PN-ABM-926 8.166

FINAL REPORT

Period: July 13, 1988 to July 12, 1992

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

CONTROL OF BRUCHID SEED WEEVILS OF COMMON BEAN USING UNIQUE SEED PROTEINS HAVING INSECTICIDAL PROPERTIES

Principal Investigator: T.C. Osborn
Grantee Institution: Univ. of Wisconsin-Madison

Co-investigator: F.A. Bliss
Institution: Univ. of California, Davis

Co-investigator (Host Country): J. C. Rosas
Institution: Escuela Agrícola Panamericana, Honduras

Collaborator: Pedro A.A. Pereira
Institution: CNPAF/EMBRAPA, Goiania, Goias, Brazil

Project Number: 8.166
Grant Number: DPE-5542-G-SS-8030-00

A.I.D. Grant Project Officer: Allan Schowler

Project Duration: July 13, 1988 - July 12, 1992

REC'D IN R&D/R

CC: BOSTID + CDIE

1992

1

TABLE OF CONTENTS

	Page No.
EXECUTIVE SUMMARY	3
RESEARCH OBJECTIVES	4
METHODS AND RESULTS	6
Bruchid Resistance Related to Arcelin Type Concentration	6
Effect of Phaseolin Concentration on Arcel Expression, Agronomic Traits and Bruchid Resistance	6
Protection Against Bruchid Damage Imparted by Purlines and Seed Mixtures with Varyin Proportions of Arcelin-containing Seeds	8
Breeding Strategies for Developing Cultiva with Durable Resistance to Bruchid Insect	10
IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER	11
PROJECT ACTIVITIES/OUTPUT	11
Publications	11
Presentations by Project Participants	13
PROJECT PRODUCTIVITY	14
FUTURE WORK	14
LITERATURE CITED	15
TABLES OF SUPPORTING DATA	17

EXECUTIVE SUMMARY

Common beans are the eighth largest food crop world-wide, and in Latin America and East Africa the dry seeds are major sources of dietary protein. Consumption is often constrained because of inadequate supplies due to low yields and insect damage. The Mexican bean weevil and common bean weevil infest bean seeds causing an estimated $200 million loss each year. The aim of this project was to utilize the insecticidal properties of arcelin, a protein found in seeds of wild beans, in developing resistant cultivars for biological control of these bruchid insects.

Arcelin 1 type protein provided the highest resistance to Mexican bean weevil. The damage to seeds containing high levels of arcelin 1 was less than half that to normal seeds and often approached the levels of protection provided by chemical seed treatment. When stored up to 6 months in Honduras, seeds containing arcelin showed only 10-15% as much damage as seeds without arcelin.

Although pureline cultivars containing seeds with arcelin 1 provide uniform, high levels of resistance, the possibility that weevils may overcome this resistance exists. Line mixtures containing different proportions of seeds having arcelin 1, other arcelin types 2, 3, and 4, or no arcelin were tested. Mixtures with a large proportion of seeds containing arcelin 1 and the 4-component mixture provided levels of resistance high enough for good protection, and they may reduce the likelihood that resistance will be broken. Studies in Brazil showed similar results to those in Honduras, and also it was found that resistant, arcelin-containing lines had higher germination which is an added benefit to farmers using their own seed. Rodent-feeding studies conducted at independent labs and also under the guidance of the Centro International de Agricultura Tropical (CIAT) suggest no likely danger to humans consuming arcelin-containing seeds that are cooked properly. Arcelin is a form of lectin or phytohemagglutinin similar to the lectin found normally in beans seeds. Normal bean seeds cooked properly have caused no toxicity problems for consumers.

The knowledge gained and the plant materials derived from these studies are being used in Honduras and Brazil to develop bean cultivars better suited for their particular production conditions. Resistant breeding lines developed by CIAT are being released for utilization. These studies show the importance of collaborative efforts that encompass many facets of basic and applied research; important results can accrue when fundamental knowledge is applied to an important practical problem.

RESEARCH OBJECTIVES

The aim of this project was to develop methods for utilizing the insecticidal properties of arcelin, a unique protein found in seeds of some wild beans, Phaseolus vulgaris L., for control of bruchids that attack beans.

The specific objectives were to:

Perform medium-scale extraction of purified arcelin in quantities adequate for testing as a natural insecticide,

Determine the resistance imparted by each of the four arcelin types to bruchids and quantify the relationship between arcelin concentration in the seed and resistance level,

Determine the effect of genetically altering the concentration of phaseolin on the level of arcelin expression and bruchid resistance expressed in the seeds,

Study the level of insect damage to seed mixtures containing different arcelin forms in seeds grown and stored under unprotected field conditions,

Develop a breeding strategy for efficient development of cultivars that show high, stable resistance to insect attack and a minimum of seed damage.

Common bean grown for the dry, mature seeds is the eighth-largest foodcrop world-wide, based on harvested area. Beans are especially important in Latin America and East Africa, where they supply from 20 to 30% of the dietary protein, particularly for people of limited income. Availability of beans is often limited by low yields and the destruction of harvested grain by seed storage insects, especially the bruchids, Mexican bean weevil (Zabrotes subfasciatus) and common bean weevil (Acanthoscelides obtectus). It has been estimated that these insects cause at least $200 million damage world-wide each year.

Scientists at the Centro International de Agricultura Tropical (CIAT) were interested in developing bean cultivars with resistance to bruchids beginning in the late 1970's. Although they identified sources of resistance in wild beans from Mexico, it was not until the findings by the Bliss laboratory at the University of Wisconsin-Madison, that the physiological and genetical bases for resistance were understood. With this knowledge, an efficient breeding method was employed to develop potentially useful cultivars resistant to the Mexican bean weevil and perhaps the common bean weevil.

Despite the knowledge that the unique seed protein arcelin is responsible for the antibiosis resistance to bruchids, there was some uncertainty about its efficacy because all lines containing arcelin were not equally resistant. Without further knowledge, it was not obvious which of several different molecular forms of arcelin should be used for effective resistance. Insect feeding trials of artificial seeds containing purified arcelin were used to demonstrate the effects of the different arcelin types and the effects of varying concentrations. The development of backcross-derived lines having different arcelin alleles in a common background (e.g., cv. Porrillo 70 and Sanilac) was necessary to determine the most effective molecular form of arcelin to be used in developing cultivars that perform well under field conditions.

The ease of transferring resistance alleles to a wide range of different cultivars via back-crossing greatly facilitates new cultivar development. However, this easily-used system also raises the possibility that a single allele could be incorporated into many cultivars that are grown over large areas thus creating a monogenic cropping system in which new biotypes of bruchids might overcome the resistance. Therefore, studies to consider methods of optimum gene deployment via such strategies as multi-line cultivars were important to assure long-term durability of this promising type of biological control.

Bruchids are difficult to control with physical and chemical means, and the chemical pesticides are both costly and potentially dangerous to humans. Because arcelin is closely related in many ways to the seed phytohemagglutin or lectin normally found in bean seeds, resistant cultivars are unlikely to be dangerous for human consumption when beans are cooked as usual before eating. CIAT has taken the lead role in determining whether there is any appreciable risk associated with utilizing arcelin-based bruchid resistance for bean improvement. Preliminary data gathered prior to inauguration of this project showed no deleterious effects on rodents fed cooked beans. Recent studies conducted by an independent research lab show arcelin-containing seeds to pose no undue detrimental effects when consumed (C. Cardona, CIAT, personal communication). The availability of biochemical resistance represents a biologically and economically effective method for bruchid control that can save many kilos of harvested grain.

The institutions for which the investigators are employed have contributed to the support of this project. Additionally, we have had important informal collaboration with CIAT scientists throughout the course of this grant. The University of Wisconsin experiments that required insect feeding response were conducted at CIAT. Those were essential to the success of this project.

METHODS AND RESULTS

Bruchid Resistance Related to Arcelin Type and Concentration

Some wild bean (Phaseolus vulgaris L.) lines collected in Mexico were found to have varying levels of bruchid resistance by Schoonhoven and Cardona (1983). Subsequent studies of seed proteins in Bliss' lab at the University of Wisconsin-Madison showed that seeds of resistant lines contained one of four types of a unique protein, later named arcelin. Romero Andreas et al. (1986) described the first electrophoretically-defined arcelin type (Arc 1) in a single seed of the accession P.I. 325690 from central Mexico. Probably because the original collection of seeds of P.I. 325690 contained a mixture of both seeds with and without arcelin, CIAT did not initially identify this line as showing bruchid resistance. Seeds from a pure-line increase made by self pollinating the plant from the single seed identified by Romero Andreas, were tested by Dr. Guy Hallman at CIAT and found to be highly resistant to bruchids, indicating the likelihood of a cause and effect relationship. Three additional arcelin alleles (Arc 2, Arc 3, and Arc 4) were described by Osborn et al. (1988) and Arc 5 has been described by Lioi and Bollini in 1989.

Artificial seed tests were used to show that increased bruchid resistance was associated with increased arcelin concentration (Osborn et al., 1988a; Cardona et al., 1991), especially for Mexican bean weevil. However, seeds containing arcelin were not always found to have high and/or equal levels of resistance to both insects. A series of genetically-similar lines was developed by back-crossing each of the four arcelin alleles into each of two common parents, Porrillo 70 a black-seeded tropical cultivar, and Sanilac a white navy bean adapted to the temperate U.S. Harmsen (1989) compared paired backcross-derived lines and found that in all cases seeds without arcelin showed no resistance. In both the black- and white-seeded backgrounds, seeds with Arc 1 consistently showed the highest levels of resistance (Tables 1 and 2).

Based on the results of Harmsen (1989), the black-seeded lines containing Arc 1 alleles in the Porrillo 70 background were chosen as the most promising materials for field tests in Brazil and Honduras. Black beans are a major production class in Brazil and one of several important classes in Honduras, were they are grown sometimes also for export.

Effect of Phaseolin Concentration on Arcelin Expression, Agronomic Traits and Bruchid Resistance

Bean seeds that contain arcelin protein have up to 50% less phaseolin (normally the major storage protein) compared to seeds without arcelin (Romero Andreas et al., 1986). Similarly, phytohemagglutinin and phaseolin levels are also negatively

correlated in common bean (Osborn and Bliss, 1985). Since the level of bruchid resistance was shown to be directly related to arcelin concentration (Osborn et al., 1988), knowledge of direct and indirect genetic effects on arcelin concentration and hence bruchid resistance, is important for implementing an optimum breeding strategy to develop resistance bean cultivars.

Bean lines containing the different forms of arcelin and either with or without phytohemagglutine and/or phaseolin were developed in Osborn's lab using a standard backcross procedure. The concentration of total protein was similar in lines either with or without phaseolin. In most of the lines both the quantities of arcelin dimer and phytohemagglutinin, measured by rocket immunoelectrophoresis, were higher than in lines where phaseolin was expressed, however in some lines no differences were observed. Thus it appears that presence or absence of a functional arcelin gene plays the largest role in determining the amount of arcelin in the seeds. As was the case in previous studies (i.e., Burow, 1990), genetic removal of protein fractions had varying effects on agronomic traits. Often, but not always, lines without phaseolin displayed lower seed yields, and in general showed later maturity.

In related studies, Hartweck, Osborn and Cardona (unpublished results) determined Z. subfasciatus and A. obtectus resistance in bean lines with and without the three protein constituents arcelin, phytohemagglutinin and phaseolin. They found that resistance based on percentage emergence (% E), days to adult emergence (DAE) and index of susceptibility (IS) was related primarily to the type and concentration of arcelin protein. Lines containing phytohemagglutinin were resistant to neither insect. Although concentrations of seed proteins were not important resistance factors, of the lines with arcelin, those without phaseolin were significantly more resistant to A. obtectus than those with phaseolin, perhaps reflecting a higher arcelin concentration. One line, containing arcelin-1 protein and having no phaseolin, showed the highest levels of resistance to both types of bruchids of any large-seeded bean lines developed to date (Table 3).

Protection Against Bruchid Damage Imparted by Purelines and Seed Mixtures With Varying Proportions of Arcelin-containing Seeds

Evaluation of the level of protection provided by the mixtures of seeds with and without the different arcelin types against bruchids was made with both natural and artificial infestations. In all cases unless stated otherwise, infestations were predominantly by the Mexican bean weevil. Usually the level of natural infestation was only moderate and quite variable which made it difficult to obtain statistically significant differences. Therefore, the most definitive results were obtained by using artificial infestations with Mexican bean weevil. The results were

generally similar for both natural and artificial infestation in both Brazil and Honduras.

The highest consistent level of resistance was provided by seeds containing arcelin 1 protein and to a lesser degree by arcelin 2. Arcelin 3 and 4 which are biochemically similar (Hartweck et al., 1991) provided little if any protection against bruchid attack. A total of seven trials were conducted in Honduras and four in Brazil. The results of one such trial conducted in Honduras during the second season of 1990, and which was representative, are shown in Tables 4, 5, and 6.

The important agronomic characteristics of the pure lines and mixtures were not substantially different from the Arc- line (Porrillo 70), demonstrating that there were no large pleiotropic affects or linkages between the Arc structural gene and deleterious traits (Table 4). The susceptibility index (SI) of the Arc 1 pure line was the lowest indicating the highest resistance based on the different parameters of seed damage and insect dry wt. However, the seed mixtures that contained a large proportion of Arc 1-containing and Arc 2-containing seeds also showed good levels of protection (Table 5). The most promising mixtures were those that contained 25% of each of the four arcelin types, and either 80% or 50% Arc 1-containing seeds.

The presence of any of the four arcelin proteins causes a dramatic change in the pattern of emergence of the hatching adults (Table 6). In addition to emergence of fewer adults over a 56 day period, the time of emergence is significantly delayed by the presence of arcelin, with the largest effect by either Arc 1 or Arc 2. In seeds without arcelin, i.e., Porrillo 70 and Danli 46, adults emerge rapidly, and if left in the sample as would be the case with normal storage, they would proceed through several cycles to completely destroy the seeds. Because the adults were removed in these experimental trials, few were seen after 44 days. The significant point was that in seed lots with a high proportion of arcelin-containing seeds, there were greatly reduced total damage and greatly altered life cycles.

The agronomic performance and levels of protection provided by the pure lines and mixtures in six experiments in Honduras are shown in Table 7 and 8. The susceptibility indices of seed lots with a high proportion of either Arc 1 or Arc 2, which reflects the three damage parameters, was substantially lower than either Porrillo 70 or Danli 46, the red-seeded local cultivar. Damaged seed in the Arc 1 pure line (52%) was about one-half that of the susceptible lines (90%). The level of damaged seed in the 4-component mixture was 62% compared to 90% for the susceptible Porrillo 70.

The results of trials conducted at the National Rice and Bean Center (CNPAF/EMBRAPA) in Goiania, Brazil were similar to those obtained in Honduras. The yield differences among the pure lines and seed mixtures of different arcelin-containing alleles were non-significant and also similar to the local cultivars Rio Tibagi and Goiania Precoce (Table 11). In Brazil where natural infestations were used, there were no consistent differences in the number of eggs layed on seeds of the contrasting lines (Table 12). The number of seeds (proportion) with some damage (i.e., at least one insect perforation) was similar for all lines (Table 13). However, when multiple perforation was used as a criterion, the lines containing all seeds with Arc 1 or Arc 2 alleles or a high proportion of such seeds showed one-third to one-half the damage of the more susceptible lines (Table 14).

Another effect of arcelin-containing seeds was observed in Brazil on plant stand establishment (Table 15). Those pure lines and mixtures with a high proportion of Arc 1- and Arc 2-containing seeds had a high stand establishment (percentage) compared to those with ineffective (Arc 3 or Arc 4) arcelin and no arcelin. Seeds without arcelin suffer greater damage to the cotyledons and therefore are either inviable or produce weaker seedlings that germinate and later die. Better seeds should be an advantage to farmers producing and storing their own seeds for planting the next season.

This effect of arcelin type and presence also suggests that a change in the genetic composition of seed mixtures might be expected after several cycles of saving and planting seeds, with seeds containing the most effective types (i.e., Arc 1 and Arc 2) being favored. Only preliminary data have yet been collected and analyzed, but differential survival appears to be the case when the arcelin genotype of individual seeds is scored.

Breeding Strategies for Developing Cultivars with Durable Resistance to Bruchid Insects

All common bean cultivars that lack arcelin protein are highly susceptible to bruchid damage unless they are protected by some chemical or physical agent. Various surveys of the world collection of cultivated beans have failed to identify accessions with any useful levels of resistance. The results of a storage trial of cultivars commonly grown in Honduras are shown in Tables 16 and 17. After 55 days, all lines except the test line (Porrillo 70 Arc 1+) containing Arc 1, and the adult weevils developed and emerged very quickly (Table 17). As in previous trials, presence of Arc 1 caused a considerable delay and disruption of emergence of adults.

Commonly accepted practices are to treat bean seeds with various compounds, add cooking oils or store the seeds near heat, all of which have serious disadvantages. Many times treatment with such items as dust, ash, hot pepper etc. are only marginally effective at best. While treatment with various chemicals may provide good protection, it is expensive and dangerous. Application of oil is effective but difficult to apply to large quantities, especially if shipment is anticipated. The level of protection afforded by several common treatments in an experiment conducted in Honduras is shown in Table 18. Under the best conditions, from 5 to 7% of the seeds were damaged after several months of storage. Although the level of protection afforded by arcelin 1-containing seeds was not always as good as that provided by chemical treatment, often they were similar under conditions where infestation pressure was moderate.

Since beans are produced during distinct growing seasons in most areas, the dry seeds must be stored after harvest for a period of several months. Without some method of protection, losses due to bruchid weevils are often very high causing a shortage of seeds for consumption and for planting the next season. Large price fluctuations result, with farmers selling at low prices and often being forced to buy-back planting seeds at much higher prices. Seeds with the Arc 1 and Arc 2 proteins suffered considerably less damage during storage of up to 6 months in Honduras (Tables 9 and 10). Although differences in the number of emerged adults were evident after one month, they were especially striking after either 3 or 6 months. The percentage damaged seeds which is the most obvious defect to the farmer and consumer was greatly reduced in the Arc 1 pure line (1.4%) and in the 4-component mixture (8.4%) after 3 months in 1990B compared to over 50% for susceptible lines. Also, after 6 months the differences were of a similar magnitude.

The development of cultivars containing reduced amounts of phaseolin protein should be viewed cautiously, even though total seed protein percentage was found to be essentially unchanged. Depending on the amino acid content of the fraction replacing phaseolin, a reduction in methionine might be more disadvantageous than the small increase in bruchid resistance associated with reduce phaseolin and increased arcelin protein content (e.g., Arc 3 and Arc 4).

In summary, the arc 1 allele produces the highest level of bruchid resistance, and other alleles have lesser effects. Levels of arcelin protein and bruchid resistance can be enhanced by manipulating genes controlling other seed proteins, but only in the presence of the arcelin-expressing alleles. Although sometimes there are small deleterious effects on agronomic traits associated with altering protein expression, there are no large pleiotropic effects and only occasional deleterious effects perhaps due to linkages. The development of detailed linkage maps in bean should

be useful for designing strategies to preclude negative effects on other plant traits when breeding for increased bruchid resistance.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The findings from the research conducted in this project make important contributions toward understanding the genetical and biological basis for bruchid resistance. With this information, potential new bean cultivars with high resistance to Mexican bean weevils have been developed for use in Honduras and Brazil. Also, CIAT has developed and distributed resistant bean germplasm to national programs in several countries (C. Cardona, CIAT, personal communication). The rodent feeding studies done in this project and by CIAT indicate that there are no expected deleterious effects from consuming seeds of resistant lines. The countries where this work was done now have the capabilities to develop and utilize weevil resistant bean cultivars.

It is likely that the results of this research will be used quite widely, especially in countries in Latin America, Africa and Asia where beans are an important dietary component and protein source. People ranging from farmers to urban consumers in many developing countries should be the beneficiaries of these research results. The reduction in crop losses and reduced need for pesticides should be worth several hundred million dollars annually.

It is likely that research on developing resistant cultivars will continue in several countries including Honduras and Brazil. The development of weevil resistant lines will become part of ongoing breeding programs so that larger scale field and feeding trials will continue. Some studies on the stability of mixtures and pure lines should continue since only long-term observations will answer such questions. The scientific capabilities of collaborating scientists have been improved through the additional training of students and staff scientists in the areas of plant breeding for insect resistance.

PROJECT ACTIVITIES/OUTPUT

Publications

Altamirano, R.F. 1992. Effects of arcelin in the growth and reproductive rates of Zabrotes subfasciatus (Boheman) (Ing. Agr. Thesis, Escuela Agrícola Panamericana, Honduras, 96 p.).

Bliss, F.A. 1990. Breeding strategies for developing dry bean cultivars resistant to bruchid weevils. HortScience 25:123 (Abstr.).

Bliss, F.A. 1992. Deployment of arcelin genes for bruchial resistance in common beans. HortScience 27:597 (Abstr.).

Cárcamo, R.N. 1992. Characterization of postharvest systems of common beans at the small farmer and intermediary level in Morocelí, Honduras (Ing. Agr. Thesis, Escuela Agrícola Panamericana, Honduras, 116 p.).

de Almeida, S.C.R., E.R. Sampaio, P. Pereira, G. de Sa M.F. 1991. Purifacacas, clonagem e expressao de Arcelina em especies de feijas susceptiveis ao ataque de carunchos: estudo comparatdo da expressao obtida atraves de tecnicas de retrocruzamentos e engenharia genetica. 3ª Resiniao Brasileira sobre controle biologico de doencas, Peraccaba, S.P.

Espinal, R., F.A. Bliss, J.C. Rosas y A. Robleto. 1990. Control de bruquidos en frijol almacenado mediante el uso de semillas conteniendo proteinas que tiene propiedades insecticidas. pp. 40-43. In: Informe Anual de Investigacion 1989, Departamento de Agronomia, Escuela Agricola Panamericana, Honduras.

Harmsen, Rhea L.H. 1989. Bruchid resistance and agronomic traits of cultivated bean lines (Phaseolus vulgaris L.) containing arcelin seed protein alleles from wild beans. Ph.D. Thesis, University of Wisconsin, Madison.

Harmsen, R., F.A. Bliss, C. Cardona, C.E. Posso and T.C. Osborn. 1988. Transferring genes for arcelin protein from wild to cultivated beans. Proc. Bean Impr. Coop. 31:54-55.

Hartweck, L.M., T.C. Osborn, and C. Cardona. 1990. Development and evaluation of bean lines with arcelin and phytohemagglutinin seed proteins. Agron. Abstr. p. 92.

Hartweck, L.M., R.D. Vogelzang, and T.C. Osborn. 1991. Characterization and comparison of arcelin seed protein variants from common bean. Plant Physiol. 97:204-211.

Rodríguez, A.A. 1992. Evaluation of storage systems to control Zabrotes subfasciatus in common beans (Ing. Agr. Thesis, Escuela Agrícola Panamericana, Honduras, 87 p.).

Rosas, J.C., F.A. Bliss and G.A. Robleto. 1991. Control of Zabrotes subfasciatus in stored beans using arcelin. In: Annual Research Report 1990, Agronomy Department, Escuela Agrícola Panamericana, pp 1-5.

Rosas, J.C. F.A. Bliss and G.A. Robleto. 1992. Evaluation of the stability of the resistance to Zabrotes subfasciatus conferred by arcelin. In: Annual Research Report 1991, Agronomy Department, escuela Agrícola Panamericana, Honduras, pp 11-15.

Robleto, A., R. Espinal y J.C. Rosas. 1990. Metodos tradicionales de control del gorgojo de frijol comun en almacenamiento. pp. 44-47. In: Informe Anual de Investigacion 1989, Departamento de Agronomia, Escuela Agricola Panamericana, Honduras.

Robleto, A. 1990. Comparacion de metodos de almacenamiento para el control de Zabrotes subfasciatus en frijol comun. Tesis Ing. Agr., Escuela Agricola Panamericana, Honduras, 58 p.

Robleto, A., R. Espinal y J.C. Rosas. 1990. Metodos tradicionales de control del gorgojo del frijol comun en almacenamiento. XXXVI PCCMCA Meeting, San Salvador, El Salvador, March 26-30.

Teck, N.S. 1992. Comparison of the biology of Zabrotes subfasciatus and Acanthoscelides obtectus in stored grain of common bean (Ing. Agr. Thesis, Escuela Agrícola Panamericana, Honduras, 83 p.).

Presentations by Project Participants

Bliss, F.A. 1988. Utilization of genetic resources for improvement of common bean. Symposium on Genetics and Germplasm Resources in Crop Improvement. Davis, CA, August, 1988.

Bliss, F.A. 1989. Genetic control of quantitative expression of seed protein. Purdue University, March, 1989.

Bliss, F.A. 1989. Genetic alteration of legume seed proteins. 2nd International Symposium on Horticulture and Human Health. Alexandria, VA, November, 1989.

Bliss, F.A. 1990. Genetic control of seed protein expression in common bean. University of California, Riverside, February, 1990.

Bliss, F.A. 1992. Resistance to bruchid weevils in common bean: Genetic control and deployment strategies. University of California, Riverside, January, 1992.

Robleto, G.A., R. Espinal and J.C. Rosas. 1990. Traditional methods for controlling common bean weevil in storage. XXXVI Annual PCCMCA (Central American Cooperative Program for the Improvement of Crops and Animals) Meeting, San Salvador, El Salvador, 26-30 March, 1990.

Robleto, G.A., J.C. Rosas and F.A. Bliss. 1992. Use of arcelin as a source for resistance to Zabrotes subfasciatus in common beans. XXXVIII Annual PCCMCA Meeting, Managua, Nicaragua, 23-27 March, 1992.

Rosas, J.C., G.A. Robleto and F.A. Bliss. 1991. Control of Zabrotes subfasciatus in stored beans using arcelin. XXXVIII Annual PCCMCA Meeting, Panamá, 18-22 March, 1991.

Wright de Malo, V., L.A. Pinel, J.C. Rosas and R. Espinal. 1992. The dry bean storage system in Honduras. International Symposium on Stored Grain Ecosystems, Winnipeg, Canadá, June 1992.

PROJECT PRODUCTIVITY

The proposed objectives were realized with the following exceptions:

Methods for medium-scale extraction of purified arcelin were developed by researchers at the Biotechnology Center at U.W.-Madison. Purified arcelin was utilized in various subsequent studies, but we chose not to conduct studies of purified arcelin applied to seeds as a natural pesticide because it would have required protocols to safe-guard researchers for which we did not have suitable personnel and expertise. This is an area of research that may be worthy of pursuit but future researchers.

We had intended to conduct field studies in Tanzania similar to those done in Brazil and Honduras, using white navy beans instead of black beans. However, the experimental lines of white beans intended for those studies were susceptible to plant pathogens endemic to Tanzania and could not be used. Dr. Susan Nchimbi, the intended collaborator hoped to obtain red bean lines being developed for East Africa by CIAT, but apparently was unable to do so. Consequently, the field studies in Tanzania were not done. Never-the-less, the data obtained should be applicable to addressing problems of bean losses to bruchids regardless of where they occur, including East Africa.

FUTURE WORK

It is expected that research and development of bruchid resistant common bean cultivars will continue at both the Escuela Agricola Panamericana in Honduras and CNPAF/EMBRAPA in Brazil. At both places there are ongoing breeding programs that have incorporated the idea of breeding for bruchid resistance into their overall goals and objectives. These programs are of national importance to their respective countries. There is an active

program for developing bruchid resistant bean germplasm at CIAT that will undoubtedly continue.

LITERATURE CITED

Burow, M.D. 1990. Biochemical and genetic characteristics of common bean (Phaseolus vulgaris L). lines deficient for the seed storage proteins phaseolin and lectin. Ph.D. Diss. Univ. of Wisconsin, Madison.

Burow, M.D. 1990. Biochemical and genetic characteristics of common bean (Phaseolus vulgaris L.) lines deficient for the seed storage proteins phaseolin and lectin. Ph.D. Dissertation, University of Wisconsin, Madison.

Cardona, C., J. Kornegay, C.E. Posso, F. Morales, and H. Ramirez. 1990. Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. Entomol. Exp. Appl. 56:197-206.

Cardona, C., C.E. Posso, J. Kornegay, J. Valor, and M. Serrano. 1989. Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. 82(1):310-315.

Cardona, C., T.C. Osborn, R.D. Vogelzang, and C.E. Posso. 1991. Effects of bean seed proteins on the Mexican bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. (in press).

Cardona, C., T.C. Osborn, R.D. Vogelzang, and C.E. Posso. 1991. Effects of bean seed proteins on the Mexican bean weevil (Coleoptera: Bruchidae). J. Econ. Entomol. (in press).

Harmsen, R.L. 1989. Agronomic traits and resistance to Mexican bean weevil (Zabrotes subfasciatus) and common bean weevil (Acanthoscelides obtectus) (bruchidae) in cultivated bean lines containing arcelin a seed protein alleles from wild beans (Phaseolus vulgaris L.). Ph.D. Dissertation, University of Wisconsin, Madison.

Hartweck, L.M., R.D. Vogelzang and T.C. Osborn. 1991. Characterization and comparison of arcelin seed protein from common bean. Pl. Physiol. 97:204-211.

Labeyrie, V. 1981. Conclusions and recommendations, p. 231-233. In: V. Labeyrie (ed.). The ecology of bruchids attacking legumes (pulses). Dr. W. Junk Publishers, London.

Lioi, L. and R. Bollini. 1989. Identification of a new arcelin variant in wild bean seeds. Annu. Rept. Bean Imp. Coop. 32:28.

Osborn, T.C. 1988. Genetic control of bean seed protein. CRC Critical reviews in plant sciences. 7(2):93-116.

Osborn, T.C.M. Burrow, and F.A. Bliss. 1988b. Purification and characterization of arcelin seed protein from common bean. Plant Physiol. 86:399-405.

Osborn, T.C., D.C. Alexander, S.M. Sun, C. Cardona, and F.A. Bliss. 1988a. Insecticidal activity and lectin homology of arcelin seed protein. Science 240:207-210.

Osborn, T.C., T. Blake, P. Gepts, and F.A. Bliss. 1986. Bean arcelin 2. Genetic variation, inheritance and linkage relationships of a novel seed protein of Phaseolus vulgaris L. Theor. Appl. Genet. 71:847-855.

Osborn, T.C., and F.A. Bliss. 1985. Effects of genetically removing lectin seed protein on horticultural and seed characteristics of common bean. J. Amer. Soc. Hort. Sci. 110(4):484-488.

Romero Andreas, J., B.S. Yandell, and F.A. Bliss. 1986. bean Arcelin 1. Inheritance of a novel seed protein of Phaseolus vulgaris L. and its effect on seed composition. Theor. Appl. Genet. 72:123-128.

Schoonhoven, A.V. and C.R. Cardona. 1982. Low levels of resistance to the Mexican bean weevil in dry beans. J. of Econ. Entomol. 75:567-569.

Schoonhoven, A.V. and C.R. Cardona. 1983. Resistance to the Bean weevil and the Mexican bean weevil (Colioptera: Bruchidae) in noncultivated common bean accessions. J. Econ. Entomol. 76:1255-1259.

Table 1. Mean values for percentage emergence, days to emergence, and dry weight of Zabrotes subfasciatus reared on backcross lines either containing different arcelin (Arc^+) types or deficient in arcelin (arc^-) (Source: Harmsen, 1989).

Genetic line	Arcelin type	Number of backcross lines tested	Percentage emergence	Days to adult emergence[y]	Adult dry weight ($g \times 10^{-3}$)[y]
BC_2S_2 (Porrillo 70)	arc 1-	8	92.3 e	31.3 d	1.18 c
	Arc 1+	4	7.8 a	40.3 a	.79 a
	arc 2-	9	90.2 e	31.5 d	1.17 c
	Arc 2+	4	54.9 c	34.7 c	1.18 c
	arc 3-	4	87.7 e	31.7 d	1.18 c
	Arc 3+	2	78.1 d	37.5 b	.94 ab
	arc 4-	5	89.5 e	31.4 d	1.16 c
	Arc 4+	5	80.0 d	38.4 b	.84 a
BS_3S_2 (Sanilac)	arc 1-[z]	2	82.9	34.4	1.25
	Arc 1+[z]	2	2.3	50.4	.95
	arc 2-	3	88.3 e	31.8 d	1.10 bc
	Arc 2+	10	46.1 b	35.7 c	.97 b
	arc 3-	3	88.7 e	31.6 d	1.19 c
	Arc 3+	2	85.9 de	37.9 b	.90 ab
	arc 4-	1	89.2 e	31.4 d	.94 ab
	Arc 4+	1	83.6 de	40.0 a	.78 a
G12952	Arc 4+	1	16.0	59.60	.62
Calima	arc-	1	88.0	32.40	1.36

[y]Means followed by the same letter (within the same column) are not significantly different by LSD> at P> .05.
[z]Means for Sanilac arcelin 1^+ and arcelin 1^- lines are from a previous trial (Osborn et al., 1988a).

Table 2. Mean values for percentage emergence, days to emergence, and dry weight of Acanthoscelides obtectus reared on backcross lines either containing different arcelin (Arc^+) or deficient in arcelin (arc^-) (Source: Harmsen, 1989).

Genetic line	Arcelin type	Number of backcross lines tested	Percentage emergence	Days to adult emergence[y]	Adult dry weight ($g \times 10^{-3}$)[y]
BC_2S_2 (Porrillo 70)	arc 1-	8	42.7	33.4 f	2.03 bc
	Arc 1+	3	38.8	39.8 a	1.87 a
	arc 2-	9	45.7	33.3 f	2.18 de
	Arc 2+	4	32.9	36.2 bc	2.17 de
	arc 3-	4	31.2	34.2 de	2.11 cde
	Arc 3+	2	47.0	33.6 f	1.94 ab
	arc 4-	5	44.8	33.3 f	2.10 cd
	Arc 4+	5	55.4	34.6 de	2.09 bcd
BS_3S_2 (Sanilac)	arc 1-[z]	1	51.5	35.5	2.35
	Arc 1+[z]	2	31.5	39.3	1.85
	arc 2-	3	66.3	33.7 f	2.25 e
	Arc 2+	10	65.3	36.5 b	2.13 cde
	arc 3-	3	61.7	34.1 ef	2.15 cde
	Arc 3+	2	67.4	35.5 cd	1.94 ab
	arc 4-	1	57.4	34.6 de	2.18 de
	Arc 4+	1	34.0	39.0 a	1.90 ab
G12952	Arc 4+	1	33.0	52.0	.98
Calima	arc-	1	65.0	34.6	2.18

[y]Means followed by the same letter (within the same column) are not significantly different by LSD> .05.
[z]Means for Sanilac arcelin 1^+ and arcelin 1^- lines are from insect trials conducted previously (Osborn, 1988a).

Table 3. Mean values for percentage emergence (% E) days to adult emergence (DAE) and index of susceptibility (IS) for bean seeds containing arcelin but with (Arc 1+, Pha +) and without phaseolin (Arc 1+, Pha -).

	Z. subfasciatus			*A. obtectus*		
	% E	DAE	IS	% E	DAE	IS
Arc 1+, Pha-	3.7	50.3	0.2	29.7	39.0	8.6
Arc 1+, Pha+	3.7	48.7	0.2	42.0	39.0	9.6
Sanilac (Susc)	91.3	34.0	9.0	81.3	34.3	12.8
G12952 (Res)	6.7	63.0	0.8	ND	ND	ND

Table 4. Differences in agronomic performance of four near-isogenic Porrillo 70 arcellin containing lines, six mixtures, Porrillo 70 (Arc -) and one local cultivar from the second growing season of 1990. El Zamorano, Honduras, 1990.[z]

Pureline or Mixture	DF	DM	Yield (kg/ha)	Yield components		
				PPP	SPP	SDW
Arc 1 (1.00)	39	82	2855	13	7	19.5
Arc 2 (1.00)	37	80	2823	15	6	20.3
Arc 3 (1.00)	34	71	1899	12	6	18.7
Arc 4 (1.00)	38	81	2789	15	6	19.7
Arc 1,2,3,4 (0.25 each)	38	83	3064	14	6	19.1
Arc 1 (0.50), Arc 4 (0.50)	37	81	2748	14	6	19.9
Arc 1 (0.80), Arc - (0.20)	38	82	2775	13	6	18.4
Arc 1 (0.20), Arc - (0.80)	38	82	2839	13	6	20.4
Arc 4 (0.80), Arc - (0.20)	38	80	2403	13	6	19.8
Arc 4 (0.20), Arc - (0.80)	38	81	2685	13	6	19.1
Arc - (1.00) (Porrillo 70)	38	81	3016	12	6	20.0
Danli 46 (local check	37	75	2526	13	5	19.0
ANOVA LSD (5%)	* 1.0	* 1.9	* 373.9	ns	ns	ns

[z]Planting date: 24 Sep 90. DF = days to flowering; DM = days to physiological maturity; PPP = pods/plant; SPP = seeds/pod; SDW = 100 seeds dry wt.

* and ns Significant at P<.01 and non-significant, respectively.

Table 5. Number and percentage of emerged adults (F_1 generation), days to emergence, damaged seed, total and individual adult dry wt. and susceptibility index from samples of four near-isogenic Porrillo 70 arcelin lines, six mixtures Porrillo 70 (Arc -), and a local cultivar harvested from the second growing season of 1990 and stored under artificial infestation with Zabrotes subfasciatus for 56 days. El Zamorano, Honduras, 1990.

Pureline or Mixture	Adult Emergence[z]			Adult dry weight (mg)		Damaged seed[x]	Suscept.[w] index
	Total	Percent[y]	Days	Total	Mean		
Arc 1 (1.00)	12	9.1	43.0	11	1.11	15	0.25
Arc 2 (1.00)	24	19.3	38.5	34	1.57	15	1.17
Arc 3 (1.00)	76	91.7	40.5	94	1.33	28	2.33
Arc 4 (1.00)	116	92.2	40.4	142	1.20	41	2.97
Arc 1,2,3,4 (0.25 each)	49	36.2	39.4	55	1.08	18	2.00
Arc 1 (0.50, Arc 4 (0.50)	45	41.3	37.2	54	1.25	16	2.06
Arc 1 (0.80), Arc - (0.20)	72	58.0	34.2	94	1.27	22	2.72
Arc 1 (0.20), Arc - (0.80)	111	92.8	32.2	141	1.26	37	3.60
Arc 4 (0.80), Arc - (0.20)	61	97.3	38.0	75	1.29	28	2.18
Arc 4 (0.20), Arc - (0.80)	136	93.3	32.6	178	1.31	35	3.90
Arc - (1.00) (Porrillo 70)	114	95.1	33.0	152	1.36	38	3.59
Danli 46 (Local Check)	106	64.5	33.9	163	1.59	38	3.38
ANOVA	*	*	*	*	**	*	*
LSD (5%)	57.6	15.2	2.8	76.0	0.3	16.0	1.1

[z]Oviposition (112.8 ± 45.1 eggs/sample) no significantly different. Adults (parents) were withdrawn after 16 days of infestation.
[y](Adults/eggs) x 100.
[x]After 56 days of infestation.
[w]SI = Log (Adults/7 females)/days to emergence x 100.
* and ** Significant at $P \leq .01$ and .05, respectively.

Table 6. Time sequence of adults emerged (F_1 generation) from seed samples of four near-isogenic Porrillo 70 arcelin lines, six mixtures, Porrillo 70 (Arc -) and a local cultivar harvested from the second growing season of 1990 and stored during 56 days under artificial infestation with Zabrotes subfasciatus. El Zamorano, Honduras, 1990.

Pureline or Mixture	Days after infestation[z]							
	32	36	40	44	48	52	56	Total
Arc 1 (1.00)	2	2	2	1	3	1	1	12
Arc 2 (1.00)	2	9	7	4	2	0	0	24
Arc 3 (1.00)	5	14	28	22	5	1	1	76
Arc 4 (1.00)	7	19	41	34	14	1	0	116
Arc 1,2,3,4 (0.25 each)	7	9	15	13	3	2	0	49
Arc 1 (0.50), Arc 4 (0.50)	13	13	5	7	4	2	1	45
Arc 1 (0.80), Arc - (0.20)	36	25	6	3	1	1	0	72
Arc 1 (0.20), Arc - (0.80)	73	32	5	0	1	0	0	111
Arc 4 (0.80), Arc - (0.20)	17	14	16	9	4	1	0	61
Arc 4 (0.20), Arc - (0.80)	94	33	6	3	0	0	0	136
Arc - (1.00) (Porrillo 70)	72	35	5	2	0	0	0	114
Danli 46 (Local Check)	63	31	11	1	0	0	0	106

[z]Adult counts were made every two days. Emerged adults were discarded after each count.

Table 7. Seed yields (kg ha^{-1}) of Porrillo 70 backcross-derived purelines and mixtures containing different proportions of seeds with different arcelin types, compared with Porrillo 70 (no arcelin) and the local cultivar Danli 46. Means of six experiments during 1989-1991, El Zamorano, Honduras.

Pureline or Mixture	Year and planting season of trials							
	1988	1989A[z]	1989B	1990A	1990B	1991A	1991B	Mean
Arc 1 (1.00)	2578	2062	1677	839	2855	1552	1105	1610 (100)[y]
Arc 2 (1.00)	1895	1480	1671	827	2823	1307	1323	1618 (89)
Arc 3 (1.00)	1865	2135	1224	859	1899	1535	1263	1540 (85)
Arc 4 (1.00)	2113	1556	1512	818	2789	1171	1390	1621 (90)
Arc 1,2,3,4 (0.25 each)	2208	1742	1773	823	3064	1559	1126	1756 (97)
Arc 1 (0.50), Arc 4 (0.50)	2209	1707	1656	951	2748	1419	1372	1723 (95)
Arc 1 (0.80), Arc - (0.20)	2504	1545	1577	770	2775	1460	1329	1708 (94)
Arc 1 (0.20), Arc - (0.80)	2278	1982	1567	1123	2839	1556	1642	1855 (102)
Arc 4 (0.80), Arc - (0.20)	2001	1432	1408	820	2403	1779	1453	1614 (89)
Arc 4 (0.20), Arc - (0.80)	2317	1941	1552	810	2685	1639	1322	1752 (97)
Arc - (1.00) (Porrillo 70)	2149	1871	1602	995	3016	1637	1405	1811 (100)
Danli 46 (Local check)	1905	1936	1985	832	2526	1938	1523	1806 (99)
Mean	2169	1782	1600	872	2702	1546	1354	
NOVA	*	ns	**	*	*	ns	ns	*
LSD (0.05)	364		334	167	374			139

[y]Percent of Arc - (Porrillo 70) mean
[z]A = First (June-Aug.); and B = second (Sept.-Dec.) planting seasons.
*, **, [ns]Significant at $P \leq .01$, .05, y non-significant, respectively.

Table 8. Bruchid (Zabrotes subfasciatus) adult emergence and dry weight and damage to seeds in Porrillo 70 backcross-derived pure lines and mixtures containing different proportions of seeds with different arcelin types, compared to Porrillo 70 (no arcelin) and the local cultivar, Danli 46. Means of six experiments during 1989-1991, El Zamorano, Honduras.

Pureline or mixture	Adult emergence[z]			Adult dry wt (mg)			
	Total number	Percentage[y]	Days to emergence	Total sample	Mean	Damaged seed (%)	Suscept.[x] index
Arc 1 (1.00)	58	26.0	45.3	44	1.0	52	1.8
Arc 2 (1.00)	60	31.2	40.2	70	1.3	56	2.2
Arc 3 (1.00)	177	85.8	41.9	177	1.2	88	3.2
Arc 4 (1.00)	182	86.2	42.5	197	1.1	94	3.3
Arc 1,2,3,4 (0.25 each)	93	43.5	43.4	94	1.0	62	2.4
Arc 1 (0.50), Arc 4 (0.50)	100	49.0	41.2	95	1.1	76	2.7
Arc 1 (0.80), Arc - (0.20)	101	54.6	37.4	119	1.2	60	3.0
Arc 1 (0.20), Arc - (0.80)	168	83.4	35.5	201	1.3	86	3.8
Arc 4 (0.80), Arc - (0.20)	166	90.7	39.2	176	1.2	88	3.4
Arc 4 (0.20), Arc - (0.80)	168	90.7	35.8	218	1.3	86	3.7
Arc - (1.00) (Porrillo 70)	177	88.1	35.5	231	1.4	90	3.9
Danli 46 (Local Check)	168	85.3	35.5	221	1.4	92	3.8

[z]Oviposition (eggs/sample) was not significantly different in any experiment of the six experiments.
[y](Adults/eggs) x 100.
[x]Susceptibility index = log (adults/7 females/days to emergenc x 100).

Table 9. Number of adults of Zabrotes subfasciatus counted in bean samples seeds stored 1, 3 and 6 months after infestation following two production seasons, El Zamorano, Honduras.

Pureline or mixture	1990 B			1990 A		
	Number of Months			Number of Months		
	1	3	6	1	3	6
Arc 1 (1.00)	7	16	24	5	76	13
Arc 2 (1.00)	10	78	355	9	294	14
Arc 3 (1.00)	16	636	104	7	477	29
Arc 4 (1.00)	18	1044	71	11	537	25
Arc 1,2,3,4 (0.25 each)	5	79	111	3	126	28
Arc 1 (0.50), Arc 4 (0.50)	38	142	278	23	185	20
Arc 1 (0.80), Arc - (0.20)	38	318	167	40	272	33
Arc 1 (0.20), Arc - (0.80)	130	938	257	86	440	33
Arc 4 (0.80), Arc - (0.20)	54	515	130	42	510	42
Arc 4 (0.20), Arc - (0.80)	137	676	123	64	439	40
Arc - (1.00) (Porrillo 70)	99	597	318	63	436	33
Danli 46 (Local Check)	136	749	281	72	506	25
ANOVA	*	*	*	*	*	ns
LSD (.05)	30	254	171	20	151	–

[z]Samples of 300 seeds.

Table 10. Percentage damaged seeds in bean samples stored 1, 3 and 6 months after initial infestation with Zabrotes subfasciatus following two production seasons, El Zamorano, Honduras.

Pureline or mixture	1990 B			1991 A		
	Number of Months			Number of Months		
	1	3	6	1	3	6
Arc 1 (1.00)	0.4	1.4	8.7	1.8	15.3	17.8
Arc 2 (1.00)	0.6	10.3	92.1	2.0	40.4	80.4
Arc 3 (1.00)	0.8	62.1	99.3	1.9	58.0	86.4
Arc 4 (1.00)	0.8	73.1	99.8	3.6	56.0	90.7
Arc 1,2,3,4 (0.25 each)	0.4	8.4	44.3	1.5	21.0	42.5
Arc 1 (0.50), Arc 4 (0.50)	2.0	50.7	67.2	6.8	31.8	58.8
Arc 1 (0.80), Arc - (0.20)	2.8	40.8	79.8	11.5	38.9	68.5
Arc 1 (0.20), Arc - (0.80)	8.7	68.9	98.6	23.3	66.3	94.9
Arc 4 (0.80), Arc - (0.20)	3.7	53.5	99.3	12.3	72.3	93.8
Arc 4 (0.20), Arc - (0.80)	10.8	62.7	99.8	12.8	67.1	97.2
Arc - (1.00) (Porrillo 70)	9.1	48.0	95.8	11.4	71.8	97.0
Danli 46 (Local Check)	9.4	71.8	100.0	9.8	86.9	96.8
NOVA	*	*	*	*	*	*
LSD (.05)	3.1	28.8	22.7	5.2	16.3	13.9

[z]Samples of 300 seeds.

Table 11. Grain yield of four near-isogenic Porrillo 70 arcelin containing lines, six mixtures, Porrillo 70 (Arc-) and two local cultivars grown in the field. Goiania, Brazil, 1990.

Purline or Mixture	Yield (kg/ha)
Arc 1 (1.00)	1226
Arc 2 (1.00)	1564
Arc 3 (1.00)	938
Arc 4 (1.00)	974
Arc 1,2,3,4 (0.25 each)	1510
Arc 1 (0.50), Arc 4 (0.50)	1218
Arc 1 (0.80), Arc - (0.20)	1214
Arc 1 (0.20), Arc - (0.80)	1383
Arc 4 (0.80), Arc - (0.20)	1356
Arc 4 (0.20), Arc - (0.80)	1256
Arc - (1.00) (Porrillo 70)	1318
Rio Tibagi	1273
Goiano Precoce	1427
LSD (5%)	ns

Table 12. Number of eggs layed by adult bruchids on seeds of purelines, mixtures and local cultivars.[z] Beans grown in the field. Goiania, Brazil, 1990.

Pureline or Mixture	Days after Harvest		
	60	90	150
	(Numbers of eggs)		
Arc 1 (1.00)	2.18	35.0	96.2
Arc 2 (1.00)	1.84	24.4	61.0
Arc 3 (1.00)	4.55	26.9	68.1
Arc 4 (1.00)	8.76	39.0	90.6
Arc 1,2,3,4 (0.25 each)	1.26	24.3	90.6
Arc 1 (0.50), Arc 4 (0.50)	5.34	29.5	69.0
Arc 1 (0.80), Arc - (0.20)	1.72	33.2	69.3
Arc 1 (0.20), Arc - (0.80)	1.84	36.1	102.5
Arc 4 (0.80), Arc - (0.20)	4.62	40.7	100.3
Arc 4 (0.20), Arc - (0.80)	3.65	47.5	100.3
Porrillo 70	3.71	38.4	97.6
Rio Tibagi	3.38	32.1	62.3

[z]50 sampled seeds

Table 13. Number of seeds with damage in purelines, mixtures and local cultivars of beans grown in the field. Goiania, Brazil, 1990.

Pureline or Mixture	Days after Harvest		
	60	90	150
	(Number of seeds)[z]		
Arc 1 (1.00)	0	1.82	18.5
Arc 2 (1.00)	0.22	2.19	16.8
Arc 3 (1.00)	0	2.93	16.5
Arc 4 (1.00)	0.64	3.23	16.8
Arc 1,2,3,4 (0.25 each)	0	1.60	18.0
Arc 1 (0.50), Arc 4 (0.50)	0.51	4.91	18.0
Arc 1 (0.80), Arc - (0.20)	0	1.38	15.4
Arc 1 (0.20), Arc - (0.80)	0	2.41	12.5
Arc 4 (0.80), Arc - (0.20)	0.27	3.73	11.6
Arc 4 (0.20), Arc - (0.80)	0	2.49	12.5
Porrillo 70	0.30	3.37	13.6
Rio Tibagi	0.11	3.45	15.6

[z]50 sampled seeds

Table 14. Number of seeds with multiple perforations in purelines, mixtures and local cultivars of beans grown in the field. Goiania, Brazil, 1990.

Pureline or Mixture	Days after Harvest		
	60	90	150
	(Number of seeds)[z]		
Arc 1 (1.00)	0	0.31	8.5 c
Arc 2 (1.00)	0.11	0.52	6.5 c
Arc 3 (1.00)	0	0.39	18.6 abc
Arc 4 (1.00)	0.19	1.67	22.3 ab
Arc 1,2,3,4 (0.25 each)	0	0.97	15.6 abc
Arc 1 (0.50), Arc 4 (0.50)	0.39	1.62	17.4 abc
Arc 1 (0.80), Arc - (0.20)	0	0.11	15.9 abc
Arc 1 (0.20), Arc - (0.80)	0	0.51	30.6 a
Arc 4 (0.80), Arc - (0.20)	0	0.82	28.4 a
Arc 4 (0.20), Arc - (0.80)	0.11	0.99	30.6 a
Porrillo 70	0.11	1.62	28.4 a
Rio Tibagi	0	0.72	20.5 ab

[z]50 sampled seeds

Table 15. Plant counts (percentage stand) at three different stages for purelines, mixtures and local cultivars of beans grown in the field. Goiania, Brazil, 1991.

Pureline or Mixture	Days after Planting		
	19	53	104[z]
	(Percentage Stand)[y]		
Arc 1 (1.00)	67	68	63
Arc 2 (1.00)	83	78	73
Arc 3 (1.00)	40	35	33
Arc 4 (1.00)	38	35	34
Arc 1,2,3,4 (0.25 each)	91	87	81
Arc 1 (0.50), Arc 4 (0.50)	86	72	66
Arc 1 (0.80), Arc 4 (0.20)	75	69	64
Arc 1 (0.20), Arc 4 (0.80)	36	29	29
Arc 4 (0.80), Arc - (0.20)	65	53	51
Arc 4 (0.20), Arc - (0.80)	46	41	36
Arc - (1.00)	27	24	22
Rio Tibagi	47	43	39
Goiano Precoce	25	19	18
LSD, 05	13	13	13

[y]Based on 120 seeds planted.
[z]Harvested plants as a percentage of 120 seeds planted.

Table 16. Number and percentage of emerged adults (F_1 generation), days to emergence, damaged seed, total and individual adult dry wt. and susceptibility index from samples of 11 small-red honduran genotypes and two near-isogenic Porrillo 70 arcelin lines stored under artificial infestation with Zabrotes subfasciatus for 55 days. El Zamorano, Honduras, 1991.

Line	Adult Emergence[z]			Adult dry weight (mg)		Damaged seed[x]	Suscept.[w] Index
	Total	Percent[y]	Days	Total	Mean		
Catrachita	197	87.2	35.4	288	1.45	47	4.1
Chingo R	186	90.0	35.6	277	1.49	48	4.0
Cincuenteno	195	93.7	35.1	306	1.57	49	4.1
Cuarenteno	165	90.2	35.2	242	1.48	46	3.9
Danili 46	198	90.0	37.1	277	1.41	48	3.9
Desarrural 1R	190	86.4	35.1	284	1.51	48	4.1
DOR 364	230	89.4	35.8	354	1.53	49	4.2
DOR 391	204	98.5	34.8	264	1.29	47	4.2
RAB 50	219	88.5	36.1	326	1.50	49	4.1
Zamorano	197	84.4	35.0	308	1.57	49	4.1
Frijol Chile	212	91.7	35.9	311	1.47	47	4.1
Porrillo 70 Arc-	215	89.7	39.1	274	1.28	49	3.8
Porrillo 70 Arc+1	56	25.6	47.9	53	0.95	29	1.9
ANOVA	*	*	*	*	*	*	*
LSD (5%)	39.2	8.7	1.2	0.1	0.2	3.9	0.3

[z]Planting date: 12 Sep 91. Oviposition (222.7 ± 40.3 eggs/sample) no significantly different. Adults (parents) were withdrawn after 16 days of infestation.
[y](Adults/eggs) x 100.
[x]After 55 days of infestation.
[w]SI = Log (Adults/7 females)/days to emergence x 100.
* Significant at $P \leq .01$.

Table 17. Time sequence of adults (F^1 generation) emerged from seed samples of Hondura germoplam and two near-isogenic Porrillo 70 arcelin lines, stored during 55 days under artificial infestation with Zabrotes subfasciatus. El Zamorano, Honduras, 1991.

Line	Days after infestation[z]							
	31	35	39	43	47	51	55	Total
Catrachita	49	74	50	21	4	2	0	200
Chingo R	41	73	44	26	3	1	0	188
Cincuenteno	51	73	49	19	3	0	0	195
Cuarenteno	50	54	38	19	4	1	0	166
Danli 46	21	71	55	35	12	3	0	197
Desarrural 1R	49	76	45	19	2	0	1	192
DOR 364	48	82	63	31	6	1	0	231
DOR 391	62	80	39	18	5	1	0	205
RAB 50	44	70	63	35	5	1	0	218
Zamorano	59	66	51	19	3	1	0	199
Frijol Chile	50	73	52	28	9	1	0	213
Porrillo 70 Arc-	13	72	51	33	22	20	4	215
Porrillo 70 Arc+1	1	3	3	5	10	27	9	58

[z]Adult counts were made every two days. Emerged adults were discarded after each count.

Table 18. Influence of bean genotype and method of control on the percentage of damaged seed produced by Zabrotes subfasciatus in common bean seed stored for seven months. Honduras, 1990.

Treatment	Months of storage						
	1	2	3	4	5	6	7
GENTOYPE							
Catrachita	5.7	17.8	27.7	49.0	55.8	61.4	66.4
F-Chile	5.8	10.3	23.0	27.6	31.3	33.1	33.4
Significancia	ns	ns	ns	ns	*	**	**
METHOD OF CONTROL							
Brush	6.4	26.6	56.4	82.5	83.2	85.4	86.5
Hot-pepper	5.3	19.3	41.3	64.6	84.2	91.0	90.6
Check[z]	5.2	16.8	36.3	50.9	53.9	53.2	53.8
Lime	6.0	7.4	7.1	21.6	28.7	40.6	54.1
Ash	5.9	7.5	5.5	5.5	5.8	6.9	7.6
Insecticide[y]	5.4	6.7	5.7	4.8	5.6	6.2	6.9
ANOVA	ns	**	**	**	**	**	**
LSD (5%)		13.1	29.3	36.6	30.1	21.2	13.2

[z]Seed stored without applying any control method.
[y]Pirimiphos-metilo 2%.
** and ns Significant at $P \leq .01$ and non-significant.