. When the fungus began to produce spores, the contents of the flask were pureed in a blender and diluted with distilled water. The final spore concentration was calculated using a hemicytometer. The spore solution was applied to the plants in late afternoon using a backpack sprayer. The plants were first inoculated at 59 days after planting with 7 L of spore solution at a concentration of 1.2×10^5 spores/ml. The second inoculation was at 74 days after planting with 15 L of spore solution at a concentration of 4.2×10^5 spores/ml. The third inoculation was at 92 days after planting with 20 L of spore solution at a concentration of 8.5×10^4 spores/ml.

Figure 1. Layout of field planted on the Santa Rosa Substation

	Line/Population	# planted	Line/Population	# planted	Line/Population	# planted	
2 m rows of Paragachi	Paragachi		Paragachi		Paragachi		**3 m rows of Paragachi**
	Paragachi		Paragachi		Paragachi		
	ARME-2 BC_2	15	Cocacho BC_3	16	ACE-1 BC_3	11	
	ARME-2 BC_2	15	Cocacho BC_3	17	ARME-2	8	
	ACE-1 BC_3	10	Cocacho BC_2	12	ACE-1	19	
	Genuine	21	Cocacho BC_2	13	Paragachi BC_2	19	
	Cocacho BC_2	12	Yunguilla	21	Yunguilla BC_3	9	
	Cocacho BC_2	13	Yunguilla BC_3	9	Cocacho	14	
	Genuine BC_3	15	ACE-1 BC_3	10	Yunguilla	21	
	ACE-1	18	ARME-2 BC_3	6	Genuine BC_2	22	
	Cocacho	14	Yunguilla BC_2	13	Cocacho BC_3	17	
	ARME-2	8	ARME-2	8	Cocacho BC_3	17	
	ACE-1 BC_2	15	Paragachi	21	Cocacho BC_2	12	
	ACE-1 BC_2	15	Genuine	21	Cocacho BC_2	12	
	ACE-1 BC_2	16	Paragachi BC_3	7	Genuine BC_3	16	
	ARME-2 BC_3	6	Cocacho	14	ACE-1 BC_2	15	
	Cocacho BC_3	16	Paragachi BC_2	18	ACE-1 BC_2	15	
	Cocacho BC_3	17	ACE-1 BC_2	15	ACE-1 BC_2	16	
	Paragachi	21	ACE-1 BC_2	15	Paragachi	21	
	Paragachi BC_2	18	ACE-1 BC_2	16	Paragachi BC_3	7	
	Yunguilla BC_2	13	ARME-2 BC_2	15	Genuine	21	
	Paragachi BC3	6	ARME-2 BC_2	16	ARME-2 BC_3	7	
	Genuine BC_2	21	Genuine BC_3	15	Yunguilla BC_2	13	
	Yunguilla	20	Genuine BC_2	22	ARME-2 BC_2	14	
	Yunguilla BC_3	9	ACE-1	19	ARME-2 BC_2	15	
	Paragachi		Paragachi		Paragachi		
	Paragachi		Paragachi		Paragachi		
	3 m rows		**3 m rows**		**3 m rows**		

The number of plants that were flowering in each population was recorded at 55, 62, 69, 73, 83, and 87 days after planting. The anthracnose symptoms on each plant were evaluated at 74, 88, 102 and 119 days after planting. Leaf symptoms were rated on the 1 to 9 scale described in Table 5. Plants having a rating of 1-3 were considered resistant, those with a rating of 4 to 6 were considered moderately susceptible, and plants with a rating of 7 to 9 were considered very susceptible. Pod symptoms were rated according to the 0 to 3 scale shown in Table 6. A rating of 0 was considered resistant, a rating of 1 moderately susceptible, and a rating of 2 or 3 very susceptible.

Table 5: Foliage Symptom Ratings for Anthracnose*

Rating	Symptoms
1	Plant has no visible symptoms on leaves.
2	Few small lesions in veins on back of leaf
3	Frequent small lesions in veins on back of leaf
4	Large lesions in the veins on back of leaf.
5	Large lesions visible on front and back of leaf.
6	Leaf lesions as in 5, some lesions on petioles, and stems
7	Many large lesions on leaves, petioles, and stems.
8	Many large lesions, accompanied by dead leaves, reduced growth
9	Plant is dead or nearly so.

*A rating of 1 to 3 is considered resistant; 4 to 6 is considered moderately susceptible, and 7 to 9 very susceptible.

Table 6: Pod Symptom Ratings for Anthracnose†

Rating	Symptoms
0	Plant has no visible symptoms on pods.
1	Small superficial lesions on pods
2	Large, deep, sporulating lesions on pods
3	Plant has no pods due to severe anthracnose symptoms in foliage.

†A rating of 0 is considered resistant, 1 is considered moderately susceptible, and 2 or 3 very susceptible.

Evaluation of Seed From Field Grown Populations

Seed from each of the plants grown in the field was harvested and maintained separately. Plants were classified according to their seed coat pattern and color. Some difference in seed size was also apparent. However, there was insufficient quantity of seed to evaluate this trait.

Farmers recruited from among the participants in a Bean Seed Exhibition held by PRONALEG-GA in the community of La Concepción, June 29, 2003 were asked to rank samples of the seed produced by the ACE-1, Cocacho, and Yunguilla BC_2F_2 and BC_3F_1 populations according to their preference. Ties were permitted.

For presentation to the farmers the seeds were glued onto 2 x 2" pieces of card stock. Figure 2 shows a photograph of the seed samples that were used. (Figure 2 in this thesis is presented in color.) Table 7 gives a description of each sample.

Figure 2. Seed samples farmers rated in survey

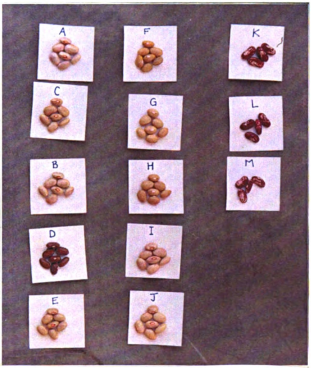


Table 7. Samples used in farmer evaluations of seed quality

Cocacho Samples		ACE-1 Samples		Yunguilla Samples	
A	white	F	tan, yellow edges	K	red/pink/white mottle*
B	white/yellow mottle	G	ACE-1, parent	L	Yunguilla, parent
C	yellow*	H	yellow*	M	red/white mottle
D	brown	I	white		
E	Cocacho, parent	J	white/yellow mottle		

* sample which most resembled the recurrent parent

Greenhouse Inoculations of Backcross Populations

The following populations were screened with the AS13 and SH18 markers for *Co-4*^{2} and inoculated with *C. lindemuthianum* under greenhouse conditions at Michigan State University: Paragachi BC_1F_2, BC_2F_2, and BC_3F_1; Cocacho BC_1F_2, BC_2F_2, and BC_3F_1; ARME-2 BC_2F_2 and BC_3F_1; and Yunguilla BC_2F_2 and BC_3F_1. Leaf tissue was collected from the plants before inoculation and DNA extraction and marker analysis were performed as previously described. According to the method described by Balardin and Kelly (1998) plants were spray inoculated at ten days after planting with a spore suspension containing $1.0x10^6$ spores/ml and 0.01% Tween 80. After inoculation, plants were maintained in a humid chamber for 48 hours.

The Paragachi and Cocacho populations were inoculated with the same Ecuadorian race 0 isolate that was used for field inoculations in Ecuador. The ARME-2 and Yunguilla populations were inoculated with a Mexican race 2 isolate. The Ecuadorian parent of the population and SEL 1308 were included in each inoculation as susceptible and resistant controls respectively. Disease reactions were evaluated 6 to 10 days after inoculation. Plants were rated as susceptible (a disease reaction like that of the Ecuadorian parent, resulting in death of the plant), intermediately resistant (more resistant than the Ecuadorian parent but exhibiting susceptible symptoms -- these plants were often able to survive inoculation), resistant (disease reaction like that of SEL 1308, no symptoms of anthracnose).

Results and Discussion

Flowering in Field Grown Populations

Days to flower (DTF) of the twelve populations and six parents are given in Table 8. There was no difference in DTF between the backcross populations and their recurrent parent. This is one indication that plants in the backcross populations resembled the recurrent parent, and may be similarly adapted to the target Ecuadorian bean production areas. DTF was longer than is typical for these varieties. The cool temperatures due to the high elevation of the field site were probably the cause of the delay in flowering as temperatures of 12 to 14 C have been shown to dramatically increase DTF in beans (Wallace et al., 1991).

Table 8. Days to flowering for the backcross populations an recurrent parents

Population	Days to Flower	Population	Days to Flower
Cocacho	62	ARME-2	73
Cocacho BC_2F_2	62	ARME-2 BC_2F_2	73
Cocacho BC_3F_1	62	ARME-2 BC_3F_1	73
ACE-1	62	Yunguilla	69
ACE-1 BC_2F_2	62	Yunguilla BC_2F_2	69
ACE-1 BC_3F_1	62	Yunguilla BC_3F_1	69
Paragachi	73	Genuine	73
Paragachi BC_2F_2	73	Genuine BC_2F_2	73
Paragachi BC_3F_1	73	Genuine BC_3F_1	73

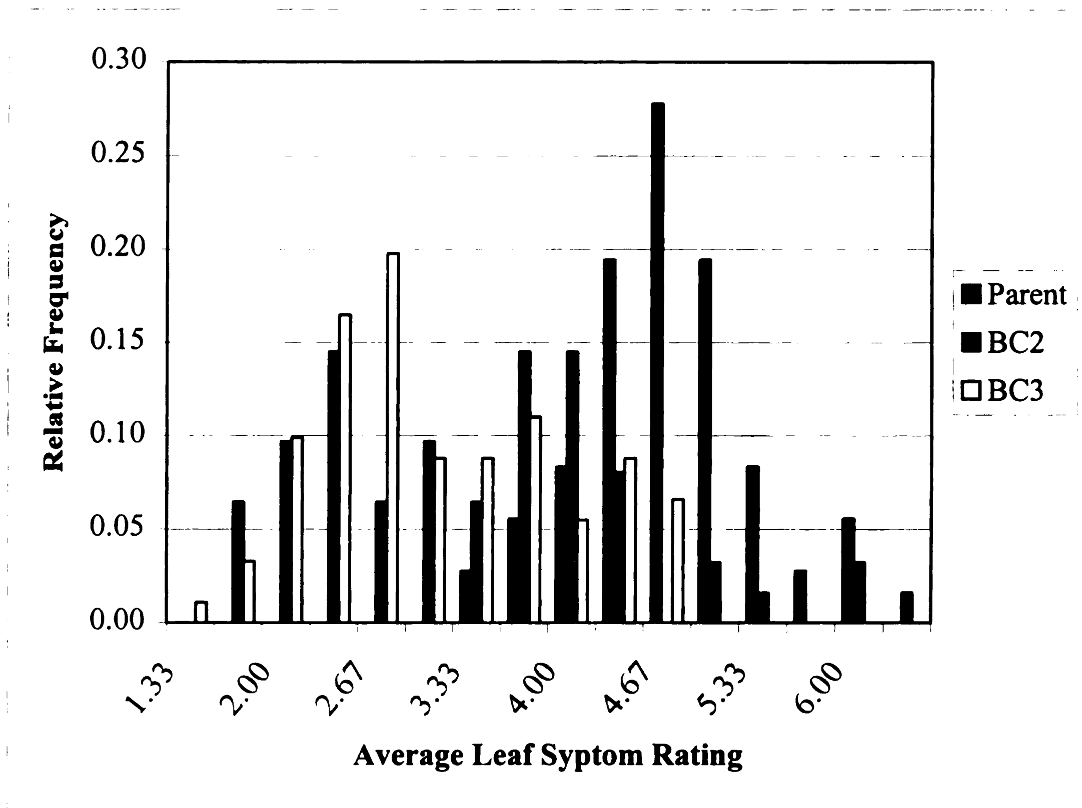
Reaction to Field and Greenhouse Inoculations

Cocacho Field Inoculation With Race 0

Figure 3 shows the average leaf symptom ratings for the two Cocacho backcross populations and the parent in response to field inoculation with race 0. The average leaf symptom rating for the parent ranged from 3.3 to 6.0. The BC_2 and BC_3 populations exhibited a larger range of leaf symptom ratings than the parent: 1.67 to 6.67 and 1.33 to

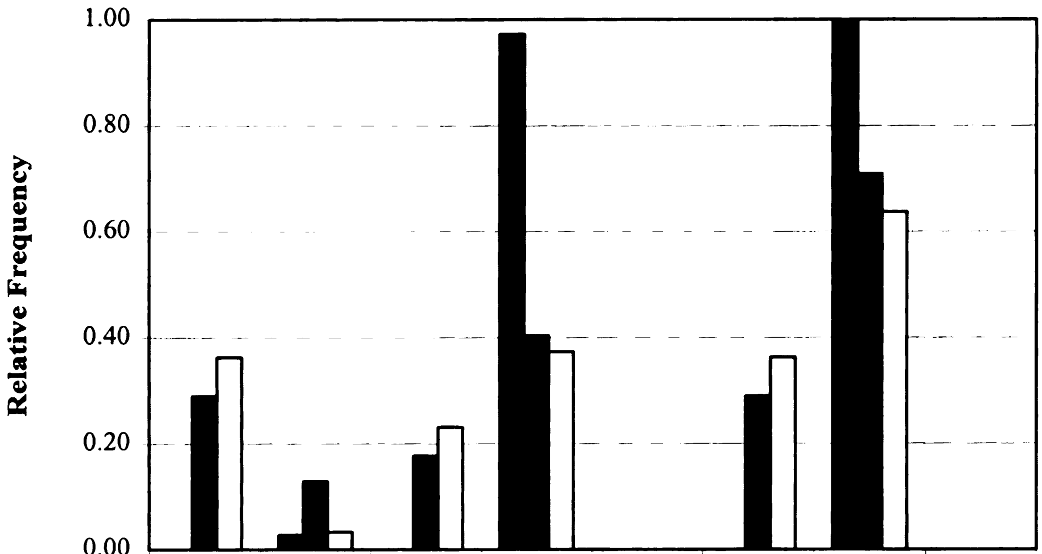
4.67 respectively. 53% of the BC2 plants and 40% of the BC3 plants had an average leaf symptom rating greater than three and were categorized as having susceptible leaves. All of the control plants (parent) had an average leaf symptom rating that was greater than 3.

Figure 3. Average leaf symptom ratings for Cocacho backcross populations and parent



Three percent of the parent plants exhibited no pod symptoms and three percent had small lesions. The remaining 94% of the plants had deep spore producing lesions on their pods. All of the parent plants exhibited either susceptible pod or leaf symptoms and were, overall considered susceptible. 58% of the BC_2 plants and 60% of the BC_3 plants had susceptible pod symptoms. Figure 4 shows the assignment of the Cocacho populations to one of two categories, resistant or susceptible, based on both pod and leaf symptoms. 80% of the BC_2 plants and 64% of the BC_3 plants had either susceptible leaves or susceptible pods and were overall considered susceptible.

Figure 4. Determination of resistance or susceptibility in Cocacho backcross and parent plants inoculated with race 0 isolate based on pod and leaf symptoms



	R pod, R leaf 0	R pod, S leaf 1	S pod, R leaf 2	S pod, S leaf 3		Resistant	Suscep-tible
■ Parent	0.00	0.03	0.00	0.97		0.00	1.00
■ BC2	0.29	0.13	0.18	0.40		0.29	0.71
□ BC3	0.36	0.03	0.23	0.37		0.36	0.64

Resistance Category

If the *Co-4*^{2} resistance gene were segregating as expected in these populations, 25% of the BC_2 plants and 50% of the BC_3 plants would be susceptible. The populations did not exhibit these expected ratios.

Cocacho Marker Analysis and Greenhouse Inoculation with Race 0

The results of marker analysis and greenhouse inoculation for the Cocacho populations are given in Table 9. In greenhouse inoculations the Cocacho parent was confirmed to be susceptible to the race 0 isolate used in the field study. Three plants from the BC_1F_2 population exhibited complete resistance to the race 0 isolate of *C. lindemuthianum*. Some additional plants in this population had intermediate resistance to the pathogen. In the BC_2F_2 and BC_3F_1 populations, none of the plants were completely resistant, although some had intermediate resistance.

Table 9. Cocacho BC_1F_2, BC_2F_2 and BC_3F_2 Marker Analysis and Reaction to Ecuadorian Race 0 Isolate

Disease Reaction		***AS13 and SH18 Markers***	
		Present	Absent
Intermediate Resistance	BC_1F_2	8	4
	BC_2F_2	2	1
	BC_3F_1	1	0
	Total	11	5
Susceptible	BC_1F_2	8	2
	BC_2F_2	9	4
	BC_3F_1	5	7
	Total	22	13

As shown in Table 10, the AS13 and SH18 markers do not appear to be associated with any of the resistance, complete or partial, observed in the Cocacho populations. Results from the χ^2 Test for Independence indicate that in each of the populations, the marker results and disease reactions are independent of one another. Moreover, odds analyses indicate that a positive marker result does not increase the chances of a plant being anthracnose resistant, as would be expected were the marker linked to the gene(s) conferring resistance.

Table 10. Cocacho BC_1F_2, BC_2F_2 and BC_3F_2 Test for Dependence of Marker and Disease Reaction

Population	X^2-value	Odds of Resistance	Odds of Resistance Given Positive Marker Result
BC_1F_2	0.49	1.20	1.20†
BC_2F_2	0.01	0.23	0.05†
BC_3F_1	1.26	0.08	0.02†
Total	0.17	0.46	0.23†

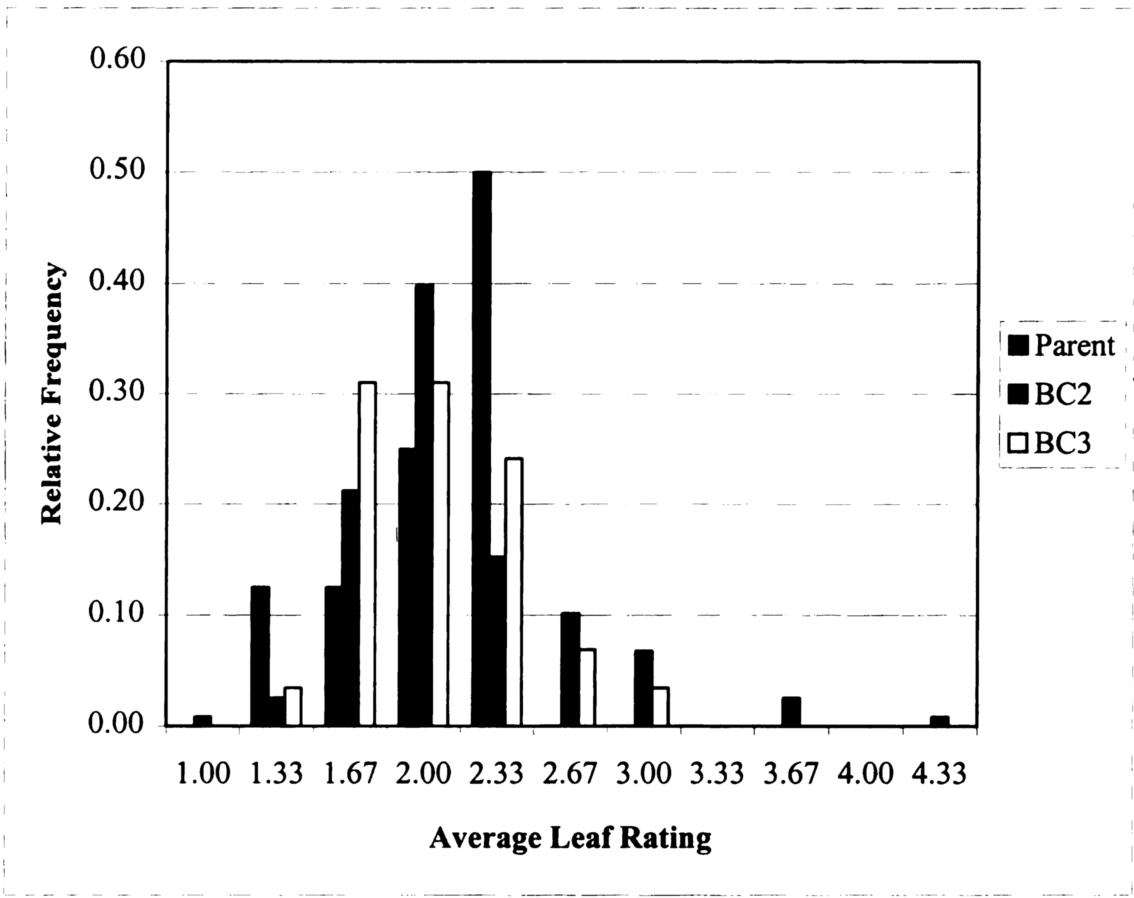
† Odds of Resistance Given a Positive Marker Result ≤ Overall Odds of Resistance indicates that a positive marker result decreases or does not affect the chances of a plant being resistant

One possible explanation for this data is that a recombination between *Co-4*^{2} and the SAS13 occurred early in the breeding program. However this is unlikely given that there is a tight linkage between SAS13 and *Co-4*^{2}, and several plants carrying the marker were used as parents in each generation. Another possible explanation is that *Co-4*^{2} does not confer resistance to anthracnose in the genetic background of Cocacho; the variety may lack certain genes that are required for *Co-4*^{2} to be expressed or to function. The anthracnose resistance genes in bean have generally been regarded as functioning independently from other genes. However in the case of Bean Common Mosaic Virus (BCMV) resistance, *bc-2*^{2}, a recessive resistance gene, requires another gene, *bc-u*, in order to function (Kelly, 1997). Additionally, genetic background has been show to affect the expression of resistance genes in wheat (van der Westhuizen et al., 1998) and oat (Wilson and McMullen, 1997). Since the *Co-4*^{2} gene is from an isolated bean race in the Middle American gene pool, it is likely that the genetic background of G2333, in which *Co-4*^{2} functions, is quite different than that of Cocacho, an Andean landrace.

ACE-1 Field Inoculation With Race 0

Figure 5 shows the average leaf symptom ratings for the two ACE-1 backcross populations and the parent in response to field inoculation with race 0. The average leaf symptom rating for the parent ranged from 1.3 to 2.3. The BC_2 and BC_3 populations exhibited a larger range of leaf symptoms ratings than the parent: 1.0 to 4.3 and 1.3 to 3.0 respectively. Three percent of the BC_2 plants had an average leaf symptom rating greater than three and were categorized as having susceptible leaves. All of the control plants (parent), and the BC_3F_1 plants had an average leaf symptom rating that was less than 3 and considered to have resistant leaves.

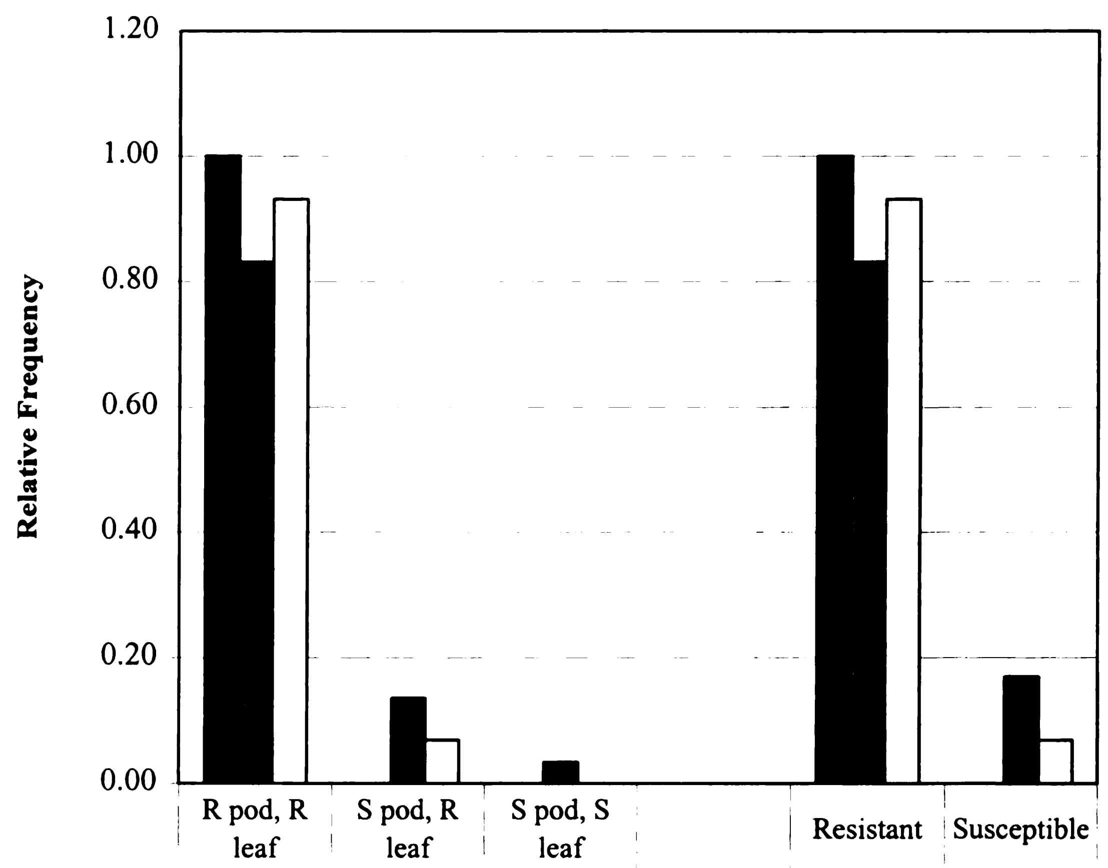
Figure 5. Average leaf symptom ratings for ACE-1 backcross populations and parent



None of the parent plants exhibited pod symptoms. The parent plants had no pod or leaf symptoms and were, considered resistant, although the sample size was very small due to poor germination. 17% of the BC2 plants and 7% of the BC3 plants had susceptible pod symptoms. These plants were considered susceptible. (3% of the BC_2

plants had susceptible leaves and susceptible pods). Figure 6 shows the determination of resistance or susceptibility in the ACE-1 backcross and parent plants based on pod and leaf symptoms.

Figure 6. Determination of resistance or susceptibility in ACE-1 backross and parent plants inoculated with race 0 based on pod and leaf symptoms



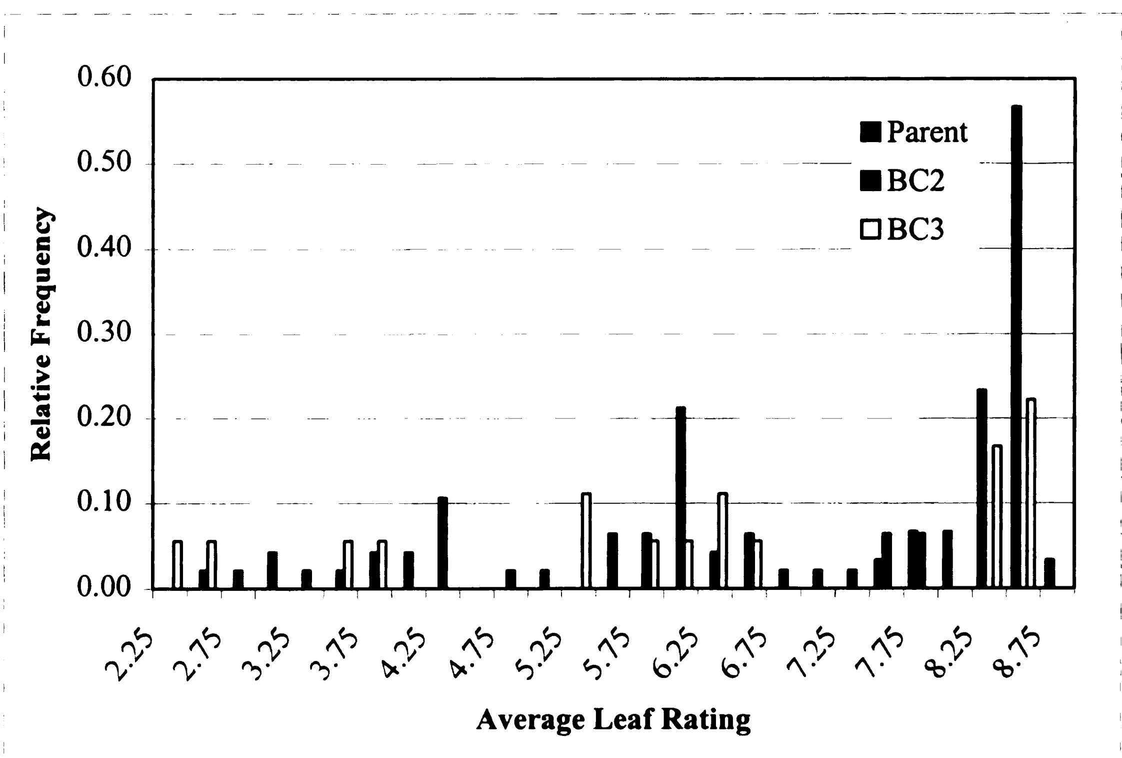
	R pod, R leaf	S pod, R leaf	S pod, S leaf		Resistant	Susceptible
■ Parent	1.00	0.00	0.00		1.00	0.00
■ BC2	0.83	0.14	0.03		0.83	0.17
□ BC3	0.93	0.07	0.00		0.93	0.07

Later greenhouse inoculations confirmed that ACE-1 is resistant to the race 0 isolate that was used in the field inoculations (see appendix A). The few susceptible individuals in the BC_2F_2 generation may be explained by loss of the parents' resistance genes through independent assortment. The presence of susceptible plants in the BC_3F_1 population suggests that the resistance in the ACE-1 parent is recessive, or that the ACE-1 parental line is segregating for resistance to this race.

Paragachi Field Inoculation With Race 0

Figure 7 shows the average leaf symptom ratings for the Paragachi backcross populations and the parent in response to field inoculation with race 0. The average leaf symptom rating for the parent ranged from 7.5 to 8.8. The BC2 and BC3 populations exhibited a larger range of leaf symptom ratings than the parent: 2.5 to 7.8 and 2.3 to 8.5 respectively.

Figure 7. Average leaf symptom ratings for Paragachi backcross populations and parent



Plants were categorized into three different leaf symptom categories. Those plants with average leaf ratings between 0 and 3.0 were considered as having resistant leaves. Plants with ratings between 3.1 and 5.5 were considered moderately susceptible and those with average ratings greater than 5.51 were considered very susceptible. All of the parental control plants had an average leaf symptom rating greater than 5.51 and were considered very susceptible. In the BC_2 population 8% of the plants had resistant leaves, 33% had moderately susceptible leaves and 58% had very susceptible leaves. In the BC_3 population 11% of the plants had resistant leaves, 22% of the plants had moderately susceptible leaves and 67% of plants had very susceptible leaves.

Forty-three percent of the parental control plants had spore-producing lesions on their pods. The remaining 57% of the control plants did not produce pods because of stress or death resulting from severe leaf symptoms. In the BC_2 populations 8% of the plants had no pod symptoms, 31% had small lesions, 50% had spore-producing lesions and 10% produced no pods due to stress or death resulting from inoculation. In the BC_3 populations 11% of the plants had small pod lesions and 61% of the plants had spore-producing lesions. The remaining 28% of the plants did not produce any pods. Table 11 shows the assignment of Paragachi backcross and parent plants to susceptibility categories based on pod and leaf symptoms. Plants with resistant leaves and those with a moderately susceptible leaf symptom rating and pods with small lesions or no lesions were considered intermediately resistant. 27% of the BC_2 plants and 22% of the BC_3 plants fell into this category.

Table 11. Determination of intermediate resistance or susceptibility in Paragachi backcross and parent populations based on pod and leaf symptoms (frequency, percent of population)

Pod Rating[a]	**Population**	**Leaf Rating**[b] **0**	**1**	**2**
0	Parent	0, 0%	0, 0%	0, 0%
	BC_2F_2	1, 21%	5, 63%	0, 0%
	BC_3F_1	0, 0%	0, 0%	0, 0%
1	Parent	0, 0%	0, 0%	0, 0%
	BC_2F_2	3, 63%	[illegible]	6, 13%
	BC_3F_1	0, 0%	[illegible]	0, 0%
2	Parent	0, 0%	0, 0%	13, 43%
	BC_2F_2	0, 0%	7, 0.15	17, 35%
	BC_3F_1	[illegible]	2, 0.11	7, 39%
3	Parent	0, 0%	0, 0%	17, 57%
	BC_2F_2	0, 0%	0, 0%	5, 10%
	BC_3F_1	0, 0%	0, 0%	5, 28%

[a] 0, no pod symptoms; 1, small lesions that did not produce spores; 2, large spore producing lesions; 3, plant did not produce pods due to stress or death as a result of disease
[b] 0, resistant, average leaf symptom rating 0 to 3.0; 1, moderately susceptible, average leaf symptom rating 3.1 to 5.5; 3, very susceptible, average leaf symptom rating 5.51 to 9.0
intermediately resistant plants
susceptible plants

Paragachi Marker Analysis and Greenhouse Inoculation with Race 0

The results of marker analysis and greenhouse inoculation for the Paragachi populations are given in Table 12. In each of the three populations some plants exhibited partial resistance to the *C. lindemuthianum* isolate, but none of the plants were completely resistant to the disease. This confirms the expression of partial or intermediate resistance observed in the field inoculations of the Paragachi backcross plants.

Table 12. Paragachi BC_1F_2, BC_2F_2 and BC_3F_2 Marker Analysis and Reaction to Ecuadorian Race 0 Isolate

Disease Reaction		*SAS13, SH18, and SBB14 Markers*	
		Present	Absent
Intermediate Resistance	BC_1F_2	14	0
	BC_2F_2	12	0
	BC_3F_1	4	1
	Total	30	1
Susceptible	BC_1F_2	5	8
	BC_2F_2	5	7
	BC_3F_1	2	2
	Total	12	17

As shown in Table 13, the SAS13, SBB14 and SH18 markers are associated with the partial resistance observed in Paragachi populations. Results from the χ^2 Test for Independence indicate that in the BC_1F_2 and BC_2F_2 populations, the marker results and disease reactions are dependent. Failure of the test to detect dependence in the BC_3F_1 population is probably due to small sample size (n=9). The odds analyses indicate that in all of the Paragachi populations, a positive marker result increases the chances of a plant being anthracnose resistant, as is expected for a marker linked a segregating resistance gene.

Table 13. Paragachi BC_1F_2, BC_2F_2 and BC_3F_2 Test for Dependence of Marker and Disease Reaction

Population	**X^2-value**	**Odds of Resistance**	**Odds of Resistance Given Positive Marker Result**
BC_1F_2	12.24****	1.08	3.02†
BC_2F_2	9.88***	1.00	2.40†
BC_3F_1	0.90	1.25	2.50†
Total	21.89****	1.07	2.67†

**** marker and disease reaction are dependent at $\alpha<.001$, *** marker and disease reaction are dependent at $\alpha=.005$,**marker and disease reaction are dependent at $\alpha=.01$ * marker and disease reaction are dependent at $\alpha=.025$

† Odds of Resistance Given a Positive Marker Result > Overall Odds of Resistance indicates that a positive marker result increases the chances of a plant being resistant

As in the Cocacho populations, only partial resistance to anthracnose was observed in the BC_2 and BC_3 populations, but in the case of the Paragachi populations, there is evidence that *Co-4*^{2} is the source of some of the partial resistance. In Paragachi's genetic background *Co-4*^{2} is not fully functional. Instead *Co-4*^{2} may act as a defeated resistance gene which confers partial resistance similar to the anthracnose resistance QTL reported by Geffroy et al. (2000). The plants that carried markers for *Co-4*^{2}, but did not express any partial resistance may represent individuals that completely lack the other genes needed for function of *Co-4*^{2}. This suggests that in a pure Paragachi genetic background *Co-4*^{2} may not function at all. In field inoculations, the most resistant plants were from the BC_2 population, perhaps because they retained a greater proportion of the donor parent's genes.

ARME-2 Field Inoculation With Race 0

The ARME-2 parent, the BC_2 and BC_3 populations were resistant to race 0 and neither the pods nor the leaves had susceptible symptoms. Since ARME-2 apparently carries resistance to race 0 it was not possible to distinguish plants in the field that were carrying *Co-4*^{2}.

ARME-2 Marker Analysis and Greenhouse Inoculation with Race 2

The results of marker analysis and greenhouse inoculation with race 2 for the ARME-2 populations are given in Table 14. ARME-2 was found to be susceptible to a Mexican race 2 isolate of *C. lindemuthianum*. Plants from the BC_2F_2 and BC_3F_1 populations exhibited a clear resistant or susceptible reaction to the isolate, with some plants from each population showing a completely resistant reaction identical to that of SEL 1308 and the remainder exhibiting susceptibility identical to the Ecuadorian parent.

Table 14. ARME-2 BC_2F_2 and BC_3F_2 Marker Analysis and Reaction to Race 2

Disease Reaction		***AS13 and SH18 Markers***	
		Present	Absent
	BC_2F_2	11	0
Resistant	BC_3F_1	6	0
	Total	17	0
	BC_2F_2	0	5
Susceptible	BC_3F_1	0	6
	Total	0	11

As shown in Table 15, positive results for the AS13 and SH18 markers are associated with resistance in the ARME-2 populations. Results from the χ^2 Test for Independence indicate that in each of the populations, the marker results and disease reactions are dependent. All resistant plants carried the two markers for *Co-4*^{2} and all of the susceptible plants tested negative for the markers, indicating that, as expected, *Co-4*^{2} confers complete resistance to race 2 in the ARME-2 genetic background. Since ARME-2 is an advanced line from the INIAP bean breeding program it may carry additional Mesoamerican genes which are necessary for *Co-4*^{2} to function.

Table 15. ARME-2 BC_2F_2 and BC_3F_2 Test for Dependence of Marker and Disease Reaction

Population	X^2-value
BC_2F_2	16.00****
BC_3F_1	12.00****
Total	28.00****

**** marker and disease reaction are dependent at $\alpha<.001$, *** marker and disease reaction are dependent at $\alpha=.005$,**marker and disease reaction are dependent at $\alpha=.01$ * marker and disease reaction are dependent at $\alpha=.025$

Yunguilla Field Inoculation With Race 0

The Yunguilla parent, the BC_2 and BC_3 populations were resistant to race 0 and neither the pods nor the leaves had susceptible symptoms. Since Yunguilla apparently carries resistance to race 0 it was not possible to distinguish plants that were carrying *Co-*4^2.

Yunguilla Marker Analysis and Greenhouse Inoculation with Race 2

The results of marker analysis and greenhouse inoculation with race 2 for the Yunguilla populations are given in Table 16. Yunguilla was found to be susceptible to a Mexican race 2 isolate of *C. lindemuthianum*. Plants from the BC_2F_2 and BC_3F_1 populations exhibited a clear resistant or susceptible reaction to the isolate, with some plants from each population showing a completely resistant reaction identical to that of SEL 1308 and the remainder exhibiting susceptibility identical to the Ecuadorian parent.

Table 16. Yunguilla BC_2F_2 and BC_3F_2 Marker Analysis and Reaction to Race 2

Disease Reaction		*AS13 and SH18 Markers*	
		Present	Absent
Resistant	BC_2F_2	12	2
	BC_3F_1	8	1
	Total	20	3
Susceptible	BC_2F_2	0	2
	BC_3F_1	0	2
	Total	0	4

As shown in Table 17, positive results for the AS13 and SH18 markers are strongly associated with resistance in the Yunguilla populations. Results from the χ^2 Test for Independence indicate that in each of the populations, the marker results and disease reactions are dependent. All plants carrying the two markers for *Co-4²* were resistant in the greenhouse inoculations, indicating that *Co-4²* functions as expected in the Yunguilla genetic background. Some of the plants from which the marker was absent were still resistant. Other anthracnose resistance genes from the Red Hawk or Je.Ma. parents, which may be segregating in the populations, could confer this additional resistance that is unlinked to *Co-4²*.

Table 17. Yunguilla BC_2F_2 and BC_3F_2 Test for Dependence of Marker and Disease Reaction

Population	X^2-value
BC_2F_2	6.85**
BC_3F_1	6.52*
Total	13.42****

**** marker and disease reaction are dependent at α<.001, *** marker and disease reaction are dependent at α=.005,**marker and disease reaction are dependent at α=.01 * marker and disease reaction are dependent at α=.025

Genuine Field Inoculation with Race 0

The Genuine parent, the BC_2 and BC_3 populations were resistant to race 0 and neither the pods nor the leaves had susceptible symptoms. Since Genuine apparently carries resistance to race 0 it was not possible to distinguish plants that were carrying *Co-4²*. The plants did suffer from bronzing of the pods and leaves, possibly due to sunscald. Genuine, which was bred for a sea level production area (Florida), may be poorly adapted to the high light intensity conditions at 2700 m.a.s.l. Consequently, Genuine may not be as useful in the Ecuadorian breeding program as was hoped.

Evaluation of Seed Appearance

Cocacho Seed Appearance

The Cocacho backcross populations produced seed in eight different seed coat pattern and color classes, which are shown in Figure 8: (1) solid yellow with darkened tan veins and an orange/brown corona, this was considered the parental class (2) yellow and white mottle with darkened tan veins and an orange/brown corona (3) solid brown with dark brown veins (4) brown and cream mottle with an orange/brown corona (5) red and dark red mottle (6) solid red (7) solid cream with tan veins and an orange/brown corona (8) gray purple with dark veins lightening to cream at edges with an orange/brown corona.

The frequency of each seed type in the two backcross populations is shown in Table 18. Certain seed types, 5, 6, 7 and 8, were present only in the BC_2F_2 population, possibly because they result from homozygous alleles from the donor parent. The yellow/white mottle pattern may segregate 1:2:1 solid yellow : yellow/white mottle : white when selfed. The yellow/white mottle seed type was the most frequent class in the BC_2F_2 population, 26%, while the parental seed type was rather infrequent, 8%. The parental type was much more frequent in the BC_3F_1 population at 41%. The second most frequent class in the BC_3F_1 population, yellow/white mottle at 39%, may segregate and produce parental type plants in future generations after further selfing.

Table 18. Seed appearance frequencies in the Cocacho populations

Seed Appearance		Frequency		% of Population	
		BC_2F_2	BC_3F_1	BC_2F_2	BC_3F_1
2	solid yellow; tan veins; orange/brown corona	5	37	8.06	40.66
3	yellow/white mottle; tan veins;orange/brown corona	16	35	25.81	38.46
4	solid brown; dark brown veins	8	10	12.90	10.99
5	brown/cream mottle; orange/brown corona	13	6	20.97	6.59
6	red/dark red mottle	3	0	4.84	0.00
7	solid red	2	0	3.23	0.00
8	solid cream; tan veins; orange/brown corona	11	0	17.74	0.00
9	gray purple; darkened veins; cream edges; orange/brown corona.	1	0	1.61	0.00
	Missing (plant did not produce seed)	3	3	4.84	3.30

ACE-1 Seed Appearance

The ACE-1 backcross populations produced seed in seven different seed coat pattern and color classes, which are shown in Figure 9: (2) solid yellow with tan veins and a tan/yellow corona, which was considered the parental class (3) solid cream with tan veins and a tan/yellow corona (4) yellow and white mottle with tan veins and a tan/yellow corona (5) brown and cream mottle with a tan/yellow corona (6) tan/yellow with yellow green edges and tan/yellow corona (7) solid brown with dark brown veins (8) sulfur yellow with tan veins and tan/yellow corona.

The frequency of each seed type in the two backcross populations is shown in Table 19. Certain seed types, 2 and 6, were present only in the BC_2F_2 population, possibly because they result from homozygous alleles from the donor parent. The yellow/white mottle pattern may segregate 1:2:1 solid yellow : yellow/white mottle : white. The solid yellow, yellow/white mottle and solid cream seed types were the most frequent class in the BC_2F_2 population, at 25%, 28% and 27% respectively. Although fairly frequent in the BC_2F_2 population the parental type was more frequent in the BC_3F_1 population at 48%. The second most frequent class in the BC_3F_1 population, yellow/white mottle at 21%, may segregate and should produce parental type plants in future generations as it is selfed. The cream-colored seed class may also be useful to breeders at INIAP, as this seed resembles Bola Blanca, another Ecuadorian commercial class.

Table 19. Seed appearance frequencies in the ACE-1 populations

	Seed Appearance	Frequency		% of Population	
		BC_2F_2	**BC_3F_1**	**BC_2F_2**	**BC_3F_1**
2	solid yellow; tan veins; tan/yellow corona	28	14	23.73	50.00
3	solid cream; tan veins; tan/yellow corona	32	0	27.12	0.00
4	yellow/white mottle; tan veins; tan/yellow corona	33	6	27.97	21.43
5	brown/cream mottle; tan/yellow corona	13	2	11.02	7.14
6	tan/yellow; yellow green edges; tan/yellow corona	1	0	0.85	0.00
7	solid brown; dark brown veins	2	0	1.69	0.00
8	sulfur yellow; tan veins; tan/yellow corona	4	5	3.39	17.86
	Missing (plant did not produce seed)	5	1	4.24	3.57

Paragachi Seed Appearance

The Paragachi backcross populations produced seed in three different seed coat pattern and color classes, which are shown in Figure 10: (1) dark purplish red, red and white mottle (2) dark purplish red and white mottle (3) white with a white corona.

Table 20 shows the frequency of each seed class in the two backcross populations. The white seed type was observed only in the BC_2 population. The red-purple and white mottle was the most frequent class in the BC_2 population at 42%. Only 10% of the plants in this population produced seed in the parental class. As expected, in the BC_3 generation the parental seed type was much more frequent at 44%.

Table 20. Seed appearance frequencies in the Paragachi populations

Seed Appearance	Frequency		% of Population	
	BC_2F_2	**BC_3F_1**	**BC_2F_2**	**BC_3F_1**
1 purplish red/red/white mottle	5	8	10.42	44.44
2 purplish red/white mottle	20	3	41.67	16.67
3 white	14	0	29.17	0.00
Missing (plant did not produce seed)	9	7	18.75	38.89

ARME-2 Seed Appearance

The ARME-2 backcross populations produced seed in three different seed coat pattern and color classes, which are shown in Figure 11: (2) dark purplish red, light red and white mottle (3) dark purplish red and white mottle (4) white with a white corona.

Table 21 shows the frequency of each seed class in the two backcross populations. The white seed type was observed only in the BC_2 population. The red-purple and white mottle was the most frequent class in the BC_2 population at 52%. Twenty-two percent of the plants in this population produced seed in the parental class. In the BC_3 generation the parental seed type was much more frequent at 56%. The two colored mottle seeds (class two) may segregate 1:2:1 for three-colored mottle : two-color mottle : white, upon further selfing.

Table 21. Seed appearance frequencies in the ARME-2 populations

Seed Appearance	Frequency		% of Population	
	BC_2F_2	BC_3F_1	BC_2F_2	BC_3F_1
2 purplish red/red/white mottle	16	9	21.92	56.25
3 purplish red/white mottle	38	7	52.05	43.75
4 white	17	0	23.29	0.00
Missing (plant did not produce seed)	2	0	2.74	0.00

Yunguilla Seed Appearance

The Yunguilla backcross populations produced seed in five different seed coat pattern and color classes, which are shown in Figure 12: (2) red, pink and white mottle (3) pink and white mottle (4) white with a yellow/brown corona (5) dark purple and white mottle (6) dark purple, purple and white mottle.

Table 22 shows the frequency of each class in the two backcross populations. The white and two color purple mottle seed types were observed only in the BC_2 population. The white seed type was the most frequent class in the BC_2 population at 29%. Only 16% of the plants in this population produced seed equivalent to the parental class. In the BC_3 generation the parental seed type was much more frequent at 43%. The two colored mottle seeds (classes 2 and 5) may segregate 1:2:1 for three-colored mottle to two-color mottle to white upon further selfing. The three-color dark purple mottle seed may also be useful in INIAP's bean breeding program, as this seed resembles another Ecuadorian commercial class, Calima Negro.

Table 22. Seed appearance frequencies in the Yunguilla populations

Seed Appearance	**Frequency**		**% of Population**	
	BC_2F_2	**BC_3F_1**	**BC_2F_2**	**BC_3F_1**
2 red/pink/white mottle	5	9	16.13	42.86
3 pink/white mottle	6	10	19.35	47.62
4 white; yellow/brown corona	9	0	29.03	0.00
5 dark purple/white mottle	6	0	19.35	0.00
6 dark purple/purple/white mottle	2	2	6.45	9.52
Missing (plant did not produce seed)	3	0	9.68	0.00

Genuine Seed Appearance

Both of the Genuine backcross populations produced only white seed that resembled seed of the parent as shown in Figure 13.

Figures 8 through 13 in this thesis are presented in color.

Figure 8. Seed of Cocacho parent (1) and BC_2 and BC_3 plants

Figure 9. Seed of ACE-1 parent (1) and BC_2 and BC_3 plants

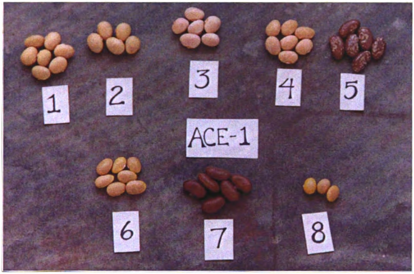
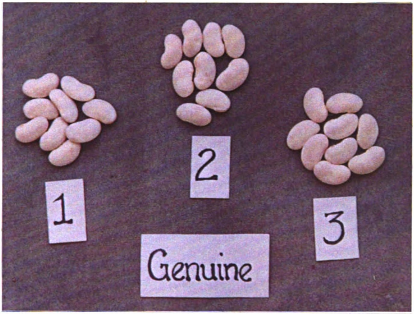


Figure 10. Seed of Paragachi BC_2 and BC_3 plants

Figure 11. Seed of ARME-2 parent (1) and BC_2 and BC_3 plants

Figure 12. Seed of Yunguilla parent (1) and BC_2 and BC_3 plants

Figure 13. Seed of Genuine parent (1) and BC_2 (2) and BC_3 plants (3)



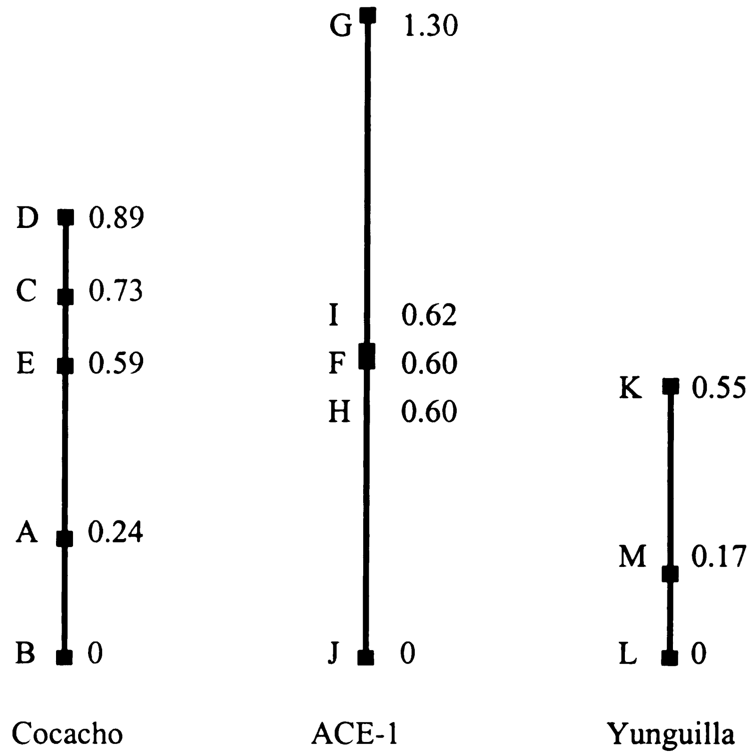
Farmer Evaluation of Seed from Cocacho, ACE-1 and Yunguilla Backcross Populations

Figure 14 shows the farmers' ranking of bean samples as determined using the Bradley-Terry model for pairwise comparisons modified to account for ties (Dittrich et al., 1998; Coe, 2002). From among the Cocacho derived samples, farmers preferred the brown seed type. Some farmers noted that brown beans are traditionally grown in the area, which may explain farmers' interest in this seed type. The sample resembling the Cocacho parent and the parent itself were the second and third ranked samples respectively. The majority of the farmers, 81%, tied these two samples in their ranking and some indicated that they did

see a difference between the two samples. This would suggest that the sample classified as the parental type sufficiently resembles the Ecuadorian recurrent parent for it to be considered the same commercial class.

Among the samples derived from the ACE-1 recurrent parent the parent itself received the highest ranking from farmers. The white, tan and yellow samples (I, F and H) are essentially tied for the second place ranking. Fifty-seven percent of the farmers ranked the seed of ACE-1 higher than the yellow-seeded sample and 36% tied the two samples. In this case the farmers were able to differentiate between the seed of the ACE-1 parent and the seed classified as the parental type and they preferred the seed type of the parent. This would suggest that the parental type sample used in the survey lacked some seed quality traits that are important to the farmers. The seed size of the samples was slightly different (Figure 2), and the larger size of the ACE-1 seed may be one reason that farmers preferred it. Seed which more closely resembled the ACE-1 parent (in the author's opinion) was harvested after the survey was completed (Figure9).

Figure 14. Farmers' ranking of seed samples from the Cocacho, ACE-1, and Yunguilla backcross populations and their respective Ecuadorian recurrent parent using Bradley-Terry modeling



Legend

Cocacho Samples		**ACE-1 Samples**		**Yunguilla Samples**	
D	brown	G	ACE-1, parent	K	red/pink/white mottle*
C	yellow*	I	white	M	red/white mottle
E	Cocacho, parent	F	tan, yellow edges	L	Yunguilla, parent
A	white	H	yellow*		
B	white/yellow mottle	J	white/yellow mottle		

*sample which most resembled the recurrent parent

Among the samples derived from Yunguilla, the farmers preferred the seed which was classified as the parental type and ranked the seed of the parent last (Figure 14). Fifty-three percent of the farmers preferred the parental type sample (K) and 33% tied the two samples. Some farmers commented that the three Yunguilla class seed samples were very similar, but others differentiated between them. Based on their comments, farmers differentiated between the seed samples on the basis of color. Bright colors and less white were two particular traits that farmers reported using to differentiate the samples. These results suggest that some seed from the Yunguilla derived backcross plants is acceptable to farmers and has reached commercial quality for the Calima Rojo class.

Number of Backcrosses

With the exception of the Genuine populations, the percent of plants producing seed resembling the recurrent parent ranged from 8 to 24 % in the BC_2 populations and from 41% to 56% in the BC_3 populations. In each case there was at least a two-fold increase in the percent of plants in the parental class. Two backcrosses (and one generation of selfing) were sufficient to obtain some plants with seed like the recurrent parent. However, three backcrosses resulted in a substantial increase in the frequency of the parental type seed. Three backcrosses more effectively recovered the desired seed quality of the Ecuadorian varieties than did two backcrosses. Additionally the adaptation of the backcross populations to the target environment must be considered, as BC_3 populations may be more adapted than BC_2 populations. Adaptation was not tested in this study since the field experiment location was at a higher elevation than bush beans are typically grown.

Conclusions

The objective of this study was to introduce a Mesoamerican anthracnose resistance gene, *Co-4*^{2}, into Ecuadorian bean market classes using molecular markers to select for the gene and multiple backcrosses to the Ecuadorian recurrent parent in order to obtain lines with commercial seed characteristics and local adaptation.

Six different Ecuadorian varieties were used as recurrent parents. In backcross populations generated from the varieties Yunguilla (a red Calima type) and ARME-2 (a medium red mottle type) plants carrying the markers for *Co-4*^{2} were resistant to anthracnose in greenhouse inoculations. However, the marker assisted backcrossing did not work as expected in the Cocacho and Paragachi derived populations. In the Cocacho backcross populations, plants carrying the markers for *Co-4*^{2} were no more likely to be anthracnose resistant, while in the Paragachi populations the markers for *Co-4*^{2} were associated with partial resistance but none of the plants in the populations were completely resistant to anthracnose.

One possible explanation for this observation is that in these populations a recombination between the *Co-4*^{2} gene and the markers occurred early in population development. However this is unlikely since the SAS13 is tightly linked to *Co-4*^{2}. Additionally, multiple plants carrying the markers for *Co-4*^{2} were used as parents in each generation (Table 4), which means that recombination would have had to occur multiple times.

Another explanation is that Cocacho and Paragachi lack certain genes necessary for the function of *Co-4*^{2}, possibly because of their genetic distance from G 2333, the source of *Co-4*^{2}. In support of this theory, evidence exists for the presence of independent complimentary genes for anthracnose resistance in the Andean cultivars Kaboon (Muhalet et al., 1981; Melotto and Kelly, 2000), and Algarrobo (Cardenas et al., 1964). Inoculation of resistant by susceptible crosses using these cultivars as sources for anthracnose resistance yielded resistant : susceptible segregation ratios of 9:7 or 57:7, which suggest that two independent genes are required for resistance. It is proposed that *Co-4*^{2} requires the presence of another complimentary gene(s) which is present in Middle American germplasm but absent in certain Andean genotypes. Using the molecular markers for *Co-4*^{2} resulted in selection for only this gene, so in resulting populations the complimentary gene(s) was not retained and *Co-4*^{2} was not functional (Cocacho) or only partially functional (Paragachi). ARME-2 and Yunguilla, which were recently selected from CIAT breeding lines are more likely to possess some Mesoamerican genes.

If *Co-4*^{2} requires other genes, which are absent from unimproved Andean bean germplasm, this particular gene may not be as useful a source of anthracnose resistance in certain Andean genotypes. The situation, at least, limits the usefulness of marker-assisted selection for introgression of this gene into genotypes which lack the gene(s) that compliments *Co-4*^{2}. In the case of the Paragachi and Cocacho populations described here, direct selection through disease inoculation would likely have produced useful anthracnose resistant backcross plants; whereas marker assisted selection did not. This highlights the necessity of incorporating direct selection, through disease screening, into breeding projects utilizing molecular markers to select for disease resistance genes. The apparent failure of *Co-4*^{2} in certain genetic backgrounds also highlights the importance of identifying new and diverse sources of anthracnose resistance.

For all of the Ecuadorian parents used in the study, two backcrosses and one generation of selfing was sufficient to obtain some plants which produced seed of commercial quality, that was acceptable to farmers. However, the proportion of plants producing parental type seed increased dramatically with the third backcross.

The plants carrying *Co-4*^{2} from the ARME-2 and Yunguilla backcross populations will be useful in the INIAP bean breeding program as potential breeding lines or new varieties with superior anthracnose resistance. Both Yunguilla and ARME-2 carry uncharacterized anthracnose resistance genes which, when pyramided with *Co-4*^{2}, should confer resistance to all reported races of the pathogen in Ecuador. The Cocacho and Paragachi backcross plants with promising agronomic qualities, parental type seed, and the markers for *Co-4*^{2} should be crossed with resistant ARME-2 or Yunguilla (and possibly ACE-1) backcross plants carrying *Co-4*^{2}. In this way the gene or genes complimentary to *Co-4*^{2} can be introduced from the ARME-2 and Yunguilla backcross plants without reintroducing genes from unadapted germplasm (i.e. SEL1308 and RH*2/SEL 1308).

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CHAPTER 2

USING PARTICIPATORY CROP IMPROVEMENT METHODS IN THE MIRA AND CHOTA VALLEYS OF ECUADOR

Introduction

Participatory Crop Improvement

Improved crop varieties are an important part of agricultural technology change, and one that can particularly benefit small-scale farmers with limited resources. For the cost of the improved seed, new varieties with higher yields, pest or disease resistance, or tolerance to stresses such as drought or cold can dramatically increase a farmer's productivity. No additional investment is required to use the new variety -- a farmer can simply plant the crop as in the past. If farmers maintain their own seed, a one time expense will provide benefit for years. In order for the potential impact of improved varieties to be realized, however, farmers must have access to varieties that will perform competitively under their environmental conditions and meet quality requirements set by the farmer or target markets. Participatory Crop Improvement (PCI), the practice of involving farmers in the development or testing of crop varieties, is one methodology which is being used to give farmers access to better crop varieties.

Farmer involvement in plant breeding can increase the effectiveness with which improved varieties are selected by 1) identifying and taking into consideration farmers' variety selection criteria and 2) providing opportunity for selection under farmers' growing conditions.

Farmer vs Breeder Selection Criteria

Plant breeders who have implemented PCI often find that their variety selection criteria differ from farmers' selection criteria. Generally, plant breeders are concerned with improving a crop's yield or stress tolerance (such as drought tolerance or disease resistance), whereas farmers tend to be concerned with more obvious traits (such as seed quality). Dissimilarity between farmer and breeder selection criteria can occur in the following circumstances:

Crop has alternative uses. Small-scale farmers sometimes have multiple uses for a crop, not all of which are obvious to plant breeders. Traits related to the crop's alternative uses may not be considered by breeders. In Morocco, barley grain is used for human consumption and the leaves, grain and straw are used as forage for sheep. When making selections from a segregating barley population, Moroccan farmers mentioned grain yield as a selection criterion 40 times and straw yield 45 times. Moroccan farmers were most interested in barley varieties with both good forage and good grain production (Ceccarelli et al., 2001).

Stringent quality requirements exist for market or home consumption. In some regions, a crop's users (be they the farm family or urban consumers) have particular quality preferences. These quality requirements are not always apparent to plant breeders but are important to farmers. In Colombia, farmers grow dry beans as a cash crop. Consumers prefer solid red or red mottle seed types, and the seed appearance determines the price a farmer gets for his crop. In a comparison of advanced lines selected by farmers and breeders (from the same initial crosses), farmer-selected lines produced larger seeds with more attractive colors and patterns. In a trial of the farmer and breeder selected lines farmers preferred varieties that produced attractive seed, which would bring a higher market price, despite the varieties' lower yields (Kornegay et al., 1996).

Certain traits are useful in view of the farmers' production practices or environment. Plant breeders cannot be fully aware of all farmers' agricultural practices and problems. Therefore, they cannot always know what traits are necessary or could be useful from the perspective of a farmer's agroecosystem. During a participatory trial of rice varieties in eastern India, farmers were interested in a particular variety which had a purple sheath. This trait allowed the farmers to differentiate between the cultivated variety and wild rice plants during weeding (Curtois et al., 2001).

Breeders and farmers differ in their understanding of traits and their inheritance. Plant breeders often focus their efforts on developing varieties with stress tolerance (such as drought tolerance or disease resistance) which are not immediately apparent to farmers. Barley breeders working with Moroccan farmers found that farmers did not realize that varieties could differ in their degree of disease resistance. Instead the farmers thought that disease occurrence depends only on weather conditions. Consequently, only a few farmers considered disease incidence when making selections from a segregating population, despite the presence of two barley diseases in the trial plots (Ceccarelli et al., 2001). Similarly, farmers in the Great Lakes Region of Africa believe the complex of diseases that affects their bean crop to result from "different types of rain" or soil fertility problems (Trutmann, 1996). Plant breeders' understanding of disease resistance genes and their inheritance allow for the development of varieties with specific disease resistance. However, farmers may not recognize the benefit of such traits unless the resistant variety's disease tolerance is proven in a field trial.

In the aforementioned examples researchers used farmer interviews to better understand farmers selection criteria and variety preferences. With such information breeders can (a) incorporate farmer selection criteria into a formal breeding program, (b) recommend varieties that are better suited to farmers' needs, (c) choose parental material that will produce useful variability for future breeding projects.

On-Farm vs. Research Station Selection Environment

PCI often involves testing varieties or making selections under farmers' field conditions. The environment in which selection occurs has a substantial impact on the resulting varieties due to genotype by environment interaction. A variety selected in one environment may not perform equally well in a location with different conditions. In mountainous areas with many different microclimates, the diversity of environmental conditions can make breeding adapted varieties more difficult. Through on-farm testing of varieties, farmers and plant breeders can select those varieties which perform best under farmers' agricultural conditions. Australian wheat breeders found that they were better able to increase yields under low, input rainfed conditions when they moved their trials from research stations to farmers fields (Cooper et al., 1997; Bänziger and Cooper, 2001).

On-farm testing and a PCI approach allow farmers and breeders to better take advantage of repeatable genotype by environment interaction than is possible with formal plant breeding. Formal plant breeding programs tend to select varieties with broad adaptation, which will perform well in many environments. Meanwhile, varieties with specific adaptation, which perform very well in some environments but not others, are often overlooked.

Despite the opportunity for very specific adaptation through PCI, varieties selected by this method are often more broadly adapted than might be expected. Examples of extremely narrowly adapted varieties are rare. However, such varieties are adopted when environmental constraints severely restrict the number of compatible genotypes (Atlin et al., 2001). Varieties that have tolerance to particular stresses but maintain a broader adaptation are more commonly chosen through PCI. Additionally, there are instances of very broadly adapted varieties that were developed through PCI. The three following examples illustrate the range of adaptation in varieties developed through PCI.

A narrowly adapted Nepalese cold water rice variety. In a PCI program aimed at developing rice varieties for high altitude areas of Nepal, one participating farmer chose a plot with cold water and high nutrient conditions to test the segregating bulk variety, Nilgiri-1, that he received from researchers. Rice tends to produce sterile spikelets under such conditions and, in fact, the farmer had previously tried to grow rice in this field, but it never produced any grain. Unexpectedly, the variety Nilgiri-1, yielded 3.3 tons of grain per hectare in the problem field. The farmer continued to grow his selections from the Nilgiri-1 bulk, but only in the problem field because the grain was prone to shattering and had a poor taste (Sthapit et al., 1996). Nilgiri-1 is an example of a very narrowly adapted variety. Its desirable ability to withstand unfavorable environmental conditions but undesirable taste and shattering make it useful in very few instances.

More broadly adapted Nepalese rice varieties bred for cold tolerance. In the same Nepalese rice improvement program, two of the farmer selected varieties were more broadly adapted than Nilgiri-1. The variety M-3 was selected by farmers from segregating bulk varieties that they planted in their own fields. The variety M-9 was selected by farmers from on station and on-farm variety trials. These two varieties were adopted by farmers from villages at a range of altitudes, although the varieties were most widely adopted in mid-altitude villages where they yielded more than the local landraces. This is particularly interesting since M-3 was originally selected by farmers in high altitude villages where it did not yield better than the local red grained landraces but did have a preferred white grain color. The two varieties were not widely adopted in low altitude villages where they did not yield as well (Joshi et al., 2001). M-3 and M-9 are examples of more broadly adapted varieties. They were bred for a high altitude environment, but also performed well at mid-range altitudes. However, M-3 and M-9's lower yields and adoption rates at low altitudes show that the varieties are not adapted to these conditions.

A very broadly adapted Brazilian maize variety bred for low nitrogen tolerance. Brazilian farmers and plant breeders developed a maize variety, Sol da Manhã, which yields well under different nitrogen conditions. The farmers and breeders' goal was to develop a variety that would tolerate low nitrogen soils. However, by making selections from populations grown in fields with varying nitrogen levels, the resulting variety is an efficient nitrogen user and yields well in both high and low nitrogen conditions. In addition, Sol da Manhã can use both ammonium and nitrate forms of nitrogen, an ability that some other modern maize varieties lack (Machado and Fernandes, 2001). Sol da Manhã is an example of a very broadly adapted variety. Although the objective of the farmers and breeders was to develop a variety that was tolerant of low nitrogen, selections were made in a variety of nitrogen conditions to ensure that the resulting variety would not be adapted only to low nitrogen soils.

PCI has been promoted as a means to develop or identify locally adapted varieties for farmers in marginal environments (Atlin et al., 2001), but one possible drawback of this approach is that the products of PCI may be so narrowly adapted that they will be useful in only a few locations. This would make PCI rather inefficient at meeting the needs of the world's many poor farmers. However, as the three examples illustrate, the varieties developed through PCI are not exclusively narrowly adapted, nor are farmers specifically looking for varieties with narrow adaptation. The same Nepalese rice breeding program produced varieties with both broad and narrow adaptation, and farmers found both types of varieties useful. Either broad or narrow adaptation may be desirable, depending on the situation.

Implementation of PCI

PCI has been used effectively to develop or identify new varieties that benefit small-scale farmers. Additionally, the new varieties have, in many cases, been available to farmers much faster than they would have if developed though formal plant breeding, since formal release of varieties takes years in some countries. However, the use of PCI does not guarantee success. Successful PCI projects have some common traits that may be considered guidelines when deciding if PCI is an appropriate solution to farmers problems: (a) farmers' needs are not met by their current crop varieties, (b) an improved crop variety is an appropriate solution to the problem, (c) the crop being improved is important to the farmers, (d) farmers have a tradition or prior knowledge of seed selection, (e) farmers maintain their own seed over the long term.

One example of a PCI project which has each of these five traits is the effort by the Institut des Sciences Agronomiques du Rwanda (ISAR) and the International Center for Tropical Agriculture (CIAT) to improve Rwandan bean varieties. Rwandan farmers were in need of higher yielding varieties as Rwanda's population was growing, the country's soil fertility was declining, and landholding size was decreasing (Sperling, 1992). Yield and stress tolerance, two problems that can be addressed through breeding, were the main concerns of farmers (Sperling, 1992). Beans are an important subsistence crop in Rwanda where they are grown by 97% of farmers (Sperling, 1992) and provide 32% of the caloric intake (Sperling and Scheidegger, 1996). Women farmers are skilled in maintaining and using bean variety mixtures, which are altered depending on the agro-ecological conditions (Sperling and Scheidegger, 1996). The bean breeders asked Rwandan women who were considered "bean experts" to make selections from bean populations grown on the research station, and then test these selections in their own fields (Sperling, 1992). During the first two years of this project farmers selected and adopted 21 new varieties, which was equal to the total number of varieties released by the national bean breeding program in the previous 25 years (Sperling and Scheidegger, 1996).

Timing of Farmer Involvement

As a part of PCI projects, farmers have been involved in various stages of the plant breeding process (modified from Sperling et al., 2001):

1. Planning and goal setting
2. Generating variation through crossing
3. Early generation selection (from large segregating populations)
4. Late generation selection (from smaller segregating populations)
5. Variety testing
6. Seed multiplication and distribution

The majority of PCI experiences previously reported have involved farmers in the last two stages of the plant breeding process, which is sometimes referred to as Participatory Varietal Selection or PVS. Some have suggested that since PVS is simpler and quicker than involving farmers in earlier stages of plant breeding, this approach should by tried first. Then if no useful varieties are identified through this method it may be necessary to involve farmers in the earlier steps of plant breeding (Almekinders and Elings, 2001; Witcombe and Virk, 2001).

In projects where farmer involvement began earlier, they are most often involved in planning, late generation selection, variety testing and seed multiplication. This approach allows farmers to have input into the direction of the breeding program from the very beginning. Also, it allows the breeders to make some selections in the early generations before the farmers make selections later. This way, breeders can eliminate obviously unacceptable plants and those which may have undesirable traits that will not be as apparent to farmers (such as disease susceptibility) (Sperling and Scheidegger, 1996). In only a few cases have farmers made selections in early generations as a part of PCI (Kornegay et al., 1996; Ceccarelli et al., 2001; Humphries, 2001). This may be because it is difficult to produce enough seed of early generations to allow for selection in very many sites. Farmers have rarely been involved in crossing to generate genetic variation. This step has mostly remained the responsibility of plant breeders.

Farmer Recruitment

For the Rwandan PCI project the plant breeders chose and recruited expert farmers to participate in the crop improvement process. This approach has been used in a number of PCI projects (e.g. Kornegay et al., 1996; Sthapit et al., 1996; Smith et al., 2001), and has some advantages. Researchers can choose farmers who are knowledgeable about the crop, communicate well, and are socially connected (to facilitate the spread of information and varieties). However, choosing only expert farmers to participate can result in a bias toward wealthier or more educated farmers. Meanwhile, the poorer farmers' crop improvement needs may remain unmet. Additionally, this approach does not give the farmers much power in the decision making process.

One way in which farmers have been given more power in the PCI process is the establishment of local farmer research committees (known by their Spanish acronym, CIAL). CIAT developed this approach for farmer participatory research in general and it has been used in several Latin American countries. The CIAL consists of farmer-researchers, who are elected by community members. The CIAL oversees a community meeting where farmers decide which of their agricultural problems the CIAL should address. With the help of the supporting researchers the CIAL then develops, and carries out an experiment to address top priority problems identified by the community. At the end of the experiment the CIAL reports back to the community members (Ashby et al., 1996).

In terms of PCI, CIALs have mostly been involved in variety testing (PVS). Because the CIAL experiments last one season before the community reevaluates the research direction, it would be difficult for CIALs to address breeding problems, which require more long-term research. At least one group, however, has applied the CIAL method to more long-term breeding work. After five years of PVS trials several CIALs in Honduras are involved in the earlier stages of plant breeding in collaboration with the Panamerican Agricultural School, Zamorano (Humphries, 2001; Rosas, 2001).

One potential problem with CIALs is that they may still be biased toward the more powerful or wealthy community members, since communities may tend to elect the more wealthy and powerful individuals to the CIAL while poorer farmers problems are still neglected (Ashby et al., 1996). When the Rwandan bean researchers later turned the responsibility of choosing representative farmer experts over to the communities, they found that the representatives were often chosen based on their political and social connections rather than on their bean expertise. Consequently, they were not as skilled at selecting new bean varieties (Sperling and Scheidegger, 1996). Breeders involved in the Honduran project have attempted to make the CIALs more representative of the communities by not limiting CIAL membership to "research oriented farmers." According to socioeconomic surveys conducted in the communities, the goal of including farmers from all social strata has largely been met (Humphries, 2001).

Bean Variety Improvement for Northern Ecuador

The Mira and Chota river valleys in the northern Ecuadorian provinces of Imbabura and Carchi are among the most important bean growing regions in the country, and constitute an estimated 40% of Ecuador's total bush bean production area. In 2002, INIAP founded a CIAL in La Concepción, a community in this region, with the goal of improving bean production, in part through PCI. Bean variety improvement decisions for the region should also be guided by survey data recently collected from bean growers in the Mira and Chota valleys.

Three surveys of bean producers have recently been conducted in the area: a study of bean production, post-harvest and marketing practices in the Chota Valley, concluded in 2000; a participatory rural assessment of bean growers in the Mira Valley, concluded in 2002; and a study of bean production practices and problems, marketing and variety preferences in the Mira and Chota valleys in 2003. Findings from the 2000 and 2002 surveys have been published as project reports by PRONALEG and are summarized here. The findings from the 2003 survey are presented in this thesis.

Geography of the Mira and Chota River Valleys

The Mira and Chota Valleys are located in the highland region of northern Ecuador. The median annual temperature is 19.5 C and varies little throughout the year. Rainfall varies substantially depending on location, however the climate is generally dry and agriculture in some areas is reliant upon irrigation. Most of the rainfall occurs between October and May. A large percentage of the population is of African descent, although in the country as a whole, blacks are an ethnic minority and account for less than 10% of the population.

Study of Production, Post Harvest and Marketing Practices of Bean Producers in the Chota Valley, 2000[1]

For this study, researchers selected, from bean growing communities in the Chota Valley, 105 farmers who owned more than 0.5 ha of land. The farmers were divided into three categories depending on their use of five recommended bean production practices: use of improved varieties, use of quality seed, application of recommended levels of fertilizer, proper timing of fertilizer application, and proper use of pesticides.

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[1] A summary of INIAP Project Report (2001) Estudio de la Producción, Poscosecha, Mercadeo y Consumo de Fréjol Arbustivo en el Valle del Chota, Ecuador. Quito, Ecuador

Those farmers who were classified as Type 1 used, on average, two of the five recommended practices. Sixty percent of the Type 1 farmers owned their land. The average land holding size was 3.5 ha and an average of 2.2 ha were planted to bean. Farmers in this category cultivated both improved varieties and landrace varieties in the Calima Negro, Calima Rojo, Medium Red Mottle, and Panamito commercial bean classes. None of the farmers used quality seed. They tended to use less than the recommended fertilizer levels and applied all of the fertilizer at planting. (The recommended practice is to fertilize 15 to 30 days after planting.) All of the farmers in this group used pesticides and made an average of three applications per growing cycle. However, they tended to apply less than the recommended dose of the products they used. The Type 1 farmers all marketed their beans through intermediaries who then sold to local vendors or exported to Colombia.

The farmers who were classified as Type 2 used an average of three of the five recommended practices. Eighty-five percent of the Type 2 farmers owned their land. The average land holding size was 4.9 ha and an average of 2.2 ha were planted to bean. Like the Type1 farmers, those in the Type 2 category cultivated both improved varieties and landraces varieties in the Calima Negro, Calima Rojo, Medium Red Mottle, and Panamito commercial classes. Thirty percent of the farmers used quality seed. Some farmers in this group used adequate fertilizer and others used less than the recommended fertilizer levels. All of the farmers applied half of the fertilizer at planting and half about 20 days after planting. All of the farmers in this group used pesticides and made an average of two applications per growing cycle. Pesticide use among the Type 2 farmers was considered acceptable. Twenty percent of the farmers sold their beans in the

Colombian market directly and the remaining 80% marketed their beans through intermediaries who then sold to local vendors or exported to Colombia.

The farmers in the Type 3 group used an average of four of the five recommended practices. Sixty-seven percent of the Type 3 farmers owned their land. The average land holding size was 30.3 ha and an average of 4.8 ha were planted to bean. Farmers in this group cultivate almost exclusively improved varieties in the commercial classes for Colombian export: Calima Negro, Calima Rojo, and Medium Red Mottle. All of the farmers used quality seed when it was available. Some farmers in this group used adequate fertilizer and others used less than the recommended fertilizer levels. All of the farmers applied fertilizer at the recommended 15 to 30 days after planting. The farmers in this group all used pesticides and made an average of 3 applications per growing cycle. Pesticide practices among the Type 3 farmers were considered appropriate. All of the farmers in this group sold their beans in the Colombian market. Across the farmer groups about one third of the producers grew only bean. Others grew additional crops, including maize, sweet pepper, hot pepper, cassava, onion, tomato, pea and sugar cane.

In all of the farmer groups, the majority considered pests and disease to be their biggest bean production problem. The most problematic diseases of bean that farmers reported were anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), and mildew (*Erysiphe polygoni*). Farmers reported many pest problems, including whitefly (*Trialeurodes vaporariorum*), thrips, spider mite (*Tetranychus* sp. and *Polyphagotasonemus latus*), leaf miner (*Liriomyza* sp.), crickets, and cut worms. For disease control the most common active ingredients used were mancozeb, cyproconazole, sulfur, and maneb. The most common active ingredients for pest control were methamidophos, profenofos, and cypermethrin.

Other production problems that farmers reported included lack of water, commercialization, and poor quality seed. The researchers also regarded the farmers' post harvest practices as problematic. They noted that the farmers in this region thresh their beans by piling them in the road so that they will be run over by large trucks. This method damages the seed and can result in a 20% loss depending on seed moisture.

Participatory Rural Appraisal of Seven Communities in the Parish of La Concepción, 2002[2]

La Concepción is located in the Mira River Valley. In conjunction with the founding of a CIAL in the community of La Concepción, INIAP researchers interviewed a total of 80 households from La Concepción and the nearby communities of El Empedradillo, La Convalencia, El Rosal, El Chamanal, Santa Lucía, and Santa Ana. Seventy-five percent of those interviewed were of African descent and the remaining quarter were Mestizo.

Agriculture is the primary activity of the men in the communities while women work mostly in the home. A few women (15%) devoted the majority of their time to agriculture. Most families have small land holdings --the majority (56%) owned 1 ha of land or less, another 24% owned between 1 and 2 ha, and 20% owned more than 2 ha. Beans are the most important crop in this area and are grown for home consumption and for sale. Of the farmers interviewed, 75% were growing beans at the time and 50% had grown beans during the previous cropping cycle. The second most important crop is

[2] A summary of INIAP Project Report (2003) Género e Investigación Participativa en el Mejorameinto de Variedades de Fréjol y Sistemas de Producción de Semillas en la Sierra de Ecuador. Quito Ecuador

maize which was being grown by 5% of the farmers at the time of the interview but had been grown by 39% in the previous crop cycle. Other crops include tomato (9% present cycle, 5% previous), cassava (5%, 4%), sugar cane (3%, 0%), sweet pepper, banana, and hot pepper.

The beans grown the parish of La Concepción belong to the Calima Negro, Calima Rojo, Cranberry, Small Black, Panamito, Medium Red Mottle, and Canario market classes. Some farmers also reported growing bean variety mixtures. Beans are sown twice during the year, typically from February to March and from August to September. However, for farmers without irrigation, planting depends on the timing of rainfall. The majority (80%) of the farmers save their own seed, the rest either purchase seed (18%) or obtain it through exchange (2%). Due to poor seed quality, farmers plant from 2 to 5 seeds per hill in order to get an acceptable plant stand. About half of the farmers fertilized their bean crop.

The bean pest and disease problems are rust, whitefly and bean weevil (*Acanthoscelides obtectus*). Farmers used products containing the following active ingredients to control bean pests and disease: carbofuran, chlorothalonil, cypermethrin, lambda cyhalothrin, malathion, mancozeb, maneb, metalaxyl, methamidophos, profenofos, propineb, and sulfur. Pesticide use in La Concepción is minimal, with farmers making at most two pesticide applications and some using no pesticides at all. Seventy-four percent of the farmers use some method for post-harvest control of the bean weevil. Chemical controls included aluminum phosphide fumigation, or malathion applied to the seed. Other farmers used garlic, eucalyptus, or ash to control weevils.

Some farmers reported treating beans for consumption with malathion and then washing them before cooking.

Bean producers in La Concepción thresh beans by beating the harvested plants with sticks. This method does not damage the seed as threshing in the road does. One third of the bean growers in the region grow only for home consumption. The majority (60%) sell to intermediaries who come to the community and a small number (7%) take their beans to Ibarra or Mira to sell to vendors directly.

A Brief Comparison of the Two Valleys

Although farmers from both valleys sell beans, production in the Chota Valley is more commercialized. Farmers use more inputs, such as fertilizer and pesticides, and their proximity to the Pan-American Highway makes markets more accessible. Some of the farmers in the Mira Valley grow beans only for home consumption and they grow a larger variety of bean classes. The predominant classes in the Chota Valley were those grown for export to Colombia. Soon after the study in the Chota Valley was completed, however, Plan Colombia effectively ended all export of beans from this region of Ecuador to Colombia. Some results of this change in the market are reflected in the most recent survey findings, which are presented in this thesis.

Materials and Methods

Farmer Survey

A questionnaire was developed to gather information that would be useful in setting breeding goals. The questionnaire that was used appears in Appendix B, Figure B1. An English translation of the questionnaire is in Appendix B, Figure B2. For a part of the interview farmers were presented with photographs of the following bean pests and diseases mounted on 5 x 8" cards: whitefly (*Trialeurodes vaporariorum*), leaf miner (*Liriomyza* sp.), spider mite (*Tetranychus* sp.), bean weevil (*Acanthoscelides obtectus*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*), angular leaf spot (*Phaseoisariopsis griseola*), powdery mildew (*Erysiphe polygoni*), and bean common mosaic virus (BCMV). Farmers were asked to identify those bean pests and diseases that were problematic in their crop, and farmers were questioned further about the particular pests or diseases that they identified as problematic.

Fifty-six bean growers from 10 different communities in the Mira and Chota Valleys of Ecuador were interviewed in May of 2003. Recruitment for the interviews was accomplished by approaching people in the communities, especially those who were working in bean fields or threshing beans. Individuals were only interviewed if they were currently growing beans.

Data from the interviews was compiled in SPSS.

Results

Age, Education and Family Size of the Bean Producers Interviewed

A total of 56 bean producers were interviewed, 10 women and 46 men. They ranged in age from 23 to 83 years old. As shown in Table 23, the largest group (41% of the farmers) was 36 to 50 years old. Approximately equal numbers of farmers were between 20 to 35 years old and 51 to 65 years old.

Table 23. Age distribution of farmers interviewed

Age Category	# of Farmers	Percent of Farmers Interviewed
20 to 35 years old	12	21.4
36 to 50 years old	23	41.1
51 to 65 years old	13	23.2
>65 years old	8	14.3

The level of schooling completed by the farmers interviewed is shown in Table 24. Most of the farmers interviewed (63%) had attended only primary school, and 23% had attended secondary school. Only 5% had had no schooling and another 5% had attended university.

Table 24. Level of schooling completed by farmers interviewed

Level of Instruction	# of Farmers	Percent of Farmers Interviewed
No Schooling	3	5.4
Primary School	35	62.5
Secondary School	13	23.2
University	3	5.4
Not Responding	2	3.6

Table 25 shows the distribution of family sizes of the farmers interviewed. Sixty-two percent of the farmers interviewed had five or more family members living at home. Only 9% were members of 1 or 2 person households.

Table 25. Family size of farmers interviewed

Number of Family Members	# of Farmers	Percent of Farmers Interviewed
1 to 2	5	8.9
3 to 4	15	26.8
5 to 6	18	32.1
7 to 8	14	25.0
>8	3	5.4
Not Responding	1	1.8

Land Ownership and Crops Cultivated by Farmers

Almost all of the farmers interviewed (93%) owned the land they cultivated. The remaining 7% of the farmers rented land. The size of farmers' land holdings ranged from 0.5 to 30 ha. The median land holding size was 2 ha. However, 39% of the farmers interviewed owned 1 ha or less and 64% owned less than 2 ha. Only 14 % of the farmers owned more than 5 ha. Those farmers who rented land rented between 1 and 2 ha.

Although the interviews were conducted in a mountainous region, excessive slope was not a problem for most farmers. The majority of the growers (59%) farmed only flat fields (usually located in the river valley), and 68% had at least one flat field. A few, 7%, owned only very sloped fields.

Within the two years preceding the interview most of the farmers had grown two or three different crops, including bean. Twenty-nine percent had grown four or more different crops. As shown in Table 26, farmers grew a variety of crops in addition to bean. Beside bean, tomato was the crop farmers most frequently reported growing. Peppers, maize, cassava, sugar cane and onion were also frequently grown.

Table 26. Crops grown by farmers interviewed

Crop	Percent of Farmers Growing Crop
Bean	100%
Tomato	39%
Sweet Pepper, Maize	20 to 30%
Cassava, Sugar Cane, Hot Pepper, Onion	10 to 20%
Anise, Camote, Pea	5 to 10 %
Alfalfa, Avocado, Carrot, Papaya, Pigeon Pea, Prickly Pear, Potato, Blackberry	>5%

Table 27 shows the distribution of land area that farmers planted to bean. Most frequently farmers planted between 0.5 and 1 ha of bean. About 20% planted less than 0.5 ha and another 20% planted between 1 and 2 ha. A few of the farmers, with larger land holdings, planted more than 3 ha of beans. Ten hectares was the largest area that a farmer reported planting to beans.

Table 27. Area farmers interviewed planted to beans

Hectares Planted to Bean	# of Farmers	Percent of Farmers Interviewed
≤0.5	11	19.6
0.51 to 1	26	46.4
1.1 to 2	11	19.6
2.1 to 3	4	7.1
>3	4	7.1

Data for bean consumption by the farmers interviewed suggest that the consumption rate in this region is much higher than the national average of 6 kg/year. Farmers reported eating beans an average of 5 times per week, and the consumption rate was calculated to be 38.1 ± 6.68 kg/year.

The Bean Classes Grown by Farmers

Overall, the farmers interviewed reported growing thirteen different classes of beans. However, even with this diversity, most farmers grew only one or two classes of beans (Table 28). The maximum number of classes that any farmer grew was four.

Table 28. Number of classes of beans farmers reported growing

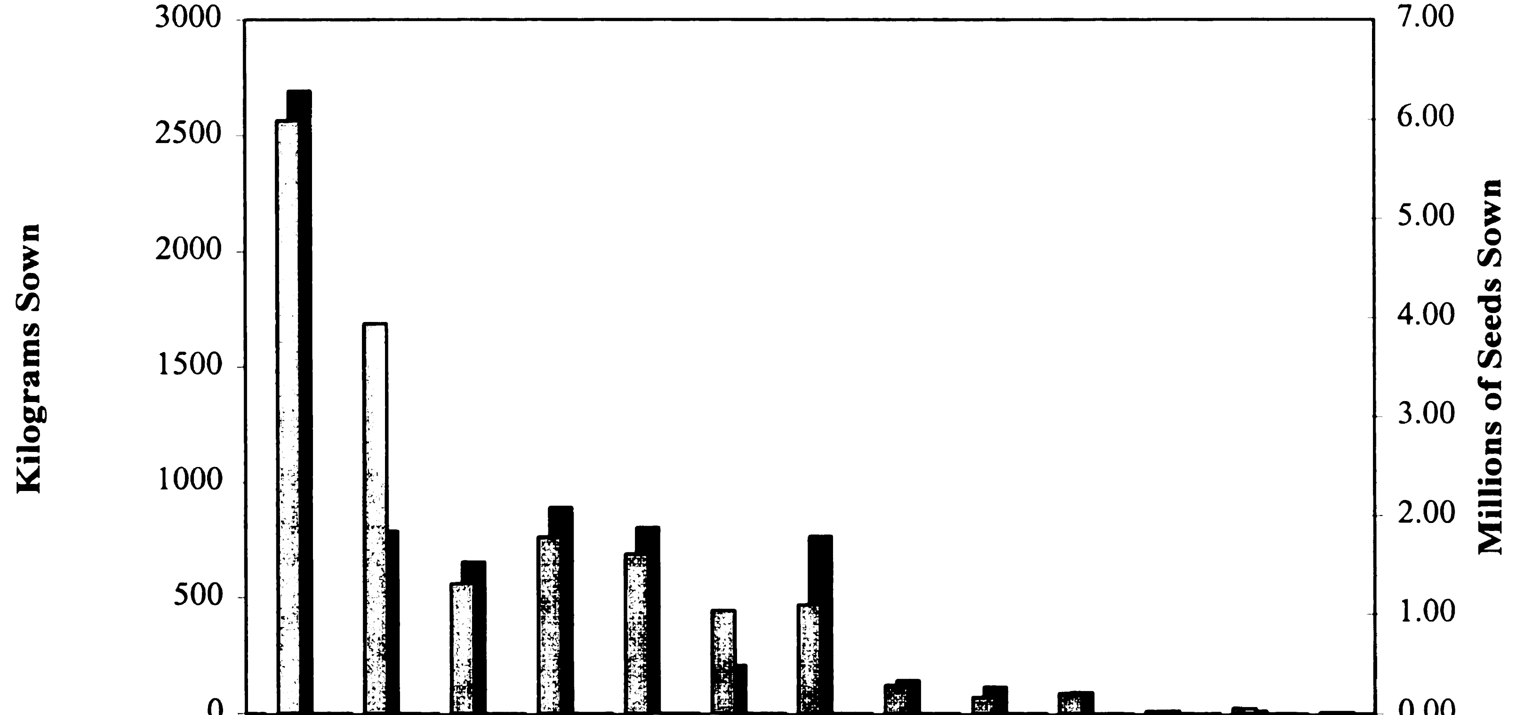
Number of Classes Grown	# of Farmers	Percent of Farmers Interviewed
1	31	55.4
2	16	28.6
3	6	10.7
4	3	5.4

Figure 15 shows the number of farmers growing each commercial class and the total quantity sown in kilograms reported by farmers and in millions of seeds. The calculation of millions of seeds sown was made to adjust for differences in seed weight among the classes. Medium seeded red mottles were the most frequently grown class. Other classes that were frequently grown were small black, Blanco de Leche, Calima Negro and Calima Rojo. A few farmers grew Panamito and yellow seeded Canario and Matahambre classes. The most rarely reported classes were Bayo Blanco, Vaquita, solid red, Musgo and snap beans. A description of each class, its distribution, uses, prices (Figure 16), and farmers' comments follow.

Medium Red Mottle

Farmers call medium seeded red mottle (MRM) beans Injerto, Rojo Injerto, or by the names of several released varieties in this class -- Cargabello, Paragachi, and Selva. Seed of this class is medium in size and has a mottled pattern of dark and light red and white. Farmers reported growing MRM varieties with both determinant (type I) and type II indeterminate growth habits.

Figure 15. Amount of each bean class sown by the farmers interviewed and number of farmers growing each class



	Med. Red Mottle	Small Black	Blanco de Leche	Calima Negro	Calima Rojo	Pana-mito	Ca-nario	Mata-ham-bre	Bayo Blanco	Va-quita	Rojo	Mus-go	Vaiñita
# Farmers Growing	25	16	12	11	10	6	4	3	2	1	1	1	1
■ Total Kg Planted	2693	788	653	890	803	207	765	140	113	90	11	11	6
□ Millions of Seeds Planted	5.98	3.94	1.31	1.78	1.61	1.04	1.09	0.28	0.16	0.20	0.02	0.06	0.01

Figure 16. Maximum, minimum and mean prices for bean classes

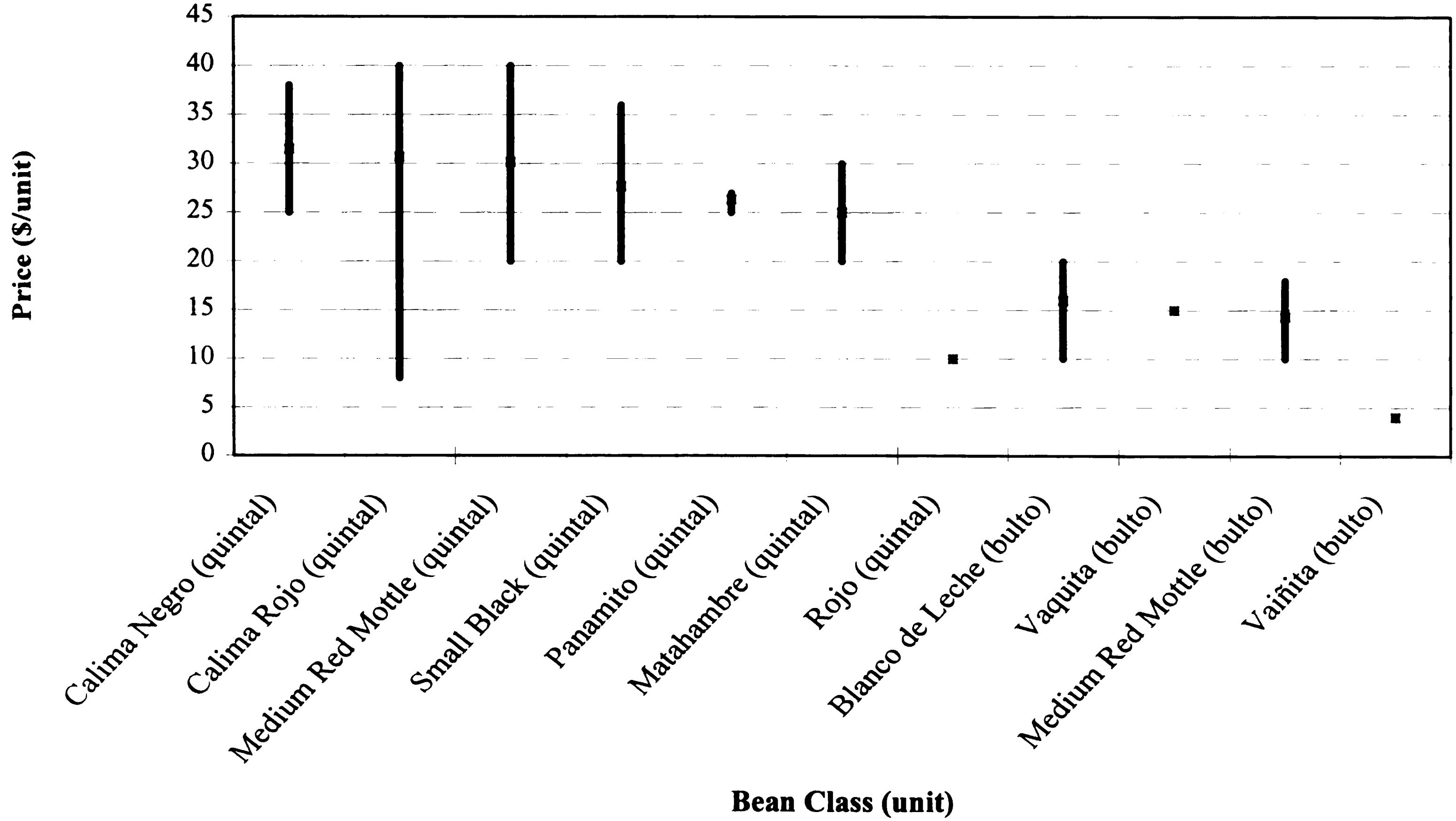
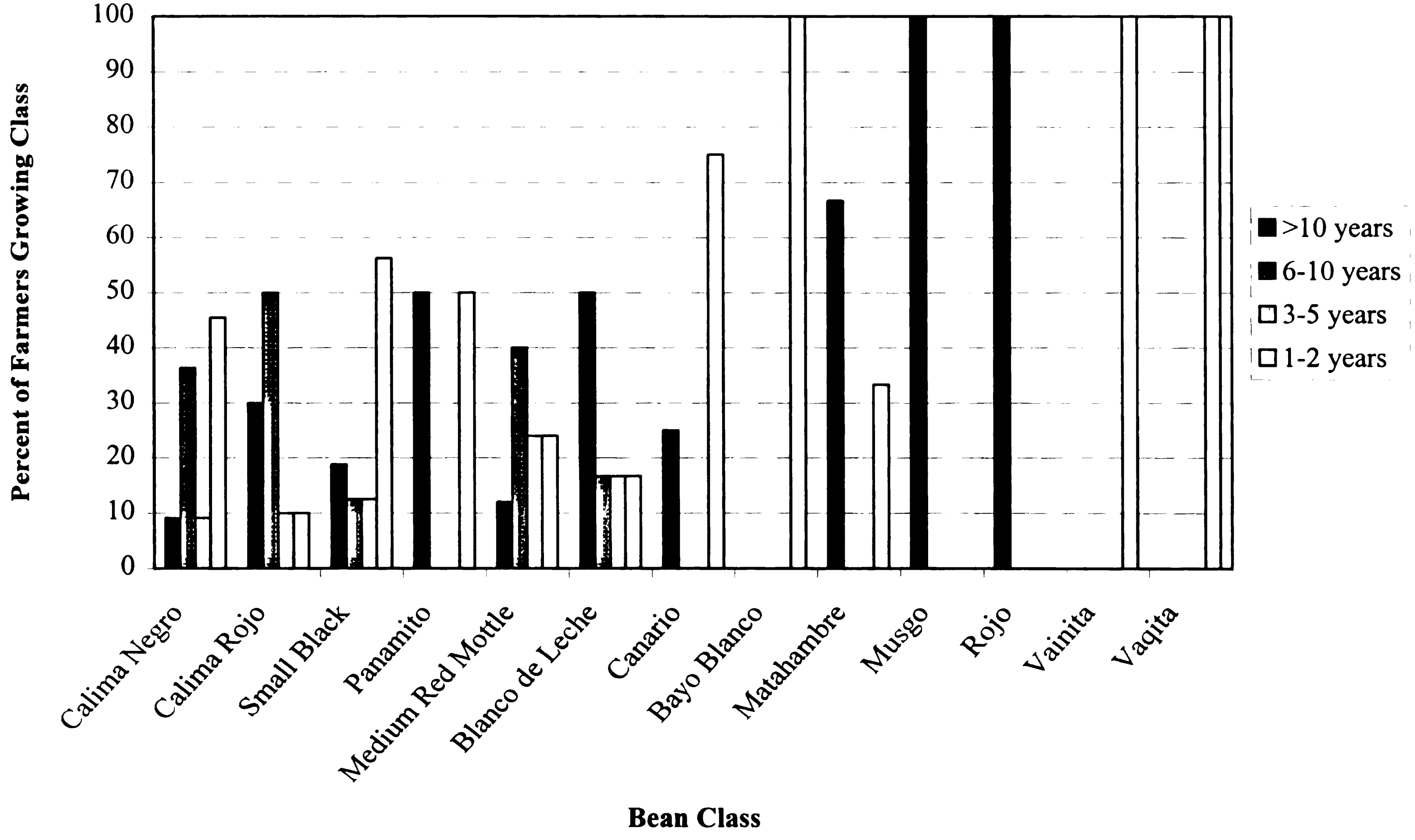


Figure 17. Number of years farmers had grown bean class



MRMs rank first among the classes in number of farmers cultivating the class, millions of seeds sown and in amount of seed sown. Twenty-five farmers, 45% of those interviewed, reported growing this class within the past year. As shown in Figure 17 12% of the farmers growing the class had grown it for more than 10 years and 40% had grown it for 6 to 10 years. Twenty-four percent had grown the variety for 3 to 5 years and another 24% were very recent adopters, who had grown the variety for 1 to 2 years.

The majority (81%) of the farmers who had grown MRM in the past had sold some of their harvest. A few farmers (12%) grew the class only for sale and did not consume it themselves. Farmers reported sowing a total of 2,693 kg of seed of this class within the last year. This class was more frequently grown by farmers from higher elevation communities between 1800 and 2460 m.

MRMs are sold and consumed both dry and *in tierno*. Of the farmers who had sold this class in the past, 76% had sold the beans dry and 59% had sold them as shell beans, with some farmers selling both dry and *in tierno*. Of the farmers who had consumed MRMs in the past year, the majority, 72%, used them both dry and *in tierno*. Twenty-two percent consumed the class only as dry beans and 5% only *in tierno*.

The average price farmers reported receiving for dry MRMs was $30/quintal (about 45 kg or 100 lbs), with a minimum of $20/quintal and a maximum of $40/quintal. The mean price for MRM *in tierno* was $14/bulto, with a minimum of $10/bulto and a maximum of $18/bulto. (A bulto, the typical unit for unshelled beans *in tierno*, is a measure of volume. One bulto of beans *in tierno* weighs between 30 and 35 kgs). Of the farmers selling MRMs, 47% sold their crop in their own community, 35% sold MRM in the town of Pimanpiro and 18% sold MRM in the city of Ibarra.

Most farmers growing this variety do not save their own seed. The majority, 84%, of farmers growing MRMs had obtained their seed from an outside source -- either purchased (72%), exchanged (4%) or obtained from their landlord (8%). Only 16% used seed they had saved themselves.

Farmers had much praise for MRMs and few complaints. They regard the class as resistant to diseases, especially rust, and high yielding. They also complimented its flavor, and high quality seed for *tierno*, which brings a good price. Most farmers did not mention any negative qualities for this class, although three farmers thought MRMs took too long to mature.

Small Black

Farmers call small seeded black beans Negro Pequeño or simply Fréjol Negro. One farmer used the Colombian name for the class, Caraota. The black bean varieties farmers were growing had a type II indeterminate growth habit. This class was more frequently grown by farmers from lower elevation communities between 1340 and 1700 m.a.s.l.

Small blacks are second among the classes for number of farmers cultivating the class and for millions of seeds sown. Sixteen farmers, 29% of those interviewed, reported growing this class within the past year. Nineteen percent of the farmers growing the class had grown it for more than 10 years and 13% had grown it for 6 to 10 years. Thirteen percent had grown the variety for 3 to 5 years and another 56% were very recent adopters, who had grown the variety for 1 to 2 years.

Of the farmers who had grown small blacks in the past, 82% had sold some of their harvest. None of the farmers interviewed grew this class only for sale -- all those who grew small blacks also consumed them.

The farmers interviewed sold small blacks exclusively as dry beans. The majority also consumed small blacks only as dry beans, although 25% consumed small blacks dry and *in tierno*.

The average price farmers reported receiving for small blacks was $28/quintal(45 kg), with a minimum of $20/quintal and a maximum of $36/quintal. Of the farmers selling small blacks, one thirds sold their crop in their own community, one third sold them in the city of Ibarra, and one third sold them to a cooperative.

The majority, 88%, of the farmers growing small blacks had obtained their seed from an outside source. However, this figure may be a bit misleading since a number of farmers were growing the class for the first time. Fifty-six percent of the farmers purchased seed, and 31% got seed from a cooperative or NGO. (Several groups were promoting black beans, which have a domestic market, as an alternative to the Calima classes.) Only 12% of black bean growers planted seed they had grown and saved themselves.

Of all the classes, small blacks garnered the most positive comments on their flavor. Farmers also commented that this class is high yielding, disease resistant and has a local market. Only two farmers had negative comments for the class. One thought it took too long to mature and the other found it difficult to thresh since the small seeds fall into cracks in the road and cannot be picked up.

Blanco de Leche

The class known to farmers as Blanco de Leche produces large, white, kidney shaped seeds. The plants are determinant.

Blanco de Leche is third among the classes in both number of farmers cultivating the class but only sixth in total kilograms of seed sown of the class, indicating that the farmers who grow Blanco de Leche plant less of it relative to other varieties. Twelve farmers, 21% of those interviewed, reported growing this class within the past year. Fifty percent of the farmers growing the class had grown it for more than 10 years and 17% had grown it for 6 to 10 years. Seventeen percent had grown the variety for 3 to 5 years and another 17% were very recent adopters, who had grown the variety for 1 to 2 years. None were growing it for the first time.

The majority (92%) of the farmers who grow Blanco de Leche sold some of their harvest. Twenty-five percent of the farmers grew the class only for sale and did not consume it themselves. Farmers reported sowing a total of 653 kg of seed of this class within the last year. This class was grown at lower elevations almost exclusively by farmers from two neighboring communities: Tumbatu and Pusir Grande.

Blanco de Leche is nearly always sold and consumed *in tierno.* All of the farmers who had sold this class, sold it *in tierno.* One farmer reported also selling Blanco de Leche dry, for seed. Of the farmers who consumed this class, all ate it in the *tierno* stage, and one farmer also consumed it as a dry bean.

The mean price for Blanco de Leche *in tierno* was $16/bulto, with a minimum of $10/bulto and a maximum of $20/bulto. Of the farmers selling Blanco de Leche, 18%

sold their crop in their own community, 18% sold it in the town of Pimanpiro and 64% sold it in the city of Ibarra.

The majority, 75%, of the farmers growing Blanco de Leche saves their own seed. The 25% of farmers who got their seed from an outside source purchased it or obtained it through exchange.

Blanco de Leche is grown for sale *in tierno* during Holy Week prior to Easter, and is a part of the traditional dishes for this celebration. Farmers reported that they like Blanco de Leche because it has a short growing cycle, it brings a very good price during Holy Week, and it yields well. However, the said it is also very susceptible to disease and that prices drop substantially after Holy Week. Although grown by a relatively large number of farmers, Blanco de Leche can be a risky investment, and this probably explains why farmers plant less of it, relative to the other classes.

Calima Negro

Calima Negro, sometimes called Mil Uno, the name of a released variety in this class, produces large seed with a mottled pattern of dark and light purple and white. The Calima Negro varieties grown by the interviewed farmers were all determinant.

Calima Negro ranks fourth among the classes in number of farmers cultivating the class. Eleven farmers, 20% of those interviewed, reported growing this class within the past year. Nine percent of the farmers growing the class had grown it for more than 10 years and 36% had grown it for 6 to 10 years. Nine percent had grown the variety for 3 to 5 years and 45% were very recent adopters, who had grown the variety for 1 to 2 years.

All of the farmers who had grown Calima Negro in the past had sold some of their harvest. Twenty-two percent had grown the class only for sale and did not consume it themselves. Farmers reported sowing a total of 890 kg of seed of this class within the last year.

Calima Negro is mostly sold dry, but one farmer reported selling it *in tierno*. Of the farmers who had consumed Calima Negro in the past year about half (57%) consumed the class only as dry beans and the rest (43%) consumed them dry and *in tierno*.

The average price farmers reported receiving for dry Calima Negro was $32/quintal(45 kg), with a minimum of $25/quintal and a maximum of $38/quintal. Of the farmers selling Calima Negro, 78% sold their crop in their own community, 11% sold them in the town of Pimanpiro and 11% sold them in the city of Ibarra.

Most farmers growing Calima Negro do not plant their own saved seed. The majority, 64%, had obtained their seed from an outside source -- either purchased (54%) or through exchange (9%). Of the 36% that planted seed they had saved themselves, all had purchased Calima Negro seed within the past 3 years.

Farmers commented that they liked the seed and market quality of this class. They also liked Calima Negro's fast maturation and good yields. Some of the farmers' criticism of this class was that it required too much water to grow, its flavor and large size made it less than desirable, its price was too variable and it sometimes yields poorly.

Calima Rojo

Calima Rojo, also known as Margarita and Catio produces large seed with a mottled pattern of red, pink and white. Farmers reported growing Calima Rojo varieties with both determinant (type I) and type II indeterminate growth habits.

Calima Rojo ranks fifth among the classes in number of farmers cultivating the class. Ten farmers, 18% of those interviewed, reported growing this class within the past year. Thirty percent of the farmers growing the class had grown it for more than 10 years and 50% had grown it for 6 to 10 years. Ten percent had grown the variety for 3 to 5 years and another 10% were very recent adopters, who had grown the variety for 2 years. None of the farmers were growing the class for the first time.

All of the farmers who were growing Calima Rojo had sold some of their harvest. Ten percent grew the class only for sale and did not consume it themselves. Farmers reported sowing a total of 803 kg of seed of this class within the last year.

Calima Rojo is mostly sold dry, but two farmers reported also selling it *in tierno*. Of the farmers who had consumed Calima Rojo in the past year, 66% consumed the class only as dry beans and the rest (33%) consumed them dry and *in tierno*.

The average price farmers reported receiving for dry Calima Rojo was $31/quintal(45 kg), with a minimum of $8/quintal and a maximum of $40/quintal. Of the farmers selling Calima Rojo, 56% sold their crop in their own community, 22% sold them in the town of Pimanpiro and 22% sold them in the city of Ibarra.

The majority, 60%, of the farmers growing Calima Rojo planted their own saved seed. Thirty percent purchased seed and 10% obtained it through exchange. Of the 60% that planted seed they had saved themselves, half had purchased Calima Rojo seed within the past 3 years.

The farmers' comments about Calima Rojo were much like those for Calima Negro. They liked the seed quality, marketability, and fast maturation. Some farmers also liked its flavor. There were mixed opinions on the yields of this class, as some farmers thought it yielded well and others thought it yielded poorly. They also considered it susceptible to rust and drought.

Panamito

Six farmers, 11% of those interviewed, were growing a small seeded white bean they called Panamito. This class can have either a determinant (type I) or type II indeterminate growth habit. The Panamito class was sixth among the classes in terms of the number of farmers growing it.

Half of the farmers growing this class were growing it for the first time. The other half had been growing the class for more than 10 years. All of those who had grown it before the current cycle had sold some of the harvest, and consumed the class themselves. None of the farmers reported consuming or selling Panamito in any form other then as a dry bean.

The average price farmers reported receiving for Panamito was $26/quintal(45 kg), with a minimum of $25/quintal and a maximum of $27/quintal. Of the farmers selling Panamito, 33% sold their crop in their own community, and 67% sold it in the city of Ibarra.

Sixty-six percent of the farmers growing Panamito obtained seed from an outside source. However, as previously mentioned, half of them were growing the class for the first time. Overall, 33% of the farmers saved their seed, 33% purchased seed and 33% obtained seed from a cooperative or NGO.

Farmers like Panamito beans because they are high yielding and have a local market. However, the farmers also commented that they did not like the flavor of this class, that it is susceptible to disease, particularly rust, and takes a long time to mature.

Canario

Four farmers were growing Canarios, which have a round, medium sized, light yellow seed, and a type I or II growth habit. Canarios are seventh in terms of number of farmers growing the class.

Of the four farmers growing this class, three were growing it for the first time, the other had grown Canarios for more than 10 years. The only farmer who had grown it before the present cycle had sold it in Pimanpiro, as a dry bean and consumed it both dry and *in tierno.*

All of the farmers had obtained the seed they planted from and outside source -- two from a cooperative or NGO, one from a landlord, and one purchased.

The farmer who had grown Canarios before grew it for its flavor, others were growing the class because they believed it would yield well and bring a good price. However, two farmers thought Canarios were susceptible to disease.

Matahambre

Three farmers were growing a type II, medium, yellow seeded bean which is slightly longer and darker in color than the Canario. They called this class Amarillo or, more picturesquely, Matahambre. One of the farmers was growing the class for the first time, the other two farmers had grown the class for more than 10 years. The two farmers with more experience with Matahambre sold the class and consumed it themselves. One had sold Matahambre only as a dry bean and the other had sold it dry and *in tierno.* Both had consumed the class dry and *in tierno.* The average price farmers received for dry Matahambre beans was $25/quintal, and both had sold the beans in Pimanpiro.

All three farmers obtained seed of Matahambre from an outside source -- two purchased it and one made an exchange with another farmer. According to farmers, the outstanding quality of this class is its flavor.

Bayo Blanco

Two farmers were growing Bayo Blanco, a cream colored bean with a medium sized round seed. Both farmers were growing the class for the first time and had obtained the seed from a cooperative. The seed was a mixture, producing plants with both type I and type II growth habits. The farmers were growing the class because they believed it would bring a good price. However, one thought it susceptible to disease.

Vaquita

One farmer was growing a type II, medium seeded, cream and pink bean called Vaquita. The farmer had, within the past year, purchased seed of the variety and was growing it exclusively for sale *in tierno*. She had sold her first harvest for $15/buleto in Ibarra. The farmer thought the variety yielded well but complained that it did not mature at the same time.

Solid Red

One farmer was growing a type II, medium seeded, solid red bean simply called Rojo. She had cultivated the variety for more than 10 years. The farmer planted her own seed of the variety and grew it for home consumption and sale both dry and *in tierno*. She had received a price of $10/quintal of dry seed in Ibarra.

Musgo

One farmer was growing, Musgo, a determinant bean with a thin, medium-sized brown seed. The farmer planted his own seed of this variety, which he had grown for more than 10 years, and used it solely dry for home consumption. He thought the variety yielded well and he liked the flavor and seed size and shape.

Vaiñita

One farmer grew determinant snap beans that had a large white seed. She had begun growing the variety within the past year from seed she purchased. The farmer grew the variety for sale as a snap bean in Ibarra, and used it for home consumption dry, *in tierno* and as a snap bean for green pod consumption.

Farmers Seed Sources

Overall, only 36% of the farmers reported planting their own saved seed. Nearly all of the farmers interviewed, 91%, had obtained some seed from an off-farm source in the past three years. As shown in Table 29, farmers' decision to save or to purchase seed was not related to the amount of land they owned (a proxy for poverty). A X^2-test for independence shows that landholding size and seed saving are independent of one another.

Table 29. X^2-test for independence between landholding size and seed saving

Landholding Size	Did Not Save Seed	Saved Some Seed
0-1.0 ha	13	9
1.1-2.0 ha	9	5
2.1-3.0 ha	6	1
3.1-6.0 ha	5	3
>6 ha	3	2

X^2-value=1.71 p-value=0.79; Fail to reject H_0, conclude variables are independent

Pest and Disease Problems Reported by Farmers

When farmers were shown photographs of the bean pests whitefly, weevil, spider mite and leaf miner, 96% identified whitefly as a problem in their bean crop, 75% identified weevils as a problem, 50% identified spider mite as a problem, and 46% identified leaf miner as a problem. When shown photographs of bean diseases, 86% identified rust as a problem in their bean crop, 59% identified root rot as a problem, 52% identified anthracnose as a problem, 48% identified mildew as a problem, 48% identified common bacterial blight as a problem, and 23% identified angular leaf spot as a problem. Additional data on the severity of each production problem, farmers' names for the pest or disease, and farmers' control methods follows. A complete list of farmers' names for the production problems is given in Table 30. A comparison of the incidence and severity of the production problems is shown in Figure 18. Table 31 lists all of the active ingredients in the products that farmers reported using, the problems the products were used to treat, and the World Health Organization's acute toxicity rating for the each active ingredient (IPCS-IOMC, 2000). A list of all the active ingredients of pesticides and their Ecuadorian trade names is found in Appendix C.

Table 30. Farmers' names for production problems and number of farmers using each name

Whitefly	# Farmers	Leaf Miner	# Farmers	Spider Mite	# Farmers
Mosca blanca	25	Minador	9	Araña roja	16
Palomilla	12	Toston	5	Arañita roja	4
Mariposita	10	Enrollador	2	Arañita	3
Mariposa blanca	3	Lancha	2	Acaro	1
Plumilla	1	Rollador	1	No Name	4
Barreno	1	Araña negra	1		
Araña	1	Hongos	1		
No Name	3	No Name	8		

Weevil	# Farmers	Rust	# Farmers	ALS[a]	# Farmers
Gorgojo	27	Roya	42	Laucha	2
Redondilla(o)	9	Eumollador	1	Lancha	2
Polilla	5	Royal	1	Antracnosis	1
Pulgon	1	No Name	5	Mancha Negra	1
No Name	1			No Name	7

Anthracnose	# Farmers	Root Rot	# Farmers	CBB[b]	# Farmers
La Gota	8	Pudrición de raiz	8	Lada (Helada)	4
Antracnosis	6	Pudrición	2	Lancha	4
Lancha	2	Hongos de tallo	2	Bacteria	2
Laucha	2	Fusario	1	Lancha amarilla	1
Manchas	2	Hongos	1	Baterosis	1
Pudricion mata	1	Cancer de raiz	1	Laucha	1
Sancochado	1	Tizon	1	Antracnosis	1
Mancha negra	1	Trozador gusano	1	Minador	1
Septolia	1	Enrollador	1	Enrollador	1
Helada	1	Barrenador	1	Picudo	1
Enrolleador	1	No Name	3	Toston	1
No Name	6			Mosco	1
				No Name	7

Mildew	# Farmers	BCMV[c]	# Farmers
Ceniza	20	Deficiencias	1
Pudrición	1	Toston	1
Cenicilla	1	Desconocido	1
Acaros	1	Enrollador	1
Mosca	1	No Name	7
No Name	4		

a Angular Leaf Spot; b Common Bacterial Blight; c Bean Common Mosaic Virus

Figure 18. Farmer reported incidence and severity of bean pests and diseases

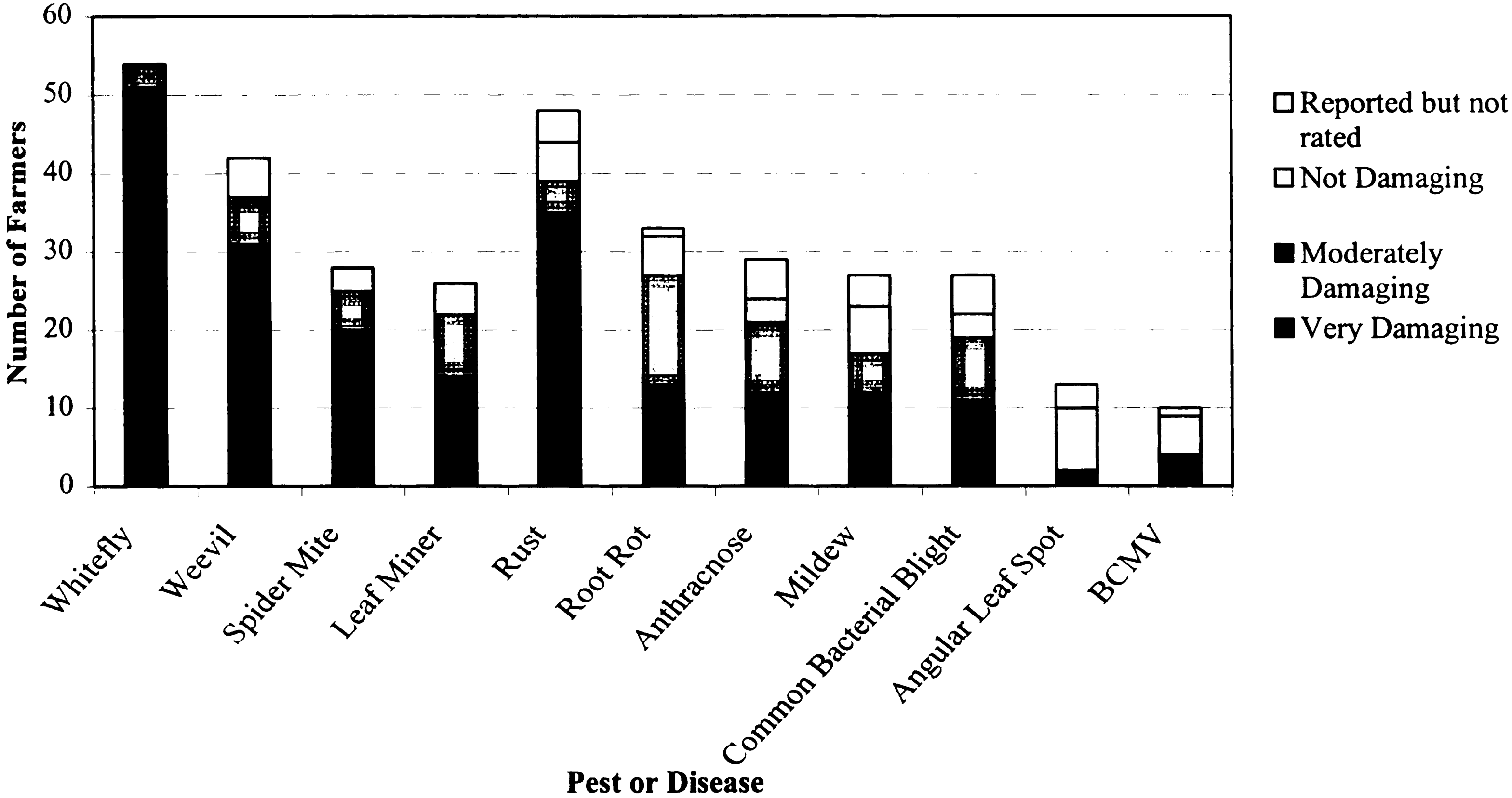


Table 31. Active ingredients (A.I.) in pesticide farmers used to control bean production problems, an problems treated

Active Ingredient Fungicides	# farmers using A.I.	WHO Rating†	Leaf Miner	White-fly	Spider Mite	Weevil	Anthrac-nose	Rust	Mildew	ALS	CBB	Root Rot	**# problems treated w/ A.I.**
benomyl	3	*	0	0	0	0	**2**	**1**	**1**	**1**	**1**	0	**5**
bupirimate	1	*	0	0	0	0	0	0	**1**	0	0	0	**1**
captan	16	*	0	0	0	**6**	0	0	0	0	0	**10**	**2**
carbendazim	6	*	0	**1**	0	0	**1**	0	0	**1**	0	**4**	**4**
carboxin	14	*	0	0	0	**5**	0	0	0	0	0	**9**	**2**
chlorothalonil	5	*	**1**	0	0	0	**2**	0	0	**1**	**3**	0	**4**
copper oxychloride	2	**	0	0	0	0	0	**2**	0	0	0	0	**1**
cymoxanil	4	**	0	0	0	0	**3**	0	0	**1**	**2**	0	**3**
cyproconazole	19	**	0	**1**	**1**	0	**1**	**16**	**2**	0	0	0	**5**
difenoconazole	1	**	0	0	0	0	**1**	**1**	0	0	0	0	**2**
folpet	1	*	0	0	0	0	0	0	0	0	0	**1**	**1**
mancozeb	11	*	**1**	**1**	0	0	**6**	**2**	**1**	**1**	**2**	0	**7**
maneb	1	*	**1**	0	0	0	0	0	0	0	0	0	**1**
metalaxyl	1	**	0	0	0	0	**1**	0	0	0	0	0	**1**
metiram	1	*	0	**1**	0	0	0	0	0	0	0	0	**1**
myclobutanil	1	**	0	0	0	0	0	0	**1**	0	0	0	**1**
oxadixyl	2	**	0	**2**	0	0	0	0	0	0	0	0	**1**
oxycarboxin	19	*	0	0	**1**	0	0	**18**	0	0	0	0	**2**
propiconazole	3	***	0	0	0	0	0	**1**	**2**	0	0	0	**2**
propineb	6	*	0	0	0	0	**4**	**1**	0	**1**	0	0	**3**
sulfur	6	*	**1**	0	0	0	**1**	0	**4**	0	0	0	**3**
thiram	14	**	0	0	0	**5**	0	0	0	0	0	**9**	**2**

† World Health Organization ratings for acute toxicity: * "unlikely to be hazardous," ** "slightly hazardous," *** "moderately hazardous"

Table 30 continued

Active Ingredient Insecticides	# farmers using A.I.	WHO Rating†	Leaf Miner	White-fly	Spider Mite	Weevil	Anthrac-nose	Rust	Mildew	ALS	CBB	Root Rot	**# problems treated w/ A.I.**
aldicarb	1	*****	0	0	0	0	0	0	0	0	0	**1**	**1**
aluminum phosphide	8	n.r.	0	0	0	**8**	0	0	0	0	0	0	**1**
amitraz	4	**	0	0	**4**	0	0	0	0	0	0	0	**1**
avermectin	1	n.r.	**1**	0	**1**	0	0	0	0	0	0	0	**2**
buprofezin	25	*	0	**25**	0	0	0	0	0	0	0	0	**1**
carbofuran	6	****	0	**1**	0	**1**	0	0	0	0	0	**4**	**3**
cartap	1	***	0	0	**1**	0	0	0	0	0	0	0	**1**
chlorpyrifos	3	***	**1**	**1**	0	0	0	0	0	0	0	**1**	**3**
cyfluthrin	1	***	**1**	0	0	0	0	0	0	0	0	0	**1**
cypermethrin	7	***	**3**	**3**	0	0	0	0	0	0	**1**	0	**3**
diazinon	1	***	0	0	0	**1**	0	0	0	0	0	0	**1**
endosulfan	10	***	**1**	**4**	**5**	0	0	**1**	0	0	0	0	**4**
lambda cyhalothrin	3	***	**1**	**1**	**1**	0	0	0	**1**	0	0	0	**4**
lufenuron	5	n.r.	**2**	**3**	0	0	0	0	0	0	0	0	**2**
malathion	14	**	**1**	0	0	**13**	0	0	0	0	0	0	**2**
methamidophos	5	****	**2**	**1**	**2**	0	0	0	0	0	0	0	**3**
methomyl	5	****	0	**4**	0	0	0	**1**	0	0	0	0	**2**
permethrin	3	***	0	**2**	0	0	0	0	0	0	**1**	0	**2**
profenofos	6	***	**2**	0	**2**	**1**	0	**2**	0	0	**1**	0	**5**
thiocyclam hydrogen oxalate	8	***	**2**	**6**	**1**	0	0	0	0	0	0	0	**3**
Overall # A.I.s used to control problem			**15**	**16**	**10**	**8**	**10**	**11**	**8**	**6**	**7**	**7**	

† World Health Organization ratings for acute toxicity: * "unlikely to be hazardous," ** "slightly hazardous," *** "moderately hazardous," **** "highly hazardous," ***** "extremely hazardous," n.r. not rated by the WHO

Whitefly

Whitefly was the most commonly reported pest of beans. Ninety-six percent of farmers reported this pest as problematic in their bean crop, and 91% of the farmers considered it very damaging to bean yield. The names farmers used for whitefly are given in Table 30. It is most commonly referred to as *mosca blanca*, *palomilla*, or *mariposita*. Only three farmers were unfamiliar enough with whitefly that they did not have a name for it and one apparently confused it with spider mite, calling it *araña*. The vast majority of producers (91%) used some sort of chemical control for whitefly. The most frequently reported active ingredient for chemical control of whitefly was buprofezin. This chemical was used by 45% of the farmers interviewed, and unlike other frequently used products, buprofezin was used to treat only one production problem.

Weevil

Weevil was the second most commonly reported pest of beans. Seventy-five percent of farmers reported this pest as problematic in their bean crop, and 55% of the farmers considered it very damaging to bean yield. The names farmers used for weevil are given in Table 30. It is most frequently called *gorgojo*. Other names include *redondilla* or *polilla*. One farmer had no name for the weevil and one called it *pulgon*, a name typically used for aphids. The majority of producers (57%) used some sort of chemical control for weevil. The most frequently reported active ingredient for control of weevil, malathion, was used by 23% of the farmers interviewed to control the pest. Farmers added the chemical to harvested seed, and a few farmers reported using malathion on seed that they later consumed. Fourteen percent of the farmers interviewed used aluminum phosphide fumigation to control weevils in harvested seed and about 9% reported using a fungicidal seed treatment containing captan, carboxin and thiram to control weevils. Weevils were the only production problem of beans for which farmers reported using some sort of alternative method for control. Such controls were usually used for seed set aside for consumption, and included mixing ash, oil, or eucalyptus leaves with the seed, sunning harvested seed, and sorting seed. Thirteen percent of farmers used at least one of the alternative methods to control weevils.

Spider Mite

Spider mite was a problem in the bean crops of half of the farmers interviewed and 36% of the farmers considered mites very damaging to bean yield. The names farmers used for spider mites are given in Table 30. It is most commonly referred to as *araña* or *arañita roja*. Four farmers did not have a name for it. Thirty-eight percent of producers applied some sort of chemical control for spider mite. Farmers reported using ten different active ingredients to control spider mite, the most common of which were endosulfan, amitraz, methamidophos, and profenofos.

Leaf Miner

Leaf miners were identified as problematic by 46% of the farmers, but only 25% considered it very damaging to their bean crop. The names farmers used for leaf miner are given in Table 30. The most commonly used names were *minador* and *toston*. Thirty-one percent of the farmers who identified leaf miner as a problem did not have a name for it. A few farmers, who used the names *lancha* or *hongos*, apparently confused the damage caused by leaf miner with fungal pathogens. Forty percent of farmers reported using some sort of chemical control for leaf miner. Farmers reported using fifteen different active ingredients to control leaf miner. The most commonly used chemicals were cypermethrin, lufenuron, methamidophos, profenofos, and thiocyclam hydrogen oxalate.

Rust

Rust was the most commonly reported disease of beans. Eighty-five percent of farmers reported this disease as problematic in their bean crop, and 80% of the farmers considered it very damaging to bean yield. The names farmers used for rust are given in Table 30. It is almost universally known as *roya,* although the names *eumollador* and *royal* are also used. Five farmers did not have a name for rust. The vast majority of producers (84%) used some sort of chemical control for the disease. The most frequently reported active ingredients for control of rust were oxycarboxin and cyproconazole, although farmers reported using nine other chemicals as well, three of which were insecticides.

Root Rot

Fifty-nine percent of the farmers interviewed identified the photographs of root rot as a problem they had had in their bean crop although only 23% considered it very damaging. Some farmers recognized the disease as being caused by a fungal pathogen while others reported seeing root damage, similar to that in the photograph, which they believed was caused by an insect larva. The names farmers used for root rot are given in Table 30. The most common name for root rot was *pudrición de raiz.* Thirty-eight percent of farmers used some sort of chemical control for the disease. The most common control reported was use of a seed treatment containing captan, carboxin, and thiram. Farmers who correctly or incorrectly believed the root damage to their beans to be caused by an insect used carbofuran, aldicarb and chlorpyrifos to treat the problem.

Anthracnose

Fifty-two percent of the farmers interviewed identified the photographs of anthracnose as a problem in their bean crop, and 21% considered it very damaging. The many names farmers used for anthracnose are given in Table 30. The most commonly used names were *la gota* and *antracnosis.* Forty-one percent of the farmers interviewed used some sort of chemical control to treat anthracnose. The most frequently used active ingredients were mancozeb, propineb, and cymoxanil.

Mildew

Forty-eight percent of the farmers reported problems with mildew in their bean crop and 21% considered it very damaging. As shown in Table 30 *ceniza* was the most common name used for mildew. Some farmers apparently confused the photograph of mildew with damage caused by spider mites or whitefly, since they called the disease *mosca* or *acaros*. Thirty-six percent of the farmers used some sort of chemical control for mildew. The most commonly used active ingredient was sulfur.

Common Bacterial Blight (CBB)

Forty-eight percent of the farmers interviewed reported that they had seen damage similar to that caused by CBB in their bean fields, and 20% believed the problem to be very damaging. The twelve different names farmers used for the damage caused by CBB are given in Table 30. Twenty-nine percent of the farmers used pesticides to treat CBB, the most common of which were chlorothalonil, cymoxanil, and mancozeb.

Angular Leaf Spot (ALS)

Only 23% of the farmers interviewed identified ALS as a problem in their bean crop and only 2% considered it very damaging. Most farmers who reported seeing damage like that caused by ALS did not have a name for the disease. Those who did report a name called it by one of the generic terms for foliar diseases, *mancha*, *laucha* or *lancha*. One farmer called the disease *antracnosis*. Only 14% of the farmers used some sort of chemical control for ALS and each farmer used a different active ingredient.

Bean Common Mosaic Virus(BCMV)

Damage like that caused by BCMV was reported by 20% of the farmers interviewed and 5% considered it very damaging. Most farmers did not have a name for the problem. Those names which farmers did report are given in Table 30. Farmers did not report using any chemical control for the problem.

Pesticide Use

Overall, 98% of the farmers interviewed applying pesticides to their bean crop. As shown in Table 32, 59% of the farmers applied chemicals classified as “moderately hazardous” or “highly hazardous” by the WHO (IPCS-IOMC, 2000). Twenty-seven percent applied only pesticides rated “unlikely to be hazardous” or “slightly hazardous.”

Table 32. Classification of farmers by most hazardous pesticide level used on bean

Pesticide Use Category	# of Farmers	Percent of Farmers Interviewed
Most dangerous pesticide used is classified "Extremely Hazardous"	1	1.8
Most dangerous pesticide used is classified "Highly Hazardous"	13	23.2
Most dangerous pesticide used is classified "Moderately Hazardous"	20	35.7
Most dangerous pesticide used is classified "Slightly Hazardous"	12	21.4
Most dangerous pesticide used is classified "unlikely to be hazardous"	3	5.4
Did not recall names of pesticides used	6	10.7
Did not apply pesticides to bean	1	1.8

Farmers' Suggestions for Bean Variety Improvement

Farmers were asked what characteristics of their current bean varieties should be improved and they were asked to list the characteristics of a good bean variety. Farmers' responses to this question are summarized in Table 33.

Yield and disease resistance were the traits that farmers most often thought needed to be improved in their current varieties. Few farmers mentioned seed characteristics in their lists of traits to improve. However, 61% of the farmers mentioned traits related to seed quality as important in a good bean variety. This suggests that most farmers do not think the seed quality of their current varieties needs to be improved. However, since seed quality is important to farmers, new bean varieties must posses a seed quality similar to that of the varieties currently grown

Table 33 . Desirable bean variety characteristics identified by farmers

Desirable Characteristic **Categorized** *Specific*	**Cited for Improvement in Current Varieties** # Farmers	% Farmers	**Listed as Important for a Good Bean Variety** # Farmers	% Farmers
Yield Characteristics	**30**	**53.6**	**25**	**44.6**
high yield	30	53.6	24	42.9
long pods	1	1.8	1	1.8
Seed Characteristics	**9**	**16.1**	**34**	**60.7**
heavy seed	4	7.1	23	41.1
seed color	0	0.0	15	26.8
shiny seed	1	1.8	7	12.5
"quality" seed	4	7.1	0	0.0
Plant Characteristics	**24**	**42.9**	**29**	**51.8**
disease resistant/healthy	20	35.7	11	19.6
large plant	2	3.6	7	12.5
vigorous/abundant foliage	0	0.0	15	26.8
abundant flowers	2	3.6	2	3.6
locally adapted	1	1.8	2	3.6
drought resistant	1	1.8	0	0.0
Harvest Characteristics	**8**	**14.3**	**8**	**14.3**
early maturity	7	12.5	7	12.5
uniformity at harvest	1	1.8	1	1.8
Flavor	**1**	**1.8**	**3**	**5.4**
Marketability	**6**	**10.7**	**7**	**12.5**

Introduction of New Bean Varieties

When asked how they hear about new bean varieties, 23% of farmers responded that they got such information from an institution or training program. The remainder of the farmers heard about new varieties from friends or neighbors (43%) and at markets from intermediaries or vendors (46%).

Farmers were also asked where they would go if they wanted to obtain seed of a new variety. Over half of the farmers (52%) said they would go to the markets to get seed of a new variety, another 11% said they would go to other farmers in the community. One quarter said they would go to an institution or non-governmental organization (NGO). Sixteen percent mentioned INIAP in particular, and 9% named other institutions (such as the Ecuadorian Ministry of Agriculture) or NGOs. Five percent of the farmers said they did not know where to get seed of new bean varieties.

Discussion

Farmer Demographics and Agricultural Systems

Most of the bean growers interviewed were 35 or older, had only a primary school education, and were members of households of five or more people. Although almost all of the farmers were land owners, most owned one hectare of land or less. Such data support the observation that most of the bean growers in the Mira and Chota valleys are practicing small scale, labor intensive agriculture, as farm sizes are small and household size is large.

The majority of the farmers reported growing only two or three different crops within the past two years. However, the diversity of crops grown in the region was quite large (Table 26) with 19 different crops reported in addition to bean. Some of this diversity is probably due to the different microclimates present in the Mira and Chota Valleys. Sugar cane and cassava, for example, were grown in lower elevation communities, while carrot, pea, and potato were grown at somewhat higher elevations.

Almost all of the crops that farmers reported growing are unquestionably cash crops (i.e. tomato, sugar cane, pepper, anise) and not subsistence crops. Only a few crops could serve as either cash or subsistence crops, specifically bean, maize, cassava, potato, pigeon pea, and camote. It seems that farmers use their limited land to grow only a few labor intensive cash crops. The fact that these farmers are not subsistence farmers, but are instead small-scale producers of labor intensive fruit and vegetable crops means that prices and markets of the crops they grow have a substantial impact on their livelihoods. Beans are uniquely suited to this type of system since they bring a good market price, yet could sustain the farmers and their families nutritionally if prices dropped so low as to make selling the crop unprofitable. Some of the other cash crops that the farmers frequently grow, such as tomato, peppers, sugar cane, and onion, do not afford this potential. The fact that beans are such an important part of the diets of the producers in the Mira and Chota valleys reinforces their dual value to farmers in this type of agricultural system.

Farmers' Variety Choices in the Mira and Chota Valleys

Previous surveys of bean growers in the Chota Valley reported that farmers were primarily growing the Calima classes for export to Colombia, with the exception of some smaller farmers who grew Panamito type beans. In the survey reported here, however, farmers most frequently reported growing medium red mottle and black beans. The data presented in Figure 17 reveal that Calima Rojo has very low recent adoption rates compared to the other important classes. In comparison, a high percent of the farmers growing the Black, Panamito, Matahambre, Canario and Bayo Blanco classes have adopted them recently. Although these are classes that have historically been grown in the region, they have not been the farmers' choice for commercialization. The farmers' comments and the survey results suggest that since exports to Colombia have been halted some bean producers are dropping the Calima Rojo class in favor of classes that have a domestic market. Classes such as MRM, Blanco de Leche, and Calima Negro, which have been grown as cash crops in the past, continue to be grown because they have a domestic market. Finally, the trend toward growing some of the previously minor classes for sale is probably not exclusive to small-scale producers in the region. Although primarily small farmers were interviewed, the producer with the largest area planted to bean (10 ha) was growing Canario, Bayo Blanco, Matahambre, and MRM beans.

Prices and Marketing

The prices that farmers reported receiving for their most recent bean harvest validate their move away from Calima Rojo. The highest prices that farmers reported for dry beans, $40/quintal, were for MRM and Calima Rojo (Figure 16). However, Calima Rojo also brought the lowest price reported for dry beans -- $8/quintal. Calima Rojo's variable price is probably an important part of farmers' decision to grow other classes. The prices reported for Calima Negro and MRM remained more consistently high, and this may explain why these two classes have more recent adopters than Calima Rojo.

On average Black beans brought lower prices than the Calima and MRM classes, but the prices were more reliable than that of Calima Rojo. Additionally, farmers reported that black beans are more disease resistant and yield better than the Calima classes, and this may make them equally, if not more, profitable than Calima. Like black beans, Panamito and Matahambre classes brought, on average, lower but stable prices. The solid red landrace grown by one farmer brought a very low price; probably because there is little market for this class.

Among the varieties sold *in tierno* Blanco de Leche, which is grown specifically for sale during Holy Week, brought the highest price. This explains why farmers risk growing the class when the window of time for marketing it is so short. However, to minimize risk they plant limited amounts of the variety. In the case of the MRM class, farmers reported that they decided whether to sell their crop *in tierno* or dry depending on current market prices. If *tierno* prices are good it is desirable to harvest the crop so that the next cycle of cropping can begin sooner. However, if *tierno* prices are low farmers can wait and hope to make more money on the dry seed. The option of selling MRM at two different stages may make the class particularly appealing to farmers since it offers some flexibility and protection against low prices.

As expected among producers growing a crop for sale, farmers' decision of which bean classes to grow seem to be based in part on the price that the class will bring and the stability of the price. Calima Rojo, a class with few recent adopters, is also the class with the most variable price. The most frequently cultivated dry bean classes, MRM, Blacks, and Calima Negro, are those with stable, acceptable prices.

INIAP bean breeders should consider that farmers' bean variety choices are based at least in part on the marketability of the class, and that farmers in the Mira and Chota valleys are changing the classes they grow as a result of changes in the market. It is important to include in the PRONALEG breeding program some of the previously minor classes, which are gaining importance. Some of the classes are basically landraces and have received little attention from breeders.

The interviewed farmers marketed beans to vendors in the city of Ibarra and the town of Pimanpiro, or through intermediaries who came to the farmers' communities. Since none of the farmers reported selling beans directly to the consumer, farmers may not have a good idea of what class and quality of bean is most marketable. Consequently, participatory bean breeding efforts would benefit from involvement of bean intermediaries or vendors, who will be knowledgeable about the local domestic market and its quality requirements.

Farmers' Seed Sources

The majority of the farmers interviewed did not plant seed that they had grown and saved. This finding is surprising considering that small-scale farmers are usually characterized as saving their own seed. Although farmers were not specifically asked why they decided to save their own seed or to buy it, there are several reasons why farmers might choose to buy seed. Some farmers commented that they thought it was good to plant seed that was grown in a different climate (i.e. a farmer living in a warmer, low elevation community should plant seed grown at a higher elevation and vice versa). Farmers thought that this exchange of seed resulted in healthier plants. Another possible reason is that farmers' stored seed may be damaged by weevils. (Some farmers reported that their weevil control method is to sell the whole harvest as soon as possible.) If a farmer does not grow beans each season, it may be desirable to purchase fresher, higher quality seed that has just been harvested. Although the high incidence of farmers purchasing seed could be a product of so many farmers switching bean classes, this is probably not the only explanation. Classes with a lower percent of recent adopters, MRM and Calima Negro, still had a majority of farmers using purchased seed.

The exceptions to the trend of purchased seed were the solid red, Musgo, and Blanco de Leche classes. The solid red and Musgo beans can be characterized as noncommercial, farmer-maintained landraces, which explains why farmers saved their own seed of these classes. Blanco de Leche, in contrast, is grown as a cash crop but unlike the other commercial classes, most farmers growing it maintained their own seed over the long term. This may be because production of the class is localized in two neighboring communities, and seed of the variety is not readily available in the markets -- especially since it is always sold *in tierno*. One of the farmers growing Blanco de Leche commented that seed of the class is very expensive.

The long-term success of PCI can hinge on whether or not farmers save their own seed. In a participatory maize breeding project in Honduras, half of the participating farmers switched to different varieties and discontinued their work with the project over four cycles of selection. The breeders concluded that "For areas where farmers are accustomed to switching varieties or 'refreshing' their seed from outside sources, even if infrequently, PPB [participatory plant breeding] may not be appropriate due to its long term nature (Smith et al., 2001)." Whether farmers select a variety through trials of advanced lines or participate in the development of a variety through selection in earlier generations, the impact of the improved variety and its usefulness to farmers depends on their access to seed of the variety year after year.

Farmers in the Mira and Chota valleys are without a formal seed system and for the most part rely on the grain markets for seed. Consequently the purchased seed that they plant is often a mixture of varieties that produce the same seed type. A study of pigeonpea in Kenya demonstrated that a variety's integrity can be maintained in such a system if it is distinctive. The particular pigeonpea variety tracked in the study was easily distinguished from local varieties by its white seeds and determinant growth habit (Jones et al., 2001). Since a difference in seed appearance is part of what allowed the pigeonpea variety to be maintained in the informal seed system, pure seed of a new variety would not likely be maintained if the seed closely resembled existing varieties. In the case of beans in Ecuador seed appearance determines marketability. Consequently the characteristic shape, size, pattern, and color of the existing commercial classes must be maintained in new varieties if they are to be marketable. Without a major change in the current seed system, the best way for farmers to assure themselves access to seed of a preferred improved variety is to plant seed they have grown themselves.

Farmers' frequent seed purchases and the current bean seed system in this region of Ecuador are, for the most part, not compatible with the PCI approach. The exception is the farmers growing and maintaining the Blanco de Leche class. This class should be considered as a candidate for improvement through PPB. Furthermore, the reasons for farmers' seed saving choices should be investigated, and any problems that lead farmers to purchase seed (i.e. weevil infestation) should be addressed.

Farmers' Production Problems in the Mira and Chota Valleys

Whitefly

Whitefly was the most frequently reported pest problem of bean in the Mira and Chota valleys. The vast majority of farmers are familiar with the whitefly, as they called the pest by one of four distinctive common names: *mosca blanca, palomilla*, *mariposita*, or *mariposa blanca.* Its prevalence and negative impact are evidenced by the fact that almost all farmers were familiar with the pest and considered it very damaging to bean yield (Figure 18). Although almost all the farmers applied some sort of chemical control for whitefly, many expressed frustration in trying to control the pest. There is evidence that whitefly in the region has already developed resistance to some pesticides, as a CIAT affiliated group of researchers working in the Chota valley reported finding whiteflys that were resistant to cypermethrin, methamidophos, and methomyl. Almost half of the farmers interviewed reported using buprofezin to control whitefly. This finding is encouraging since buprofezin is very safe (Table 30). For effective control of whitefly, however, it must be applied at a precise stage in the insect's life cycle. The aforementioned group was working to teach farmers to scout their fields for whitefly larvae and apply buprofezin at the critical time. Such efforts are imperative if farmers are to learn to safely manage this pest. Any Bean/Cowpea CRSP supported work regarding whitefly control should be planned with consideration to the research that has already been done in this area by CIAT in order to avoid a duplication of previous work.

Weevil

Weevils were the second most frequently reported pest of bean. Farmers were familiar with weevils as evidenced by the fact that most called them by one of three commonly used names for the pest: *gorgojo*, *redondilla*, or *polilla*. Farmers primarily used malathion or the fumigant aluminum phosphide to control weevil in stored seed. Disturbingly, a few farmers reported applying malathion to seed they later consumed. Although malathion has a low oral toxicity, this is probably not the safest way to control weevils in beans for consumption. Weevil was the only pest or disease for which farmers reported using some sort of non-pesticide control. Some of the non-pesticide controls farmers use, such as mixing stored seed with vegetable oil or ash, have been reported elsewhere as effective controls for the pest (Cardona, 1989).

Although practical controls for weevils exist, over half the farmers still considered the pest very damaging to bean yield, suggesting that it is not being adequately controlled. This pest problem should be addressed as a part of the Bean and Cowpea CRSP sponsored PCI initiative, since the pest may prevent farmers from saving their own seed and farmer seed saving is vital to the success of PCI. A first step would be to inform farmers of the available control options and possibly test them as a part of the CIAL activities. A suggested second step is to consider implementation of genetic resistance.

Seven different *Arc* gene alleles that encode the arcelin protein, which confers resistance to the Mexican bean weevil *Zabrotes subfasciatus*, have been characterized. It has been reported that the weevil pest in the Mira and Chota Valleys is *Acanthoscelides obtectus*. The *Arc* genes are not as effective against this species. However, effective control of *A. obtectus* has been obtained using a combination of arcelin-enriched bean varieties and the parasitoid *Dinarmus basilis* (Schmale et al., 2003).

Spider Mite and Leaf Miner

Spider mites and leaf miners are not as common as weevil and whitefly, and not considered as damaging to bean yield by farmers. Farmers were more familiar with spider mites than they were with leaf miners. Most of the farmers who reported mite problems in beans called them by one of three commonly used names: *araña roja*, *arañita roja*, or *arañita*. In contrast, a number of farmers did not have a name for leaf miner or confused it with fungal pathogens. Although only a quarter of farmers considered leaf miners very damaging to bean yield, forty percent reported using a chemical control for the pest. Leaf miners are generally considered to have little economic impact on bean yield making chemical control unwarranted (Schwartz et al., 1978). The farmers who apply pesticides to control leaf miner probably do so unnecessarily.

Rust

Rust was the most commonly reported disease of bean. Most of the farmers are familiar with rust and call it *roya*. Beside whitefly, rust is the other major production problem that farmers applied pesticides to control. Most farmers used products specifically recommended for rust: oxycarboxin and cyproconazole. Although a few farmers reported choosing to grow rust resistant varieties or classes, most farmers used fungicides to control the disease. PRONALEG's current CRSP sponsored work to develop rust resistant bean varieties for the Mira and Chota valleys could be very beneficial to farmers by reducing production costs and pesticide exposure.

Root Rot

Although over half of the farmers identified the photograph of root rot as a picture of something that is a problem in their bean fields it is uncertain that all such farmers were actually experiencing a problem caused by fungal root rot pathogens; particularly since some of the farmers identified the picture as damage caused by an insect larva that bores into the plant's stem. Despite this potential confusion, it is likely that some of the farmers are experiencing root rot infestation in their bean fields, since over half of those reporting the problem identified it as a fungal problem.

Root rot is controlled most effectively by cultural practices, particularly crop rotation, and potentially by use of resistant varieties. The seed treatment containing captan, carboxin, and thiram that many farmers reported using protects bean plants against death (damping off) from the root rot pathogens as seedlings, and would allow the plant to establish itself. However, this treatment would not continue to protect the mature plant's roots against root rot pathogens throughout the growing season.

A survey of fungal pathogens and root attacking pests in bean fields in the Mira and Chota valleys is necessary to determine the true prevalence of the problem and to identify the actual pests or pathogens present. Root rot resistant varieties may be the best long-term solution to root pathogens in this region. Crop rotation may also be a viable solution to both pest and fungal problems but depends on the practicality of the rotations on such small, intensively cultivated farms. If the root problems are primarily caused by insect larvae, farmers would benefit from recommendation of chemical control options that are less toxic than carbofuran and aldicarb, which some reported using to treat root problems (Table 30).

Anthracnose

About half of the farmers interviewed identified anthracnose as a problem in their bean crop. Farmers had many names for anthracnose, some of which were specific to the disease and others which were generic names for foliar diseases. The symptoms of angular leaf spot (ALS) are similar to those of anthracnose. However, the difference in frequency with which ALS and anthracnose were reported suggests that farmers differentiate between the two. With the exception of two cases, farmers used the most common specific names for anthracnose, *la gota* and *antracnosis*, only when referring to this disease.

Although many farmers reported anthracnose in their fields, less than a quarter considered it very damaging. Farmers probably consider anthracnose less damaging because its occurrence is intermittent and its severity depends on the presence of cool, wet environmental conditions, which are somewhat rare in this region. Nevertheless, many of the farmers interviewed used fungicides to treat the disease. If anthracnose is truly not very damaging, this may be another example of farmers applying pesticides unnecessarily.

Farmers in the Mira and Chota Valleys, particularly those growing at higher elevations where anthracnose is more common, could benefit from anthracnose resistant varieties. Such varieties would protect farmers from any losses that do result from the disease and would prevent unnecessary fungicide applications prompted by the appearance of diseased plants.

Mildew

Nearly half the farmers interviewed reported mildew as a problem in their bean crop but less than one quarter considered it very damaging. Most of the farmers who had experienced problems with the disease identified it by its common name, *ceniza*. The most commonly reported control for mildew, sulfur, is fairly safe and considered effective against the disease. Mildew is not a problem for most bean growers, and those who do see the disease in their fields control it effectively.

Common Bacterial Blight (CBB)

Almost half of the farmers reported seeing damage like that caused by CBB in their bean fields. However, as evidenced by the names given for the disease, there exists some confusion over what causes this damage. A few farmers identified CBB as a bacterial disease (*bacteria*, *bacterosis*) or gave it a unique name different than other foliar diseases (*lancha amarilla*). It is assumed that these few farmers were familiar with the disease. Other farmers called CBB *laucha* or *lancha*, which are unspecific names used for foliar diseases. Some farmers thought that the damage had been caused by freezing (*helada*) and others thought the damage was from insects (*minador, toston*, and *mosca*). It is unclear if farmers did actually have CBB in their fields or not.

Since very few farmers were familiar with CBB and only a few applied any pesticide to treat the problem, it is unlikely that CBB is currently a major bean production problem in this region. If the disease is present, however, it could be easily spread through infected seed. Farmers are probably unprepared to recognize or treat this disease since so few identified it correctly and none of the fungicides or insecticides that they reported applying for CBB are likely to control it.

Angular Leaf Spot(ALS)

Few farmers reported ALS as a problem in their bean fields and very few considered it damaging. As mentioned earlier, it seems that farmers are able to distinguish between ALS and anthracnose. None of the farmers gave the disease a distinctive name and few reported applying a chemical control for it. These data suggest that ALS is, at most, a minor bean production problem in this region of Ecuador.

Bean Common Mosaic Virus (BCMV)

A few farmers reported damage like that caused by BCMV and very few considered it damaging to yield. None of the farmers who reported BCMV-like symptoms in their bean plants identified the problem as a virus, which may indicate that the disease is not a problem in the region. Some thought the damage was caused by insects (*toston*, *enrollador*) and one identified the problem as nutrient deficiencies. Some of the reported instances could be cases of a physiological problem that is common in the variety Paragachi. BCMV is probably not a problem in this part of Ecuador, and the whitefly species present in the region reportedly does not vector the disease.

Pesticide Use Among Bean Producers in the Mira and Chota Valleys

Previous studies have found pesticide use among small-scale Ecuadorian farmers to be quite high. A study in the northern Ecuadorian province of Carchi (the Chota

Valley is in this same province) found that small-scale potato producers made frequent applications of a variety of pesticides. They also found that some pesticides that are quite dangerous, notably carbofuran and methamidophos, are in common use (Crissman et al., 1998). Pesticide use among bean growers in the Chota Valley is characterized as high in the report of the 2000 PRONALEG survey. According to this study, the most commonly used fungicides were mancozeb, cyproconazole, sulfur and maneb and the most common insecticides were methamidophos, profenofos, and cypermethrin. In the recent participatory rural appraisal done in the Mira Valley the most commonly reported pesticides were: mancozeb, maneb, sulfur, propineb, chlorothalonil, malathion, methamidophos, profenofos, cypermethrin, carbofuran and lambda cyhalothrin. The most dangerous among these are the insecticides, methamidophos, cypermethrin, and carbofuran.

Concurrent with previous findings, practically all of the farmers interviewed for this study applied pesticides to their bean crop. However, the product which farmers most frequently reported using, buprofezin, did not appear in any of the earlier surveys. As noted previously, farmers used buprofezin only to treat whitefly. It is much safer than the other insecticides used to control the pest, but requires precise timing of application. The widespread use of buprofezin is surprising, but encouraging, since it suggests that farmers are choosing less toxic alternatives to some of the products they had been using. Similarly, oxycarboxin, a fungicide used to treat rust, does not appear in the reports from previous surveys but was used by one third of the farmers interviewed in this study. According to the World Health Organization (WHO) pesticide ratings for acute toxicity oxycarboxin is safer than the other commonly used rust fungicide, cyproconazole.

Despite some indication that farmers are choosing to use less toxic pesticides, many are still using the insecticides carbofuran, methamidophos and methomyl, which are classified as "highly hazardous" by the WHO (Table 30). The observation that methomyl, and methamidophos were commonly reported for the control of leaf miner and whitefly, suggests that application of these pesticides is, in most cases, unjustified. As noted previously, leaf miner is not an economically important pest and whitefly has already developed resistance to these chemicals in some areas of the Chota Valley. Likewise, most use of carbofuran is probably unnecessary. Farmers most commonly reported using this insecticide for the control of root rot. If the problem that these farmers were experiencing was indeed fungal, carbofuran will be of little use. Although nearly all of the small-scale bean growers in the Mira and Chota Valleys continue to use pesticides some are using them more safely (and possibly more effectively) than others.

As indicated by the number of farmers using pesticides, bean producers in the Mira and Chota Valleys are willing and able to spend money on chemical control, probably because most of them sell a part of their bean harvest depend it as a source of income. Consequently, chemical inputs should not be excluded as an option for recommended bean management practices in this region. Farmers would benefit from straight-forward instruction in safe pesticide use, identification of pests and diseases in bean, and recommended control methods.

Farmers' Recommendations for Variety Improvement

According to farmers, yield and disease resistance are the two traits that most need improvement in their current varieties (Table 32). Therefore, the goals of the, Bean/Cowpea CRSP supported project, to improve yield and disease resistance fit well with farmers' needs. Although not many farmers thought that seed characteristics needed improvement, most farmers mentioned at least one seed characteristic when describing a good bean variety. This indicates that most farmers are satisfied with the seed quality of their current varieties, but new varieties must possess similar seed quality in order to be acceptable.

The farmers' preference for earliness (ready for harvest in 3 months) is contradictory to the recommendations to increase yield and plant size. It is necessary to clarify farmers' priorities regarding these traits. An economic analysis of the potential benefits and drawbacks of varieties with shorter cycles and lower yields versus those with longer cycles and higher yields in this agricultural system could help to elucidate the best combination of traits.

Conclusions

The objective of this study was to learn about the production practices, problems and variety preferences of bean growers in the Mira and Chota Valleys in northern Ecuador, with the purpose of making recommendations for developing improved varieties for the region. In the Mira and Chota valleys beans are important part of the diet and an important source of income. Most of the bean producers are intensively cultivating a few cash crops on small landholdings of less than 1 ha.

Although farmers in the Mira and Chota Valleys traditionally grew Calima type beans for export to Colombia, it appears that bean growers are responding to the loss of this market by growing different bean classes which are consumed domestically. In this survey the most widely cultivated bean class in this region was medium red mottle, with black beans as the second most common class. Other classes that farmers are adopting include Calima Negro, Panamito, Canario and Bayo Blanco. If export to Colombia remains an impossibility the aforementioned classes, in addition to MRM and small black beans, merit more attention from plant breeders than Calima Rojo. Improved Central American black bean germplasm from CIAT and the Michigan State University bean breeding program should be tested in Northern Ecuador and integrated into the PRONALEG-GA bean breeding program.

Unexpectedly growers tended not to save their own seed of the beans they grew, but rather purchased it. This practice may be incompatible with the participatory crop improvement (PCI) methodology being implemented in the area, since farmers will not maintain the selected lines or varieties themselves over the long term.

The problem using PCI and releasing new varieties in an area where farmers do not maintain their own seed should be addressed from several different angles. The question of why farmers purchase seed warrants further investigation and any problems which prevent them from saving their own seed (i.e. weevil infestation) should be addressed. PRONALEG-GA could try restricting their PCI efforts to farmers who maintain their own seed. This approach may be difficult, since so few farmers saved seed over the long term. Perhaps the most effective solution would be for PRONALEG-GA to collaborate with informal seed vendors and cooperatives that are operating in this region. At least one cooperative sold bean seed to farmers and then purchased the ensuing harvest. If the cooperative provided farmers with seed of an improved variety this would be an ideal way for PRONALEG-GA to release a new variety in this region. The cooperative would be able to maintain the new vareity reliably, by reselling some of the seed purchased from farmers to whom they had provided the improved variety.

Farmers' biggest bean production problems were whitefly and rust. Other pests and diseases of concern are anthracnose, root rot, and bean weevil. The problem of bean weevil should be addressed by characterization of the weevil species present in the two valleys followed by introgression of *Arc* genes for weevil resistance in to important commercial classes. The problem that farmers identified as root rot may be exacerbated or caused by insect pests. Further investigation is necessary to determine the cause and an appropriate control for the root problems that farmers are experiencing.

Although some farmers did report spider mites, leaf miner, and mildew these problems are probably not economically damaging. However, they do prompt farmers to apply pesticides unnecessarily. It has been noted in past surveys that farmers in this region tend to misuse pesticides. Concurrent with previous findings some of the farmers surveyed used highly dangerous pesticides while others used them unnecessarily or applied the wrong product. There are some indications, however, that farmer are choosing to use less hazardous pesticides and that, for the most common problems, they are applying an effective chemical. Since they get most of their pesticide information from chemical vendors, farmers would certainly benefit from current, straight-forward information or publications concerning pest and disease control practices. PRONALEG-GA should make it a priority to provide such information, particularly publications, to farmers.

Yield and disease resistance are the two traits that farmers think need to be improved in the varieties that they currently grow. This farmer recommendation fits well with the goals of the Bean/Cowpea CRSP project. Although they rarely cited seed traits for improvement, these characteristics are important to farmers. Therefore, any new varieties must maintain the seed quality of farmers' current varieties. In the absence of a formal seed certification and multiplication industry in Ecuador, PRONALEG-GA could take the approach of introducing improved varieties that deviate slightly from traditional varieties in seed shape, size or color. This would allow farmers to distinguish between seed of the improved variety and that of the traditional varieties. If such an approach is taken, bean breeders should consult with bean market intermediaries and vendors to make sure that the new variety maintains the marketability of the traditional varieties.

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APPENDICES

Appendix A: Results of Greenhouse Inoculations of Ecuadorian Recurrent Parents

Table A 1. Reaction of Ecuadorian recurrent parents to greenhouse inoculation with various isolates of *Colletotrichum lindemuthianum*

Parental Line	Race 0[a]	Race 2	Race 73
ARME-2	R[b]	S	S/R
Paragachi	S	S/R	R
ACE-1	R	R	R
Cocacho	S	S	--
Je.Ma.	--	R	--
Yunguilla	R	S	R
SEL 1308	R	R	R

a - the same Ecuadorian race 0 isolate that was used in field inoculations
b – R denotes resistant; S, susceptible; S/R segregating with some resistant and some susceptible plants

Appendix B: Questionnaire Used in Farmer Survey

Figure B 1. Spanish language questionnaire as it was used in farmer surveys

Preguntas Acerca de Tenencia de Tierra

¿Qué superficie de tierra posee? __________

¿Cuanta superficie está cultivada? __________

Dibuje un mapa de la propiedad que cultiva.

Lote #	¿Qué superficie tiene?	¿Cuál de estas cuatro líneas parece a la ladera de este lote?	¿Qué cultivos ha sembrado en este lote en los dos últimos años?	
			1. Fréjol *2. Tomate* *3. Yuca* *4. Pimiento* *5. Maíz* *6. Ají*	*7. Plátano* *8. Camote* *9. Hortalizas* *10. Caña* *11. Pastos* *12. Otros*

Variedades de Fréjol

Variedades de fréjol sembradas el último año.-

¿Tiene usted alguna semilla de estas variedades que pueda mostrar?

Nombre de Variedad	¿Cuántos años ha cultivado usted esta variedad?	¿Cuándo (en qué meses) sembró usted esta variedad?	¿Cuánta semilla sembró (cada ciclo)?	¿Cómo consiguió la semilla de esta variedad que sembró el año pasado? *Si es propia* ¿Hace cuántos años compró la semilla de esta variedad?		¿Qué hábito de crecimiento tiene esta variedad?	¿Cuál de estas semillas se parece más en tamaño?	¿Cuál es el color de la semilla de esta variedad?	¿Cómo se consume esta variedad?
1									
2									
3									
4									
5									
6									
7									
8									

	¿Consumió la variedad el año pasado?	¿Vendió usted esta variedad el año pasado?	¿Cuánto vendió?	Qué precio recibió?	¿Dónde vendió?	¿Qué le gusta acerca de esta variedad?	¿Qué no le gusta acerca de esta variedad?	Ordene las variedades de mejor a peor.
1								
2								
3								
4								
5								
6								
7								

En el año pasado. ¿Cultivó un mezcla de variedades de fréjol en el mismo lote? Sí / No

Si responde sí ¿Qué variedades cultivó? ______________________________

El año pasado, ¿Cultivó usted fréjol junto con otro cultivo? Sí / No

Si responde sí ¿Qué variedades de fréjol cultivó en este modo? ______________________________

Preguntas Acerca de Problemas de Producción

¿Cuáles son los problemas de producción de fréjol más importantes en su propiedad?

____

En los dos últimos años. ¿Usted vio a alguna plaga de fréjol que se asemeje a una de estas fotos?

Carta #	¿Cómo se llama esta plaga?	¿Usted controla esta plaga? *Si responde sí* ¿Cómo controla?	En su opinión, esta plaga: 1. Causa mucho daño a la cosecha de fréjol 2. Causa daño moderado a la cosecha de fréjol 3. No daña la cosecha de fréjol

En los dos últimos años. ¿Usted vio alguna enfermedad de fréjol que se asemeje a una de estas fotos?

Carta #	¿Cómo se llama esta enfermedad?	¿Usted controla esta enfermedad? *Si responde sí* ¿Cómo controla?	En su opinión, esta enfermedad: (1)Causa mucho daño a la cosecha de fréjol (2)Causa daño moderado a la cosecha de fréjol (3)No daña la cosecha de fréjol	¿Conoce a una variedad de fréjol que tenga resistencia a esta enfermedad? *Si responde sí* ¿Cómo se llama esta variedad con resistencia?

Preguntas Acerca de las Preferencias para las Nuevas Variedades de Fréjol

¿Cuáles son las características más importantes de una variedad de fréjol?

¿Qué características quisiera que se mejore en las variedades que cultiva?

¿Cómo se entera de las nuevas variedades de fréjol?

¿Adónde iría usted para obtener una nueva variedad de fréjol?

Si sembra una variedad de fréjol por la primera vez, ¿cuánto semilla quisiera sembrar?

¿Cuántos años tiene? ________

¿Qué nivel de enseñanza ha tenido usted? *1. Ninguno* *2. Alfabetización* *3. Preprimario* *4. Primario* *5. Secundario* *6. Superior*

Género (por observación): *Hombre* *Mujer*

Cuántas personas conforman su familia?______

Con qué frecuencia consume fréjol?
Diariamente (cuántas veces)?_______Semanalmente (cuántas veces)?_______

Qué cantidad de fréjol prepara para cada comida?_________

Figure B 2. English translation of questionnaire used in farmer surveys

Questions About the Farm

How much land do you own? __________

How much of the land is cultivated? __________

Draw a map of the land that you cultivate.

Field #	What is the area of this field?	Which of these four lines looks like the slope of this field?	What crops have you planted in this field in the past two years?	
			1.Bean *2. Tomato* *3. Yuca* *4. Sweet pepper* *5. Corn* *6. Hot pepper*	*7. Bannana* *8. Camote* *9. Vegetables* *10. Sugar cane* *11. Pasture* *12.Other*

Bean Varieties

Varieties of bean planted in the past year-

Do you have some seed of these varieties that you can show me?

Name of the variety	How many years have you grown this variety?	When (in what month) do you sow this vareity ?	How much seed do you sow (at each planting)?	Where did you get the seed of this variety for planting last year? *If seed was saved* How many years since you bought seed of this variety?		What is this variety's growth habit?	Which of these seeds resembles this vareity in size?	What color is the seed of this variety?	How is this variety consumed ?
1									
2									
3									
4									
5									
6									
7									
8									

	Did you consume this vareity last year?	Did you sell this variety last year?	How much did you sell?	What price did you receive?	Where did you sell it?	What do you like about this variety?	What don't you like about this vareity?	Rank the vareities from best to worst.
1								
2								
3								
4								
5								
6								
7								
8								

In the last year did you grow a mixture of bean varieties in the same field? Yes / No

If Yes What vareites did you grow in a mixture? ______________________________

In the last year did you grow beans together with another crop? Yes / No

*If yes*What bean vareites did you grow this way? ______________________________

Questions About Production Problems

What are the most important bean production problems on your farm?

In the past two years have you seen an insect in your beans that looks like one of these photos?

Card #	What do you call this insect?	Do you treat for this insect? *If yes* How?	In your opinion, this insect: 4. Causes much damage to the bean yield 5. Causes moderate damage to the bean yield 6. Does not harm bean yield

In the past two years have you seen a bean disease that looks like one of these photos?

Card #	what do you call this disease?	Do you treat this disease? *If yes* How?	In your opinion, this disease: 1. Causes much damage to the bean yield 2. Causes moderate damage to the bean yield 3. Does not harm bean yield	Do you know of a vareity of bean that has resistance to this diesase? *If yes* What is the name of this resistant variety?

Questions About Preferences for New Bean Vareities

What are the most important characteristics for a bean vareity?

What characteristics do you want in a bean variety which your current varieties don't have?

How do you hear about new bean varieties?

where would you go to obtain a new variety of bean?

If you grew a new bean vareity for the fist time, how much seed would you want to sow?

How old are you? ________

What level of education have you had? *1. None* *2. Alfabetización* *3. Preprimario* *4. Primario* *5. Secundario* *6. Superior*

Gender (observed): *Male* *Female*

How many people are in your family?______

How often do you eat beans?
Daily (How many times?)_______Weekly (How many times?)_______

What quantity of beans do you prepare for each meal?_________

Appendix C. List of Pesticides Farmers Reported Using and their Ecuadorian Trade Names

Table C 1. Fungicide products farmers reported using

Common Name (English)	Common Name (Spanish)	Commercial Name	Family
benomyl	benomil	Benomyl, Benlate, Pillarben	Benzimidazole
bupirimate	buprimato	Nimrod	Pyrimidine
captan	captan	Captan, VitaVax*	Thiophthalimide
carbendazim	carbendazin	Bavistin, Carbendazim	Benzimidazole
carboxin	carboxin	Vitavax*	Carboxamide
chlorothalonil	clorotalonil	Daconil, Fungil	Substituted benzene
copper oxychloride	oxicloruro de cobre	Sulcox	Inorganic copper
cymoxanil	cymoxanil	Curzate*, Fitoraz*	Unclassified
cyproconazole	cyproconazole	Alto 100	Azole
difenoconazole	difenoconazol	Score	Azole
folpet	folpet	Folpan	Thiophthalimide
mancozeb	mancozeb	Dithane, Mancozeb, Manzate, Triziman, Curzate*, Ridomil*, Sandofan*	Dithiocarbamate, inorganic zinc
maneb	maneb	Maneb	Dithiocarbamate
metalaxyl	metalaxil	Ridomil*	Xylylalanine
metiram	metiram	Polyram	Dithiocarbamate, inorganic zinc
myclobutanil	myclobutanil	Rally	Azole
oxadixyl	oxadixil	Sandofan*	Anilide
oxycarboxin	oxycarboxin	Plant Vax	Carboxamide
propiconazole	propiconazole	Torneo	Azole
propineb	propineb	Antracol, Fitoraz*	Dithiocarbamate, inorganic zinc
sulfur	azufre	Azufre, Cosan	Inorganic
thiram	thioram	Vitavax*	Dithiocarbamate

* product contains more than one active ingredient

Table C 2. Insecticide products farmers reported using

Common Name (English)	Common Name (Spanish)	Commercial Name	Family
aldicarb	aldicard	Temik	N-methyl carbamate
aluminum phosphide	fosfuro de alumino	Gastoxin	Inorganic
amitraz	amitraz	Mitac	Formadine
avermectin	abermectina	Avermectina	Botanical
buprofezin	buprofezim	Applaud	Unclassified
carbofuran	carbofuran	Furadan	N-methyl carbamate
cartap	cartap	Padan	Unclassified
chlorpyrifos	clorpirifos	Lorsban	Organophosphorus
cyfluthrin	ciflutrin	Bulldock	Pyrethroid
cypermethrin	cipermetrina	Cipermetrina, Master	Pyrethroid
diazinon	diazinon	Diazinon	Organophosphorus
endosulfan	endosulfan	Endosulfan, Palmarol, Thiodan, Thionex, Methofan*	Organochlorine
lambda cyhalothrin	lambda cihalotrina	Karate	Pyrethroid
lufenuron	lufenuron	Match	Benzoylurea
malathion	malathion	Malathion	Organophosphorus
methamidophos	metamidofos	Pillaron, Rector	Organophosphorus
methomyl	metomil	Methomex, Metomil, Methofan*	N-methyl carbamate
permethrin	permetrina	Permasec	Pyrethroid
profenofos	profenofos	Curacron	Organophosphorus
sulfur	azufre	Azufre, Cosan	Inorganic
thiocyclam hydrogen oxalate	thiocyclam-hydrogenoxalato	Evisect	Unclassified

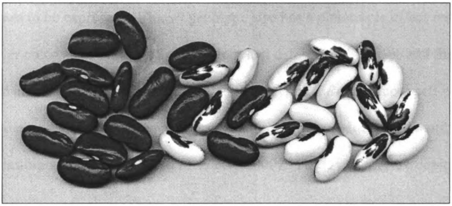
* product contains more than one active ingredient

Appendix D. Characterization of Redcoat, a Virgarcus Seed Coat Pattern Mutant of Red Hawk Dark Red Kidney Bean

Introduction

The soldier bean variety released with the name 'Redcoat' originated from a few off-type seeds found in 1999 in a Foundation Seed lot of 'Red Hawk,' a dark red kidney bean variety (Kelly et al., 1998). Unlike Red Hawk, which has totally colored seed, Redcoat possess white seed with a red virgarcus or soldier bean pattern on its ventral side (Figure D1). The only other observed phenotypic difference between the varieties is in flower color. Redcoat's flowers are pure white, while Red Hawk's flowers are white with pale red veins in the wing petals. Red veins in the flower are conferred by the recessive rk^d allele for red seed coat color.

Figure D 1. Seed of Red Hawk and Redcoat

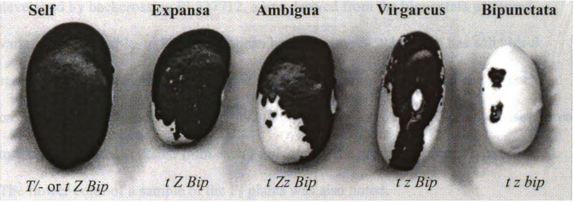


The circumstances of Redcoat's discovery aroused suspicions that it could be a seed coat pattern mutant of Red Hawk, and preliminary testing supported this possibility. When grown in the greenhouse in Winter 1999/2000, the few original Redcoat seeds produced progeny and seed that were true to type and did not segregate, indicating that the off type seeds were not the result of cross pollination. Additionally, virgarcus patterned beans are not grown commercially in Presque Isle County in Northeast

Michigan where Redcoat was first detected, making contamination of the Red Hawk seed lot with a commercial soldier bean variety unlikely. Finally, the reaction of Redcoat to inoculation with Bean Common Mosaic Necrosis Virus strain NL3 and *Colletotrichum lindemuthianum* races 7 and 73 was identical to the reaction of Red Hawk to these pathogens and different than the reactions of three other commercial soldier bean varieties. Red Hawk carries resistance genes (*Co-1*, *Co-2*, and *I*) that confer a known reaction to these pathogen isolates. Redcoat appeared to carry the same genes, which supported the theory that Redcoat is a seed coat pattern mutant of Red Hawk.

According to the work of Bassett (Bassett and McClean, 2000), the inheritance of partly colored seed coat patterns, like that of Redcoat, is controlled by at least five interacting loci: *T*, *Z*, *Bip*, *J*, and *Fib*. A dominant *T* allele results in totally colored (also called self-colored) seed, while the recessive genotype *t/t* allows the other seed coat pattern genes to be expressed. This *t/t* genotype also has a pleiotropic effect resulting in white flower color. The other seed coat pattern loci determine the shape and the extent of the colored area. With a dominant *J* allele, the genotype *t Z Bip* produces self-colored or expansa patterned seed; *t Z/z Bip* produces ambigua; *t z Bip* produces virgarcus; and *t z bip* produces bipunctata (Bassett and McClean, 2000). These patterns are depicted in Figure D2

Figure D 2. Seed coat patterns and genotypes described by Bassett and McClean (2000)



Since two different genotypes could confer Red Hawk's self-colored seed (*T/-* or *t Z Bip*), a mutation at either the T or Z locus in Red Hawk could result in the virgarcus pattern of Redcoat. This study was undertaken to elucidate Red Hawk's genotype and determine which gene in Red Hawk had mutated in order to produce the soldier pattern of Redcoat.

Materials and Methods

The seven crosses shown in Table D1 were made.

Table D 1. Crosses made between Redcoat and Red Hawk and the seed coat pattern tester lines

Female Parent		Pollen Parent
Redcoat	x	Red Hawk
		t self-colored BC_3 5-593
Redcoat	x	*t cl z g b v* virgarcus BC_3 5-593
		t z bip bipunctata BC_3 5-593
		t self-colored BC_3 5-593
Red Hawk	x	*t cl z g b v* virgarcus BC_3 5-593
		t z bip bipunctata BC_3 5-593

The lines *t* self-colored BC_3 5-593; *t cl z g b v* virgarcus BC_3 5-593; and *t z bip* bipunctata BC_3 5-593 are seed coat pattern tester stocks that were developed and provided by M. J. Bassett. The virgarcus and bipunctata patterned testers were developed by backcrossing PI 527712, a line derived from the bipunctata patterned variety Incomparable, to Florida dry bean breeding line 5-593, which has solid black seeds (Bassett, 1996). The self-colored tester line carrying *t* was developed by back crossing the variety Early Wax, to 5-593 (Bassett and Blom, 1991). For all crosses, three to four pods were harvested from each F_2 plant, and the seed coat pattern was recorded. The flower color of a sample of the F_2 plants was also noted.

Results and Discussion

Redcoat/Red Hawk Population

Of the 273 Redcoat/Red Hawk F_2 plants, 211 produced self-colored seed and 62 produced virgarcus patterned seed. This fits a segregation ratio of 3:1, self-colored to virgarcus (p=0.382) (Table D2). The 28 plants which were recorded as having white flowers with pink veins all produced self-colored seed, and the 9 plants which were recorded as having pure white flowers all produced virgarcus patterned seed. This suggests that Red Hawk carries a dominant gene conferring a self-colored seed coat, while Redcoat carries a recessive allele at this locus. When homozygous, the recessive allele in Redcoat, results in a virgarcus seed coat pattern, and has a pleiotropic effect, producing white flowers. These data support the genetic hypothesis that Red Hawk carries the dominant *T* allele, which, in this variety, masks other seed coat pattern genes that confer a virgarcus pattern (Figure D1). The dominant *T* allele in Red Hawk mutated to a recessive *t*, producing the virgarcus pattern and white flowers of Redcoat.

Table D 2. Test of fit to a ratio of 3 self-colored : 1 partly-colored in the Redcoat/Red Hawk, Red Hawk/virgarcus, and Red Hawk/bipunctata F_2 generations X^2 Goodness of Fit Test

Seed Coat Pattern	**RH/RC***		**RH/virgarcus**		**RH/bipunctata**	
	Observed	Expected	Observed	Expected	Observed	Expected
Self colored	211	204.75	110	122.25	63	63.75
Partly colored	62	68.25	53	40.75	22	21.25
X^2 (*p*-value)	**0.76**	(0.382)	**4.91**	(0.027)	**0.04**	(0.851)

* RH denotes Red Hawk; RC, Redcoat; virgarcus, *t cl z g b v* virgarcus BC_3 5-593; and bipunctata, *t z bip* bipunctata BC_3 5-593

Flower Color in Redcoat and Red Hawk by Seed Coat Pattern Tester F_2 Populations

The flower colors in the F_2 generation of the Red Hawk and Redcoat by seed coat pattern tester lines are given in Table D3. The tester lines are all white flowered since they carry the recessive *t* allele. However, the Red Hawk/self-colored and Red Hawk/ bipunctata F_2 populations included plants with violet, white, and red-veined white flowers. The Red Hawk/virgarcus F_2 plants had either white flowers or red-veined white flowers. This suggests that Red Hawk carries the dominant *T* allele, since violet flowers (conferred by *V*) would not be expressed if Red Hawk possessed *t*. Violet flowers were not present in the Red Hawk/virgarcus F_2 plants since the tester lines carries *v* and Red Hawk apparently does as well. There was no segregation for flower color in the Redcoat by seed coat pattern tester crosses, which supports the hypothesis that Redcoat carries *t*.

Table D 3. Flower Color in the Red Hawk and Redcoat by Seed Coat Pattern Tester F_2 Populations and Inferred Genotypes for Red Hawk and Redcoat

Flower Phenotypes and Genotypes	**Cross***					
	RH *T v* rk^d/			RC *t v* rk^d/		
	self *t V Rk*	vir *t v Rk*	bip *t V Rk*	self *t V Rk*	vir *t v Rk*	bip *t V Rk*
Violet *T/- V/-*	X		X			
White/Red Veins *T/- v/v* rk^d/rk^d	X	X	X			
Pure White *t/t*	X	X	X	X	X	X

* RH denotes Red Hawk; RC, Redcoat; self, *t* self BC_3 5-593; vir, *t cl z g b v* virgarcus BC_3 5-593; and bip, *t z bip* bipunctata BC_3 5-593

Red Hawk/self, Red Hawk/virgarcus, and Red Hawk/bipunctata Populations

The seed coat pattern frequencies in the Red Hawk and Redcoat by seed coat pattern tester line F_2 populations are given in Table D4 and Figure D3 depicts the seven seed coat patterns that were observed in the Red Hawk and Redcoat by seed coat pattern tester F_2 populations. Three distinct virgarcus patterns were observed in these populations: tester-like virgarcus, Redcoat-like virgarcus, and weak virgarcus.

Seed coat pattern frequencies in the Red Hawk/tester F_2 populations support the theory that Red Hawk carries *T*. If Red Hawk's self-colored seed coat were conferred by the genotype *t Z Bip*, no partly colored patterns other than expansa would be expected in the Red Hawk/self F_2 population, since the self-colored tester has this same genotype. However, a few individuals in the population expressed the ambigua and virgarcus patterns.

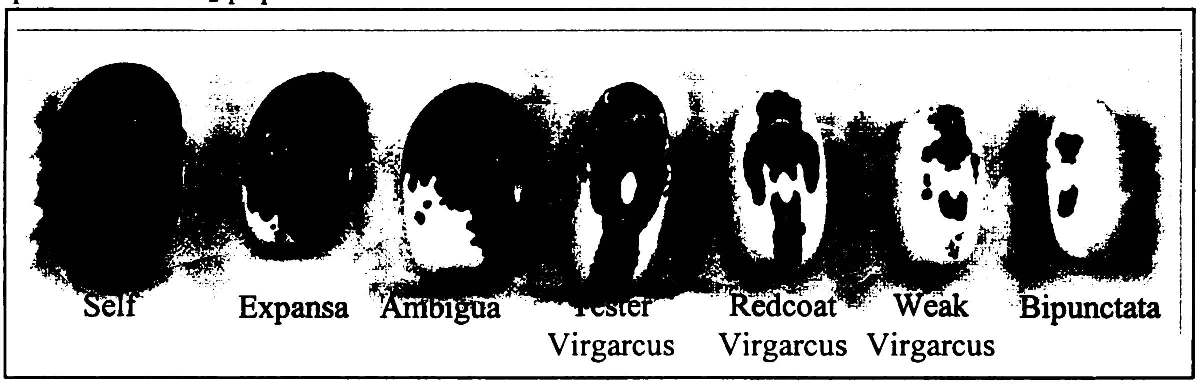
If Red Hawk carries *T*, there is an expected segregation ratio of 3 self-colored : 1 partly colored in the Red Hawk/virgarcus and Red Hawk/bipunctata F_2 populations. As shown in Table D2, the observed values for the RH/bipunctata population fit a 3:1 ratio but the data for the RH/virgarcus population, although close to this expected ratio, do not fit the expected ratio according to the X^2 goodness of fit test.

Table D 4. Seed Coat Pattern Frequency in the Red Hawk and Redcoat/Seed Coat Pattern Tester F_2 Populations

	Cross*					
Seed coat Pattern	RH/self	RH/vir	RH/bip	RC/self	RC/vir	RC/bip
Self	78	110	63	42	0	0
Expansa	4	0	0	36	0	0
Ambigua	0	17	5	26	27	0
Redcoat Virgarcus	3	11	9	13	31	58
Tester Virgarcus	2	20	0	6	24	0
Weak Virgarcus	1	5	5	5	13	40
Bipunctata	0	0	3	0	7	22
TOTAL	88	163	85	128	102	120

* RH denotes Red Hawk; RC, Redcoat; self, *t* self BC_3 5-593; vir, *t cl z g b v* virgarcus BC_3 5-593; and bip, *t z bip* bipunctata 5-593

Figure D 3. Seed coat patterns observed in the Red Hawk and Redcoat by seed coat pattern tester F_2 populations



Finally, the data in Table D4 show that Red Hawk carries the genes for Redcoat's virgarcus pattern cryptically since all of the Red Hawk/tester F_2 populations contained some plants expressing Redcoat-like virgarcus patterned seed.

Redcoat/self, Redcoat/virgarcus, and Redcoat/bipunctata Populations

The frequencies of the different seed coats patterns in the Redcoat/self, Redcoat/virgarcus and Redcoat/bipunctata F_2 populations are given in Table D4. The data from the Redcoat/virgarcus F_2 population suggest the alleles conferring Redcoat's virgarcus pattern are not the same as those carried by the virgarcus tester line, since plants in this population segregated for seed coat pattern. Unexpectedly, 27 plants expressed the ambigua pattern and seven plants expressed the bipunctata pattern from the RC/virgarcus cross. The seven bipunctata plants represent approximately 1/16 of the population, which suggests that two seed coat pattern genes are segregating.

Redcoat's seed coat pattern is different than that of the virgarcus tester. The colored area on seeds of the virgarcus tester completely encircles the hilum, while in seed of Redcoat the arcs of color, which extend from the caruncular end of the seed, do not join with the micropyle stripe (Figure D3). Since the virgarcus tester was derived from a cross between 5-593 and a plant with bipunctata patterned seed, rather than 5-593 and a plant expressing a virgarcus pattern, it is possible that this tester does not carry the same genes as a plant with the "classic" soldier or virgarcus pattern displayed by Redcoat. Plants expressing a tester-virgarcus pattern occurred in the Red Hawk/self, Red Hawk/virgarcus, Redcoat/self and Redcoat/virgarcus F_2 populations. This suggests that the *Bip* allele from 5-593 is required for the expression of tester-virgarcus, and that Redcoat and the tester differ at this locus.

My results from the virgarcus (Redcoat)/bipunctata cross are similar to those reported by Lamprecht (1940) for a virgarcus/bipunctata cross, and different than those obtained by Bassett (1996). Bassett (1996) observed a 3:1 segregation ratio for virgarcus:bipunctata in his virgarcus/bipunctata F_2 population. Lamprecht (1940), however, observed three different seed coat patterns segregating in the virgarcus/bipunctata F_2 population. The three patterns were virgarcus, bipunctata and an intermediate pattern which resembles my weak virgarcus class, in a 9:4:3 ratio. In order to explain his results Lamprecht proposed that the Bip locus interacts with alleles at an independent locus, which he called Arc. Redcoat (and Red Hawk) may carry different alleles than the tester lines at the Arc locus theorized by Lamprecht (1940).

Conclusions

Red Hawk carries the dominant *T* allele, which confers its self colored seed coat and masks the expression of other seed coat pattern genes that the variety carries. The *T* allele mutated to recessive *t* in Redcoat, resulting in a virgarcus patterned seed coat and suppressing the expression of red veins in the flowers. The genes conferring Redcoat's virgarcus pattern are different than those of the virgarcus tester. It is proposed that Redcoat (Red Hawk) and the testers differ at the Bip locus and possibly the Arc locus as well.

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