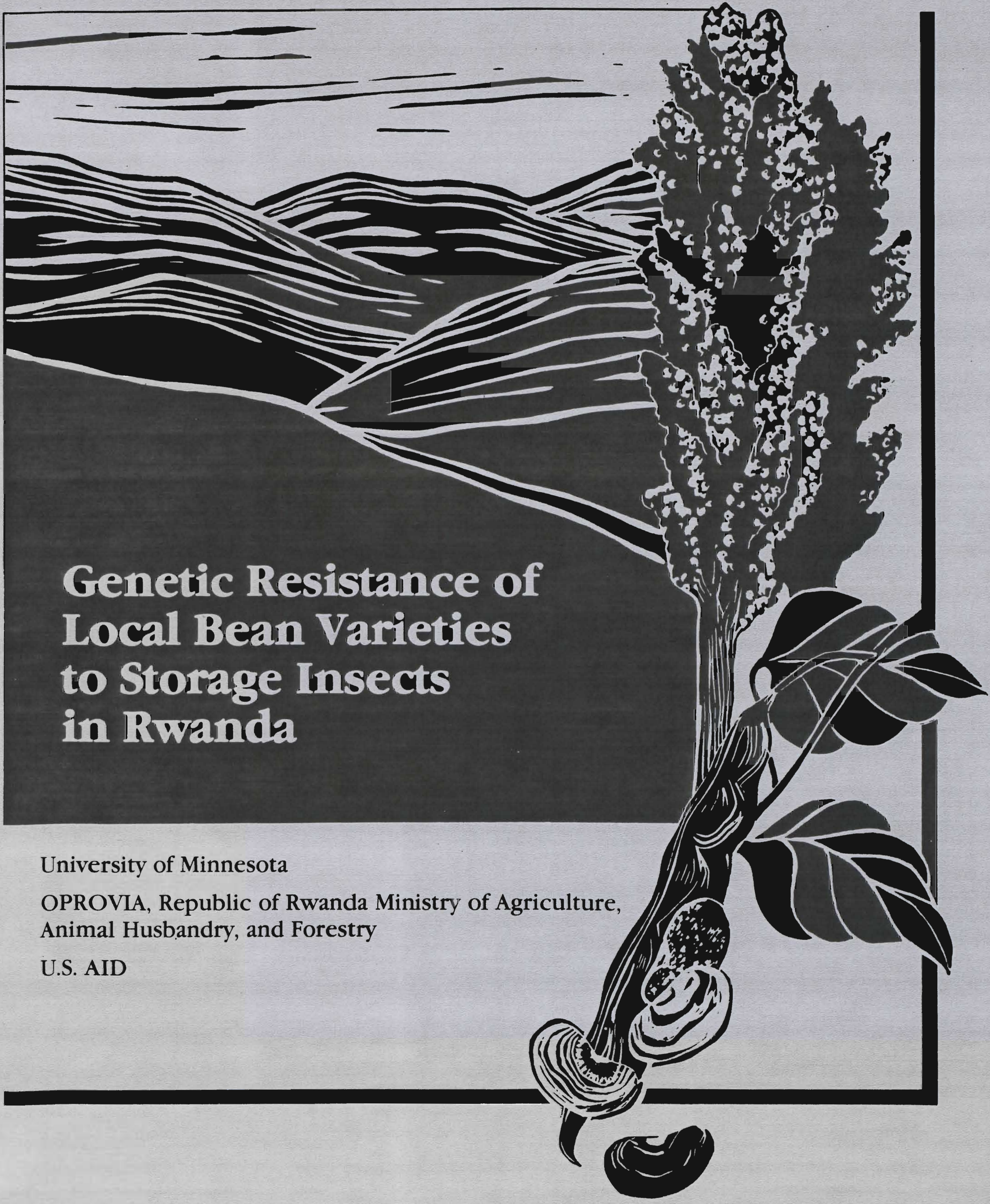

Genetic Resistance of Local Bean Varieties to Storage Insects in Rwanda

University of Minnesota

OPROVIA, Republic of Rwanda Ministry of Agriculture, Animal Husbandry, and Forestry

U.S. AID

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Genetic Resistance of Local Bean Varieties to Storage Insects in Rwanda

E.M. Lamb
Research Assistant

F.V. Dunkel
Entomologist
Technical Advisor

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Minnesota Agricultural Experiment Station, University of Minnesota

ABSTRACT

A set of experiments was carried out to examine the interrelationship of *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman with local varieties of beans (*Phaseolus vulgaris* L). A rapid screening test for determining the resistance level of these bean varieties to the two bruchid species was developed and tested on over 100 varieties. Adequate levels of resistance for the control of *A. obtectus* were found. The level of resistance to *Z. subfasciatus* in the varieties tested was not as high. Pods of certain varieties were resistant to oviposition by *A. obtectus*. The interaction of bruchids and the bean seeds during storage was affected by environmental factors such as temperature and the moisture content of the seeds. Varieties tested for resistance in long term studies, measured as the number of insects produced over a five month period, did not have the same relative levels of resistance as in the rapid screening test. In a mixture of a resistant and a susceptible variety, the presence of the resistant variety reduced the damage caused to the mixture as a whole. The level of protection depended on the percent of the resistant variety in the mixture.

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LIST OF COOPERATORS

Butare, I., Dr.	ISAR-Rubona
Chiavaroli, E.	USAID-Kigali
Clarke, S.	LCS-FSM II, Kigali
Depp, R.M.	USAID-Kigali
Dessert, K.	CIAT Great Lakes, Rubona
Dessert, M., Dr.	CIAT Great Lakes, Rubona
Dobie, P., Dr.	TDRI, Slough, England
Dupras, D.	USAID-Kigali
Edmister, J.	LCS-FSM II, Kigali
Fuchs-Carsh, M., Dr.	USAID-Kigali
Gatarasi, T., Dr.	PNAP, Ruhengeri
Gatsinzi, F.	ISAR-Rubona
Gahamanyi, L.	ISAR-Rubona
Graf, W.	CIAT Great Lakes, Rubona
Hanegreefs. P., Dr.	LCS-FSM II, Kigali
Harelimana, G.	LCS-FSM II, Rubona
Jefremovas, V.	IDRC, Rubona
Kayinamura, P	LCS-FSM II, Kigali
Kayitare, J.	ISAR-Rubona
Marekeyisoni, D.	LCS-FSM II, Kigali
Muhihu, S.K., Dr.	Nat'l Ag Labs, Nairobi, Kenya
Mwiseneza, B.	LCS-FSM II, Kigali
Nsengimana, J.C.	INRS, Butare
Ntibitura, B.	OPROVIA, Kigali
Nyabyenda, P., Dr.	ISAR-Rubona
Pere, M.	Nat'l Hort Res Sta, Thika, Kenya
Schoonhoven, A.v., Dr.	CIAT, Colombia
Thompson, D., Dr.	U of MN, St. Paul
Trutmann, P., Dr.	CIAT Great Lakes, Rubona
Ukiliho, B., Dr.	ISAR-Rubona
Uwimana, J.D.	LCS-FSM II, Rubona
Voss, J., Dr.	CIAT Great Lakes, Rubona
Wittenberger, T.	LCS-FSM II, Kigali

PREFACE

This report describes the research completed by the component `Studies of the Genetic Resistance of Local Bean Varieties to Storage Insects in Rwanda' of the GRENARWA II Research Project. The objective of this component was to "identify bean varieties resistant to stored grain pests as one means of reducing losses in beans that must be stored on farms four to five months or more."

The GRENARWA II Research Project was financed by the Grant Agreement signed June 2, 1982 between the Government of Rwanda and the Government of the USA. It was organized under Contract No. AFR-0107-C-00-4001-00 of November 4, 1983 between the University of Minnesota and the United States Agency for International Development (USAID), for a period of four years. The Project is executed under the auspices of the National Office for Development and Marketing of Food and Livestock Products (OPROVIA), especially its Food Product Division (GRENARWA), during the period November, 1983 - June, 1988.

The research described in this report was principally done by Elizabeth Lamb, Research Assistant responsible for the component, under the direction of Dr. Florence Dunkel, Entomologist and Technical Advisor. The work was done in collaboration with the Institute of Agronomic Sciences of Rwanda (ISAR). The technical personnel, Germain Harelimana, technician, and Jean Damascène Uwimana, laboratory aide, and laboratory space were provided by ISAR. Pierre Claver Munyemana, a student in the Faculty of Agronomy of the National University of Rwanda (UNR), assisted with the component during a three month trainee program during January - March, 1985.

INTRODUCTION
Background Information on Rwanda

Geographical Aspects

Rwanda is located 1 to 3° south of the equator in east central Africa and is bordered by Uganda, Zaire, Burundi and Tanzania (Map 1). Rwanda covers an area of 26,338 km^2. The topography is hilly with elevations of 950 masl (meters above sea level) in the southern region to 4500 masl in the volcanic region of the northwest. The native vegetation ranges from savannah to highland tropical forest. Most of the forest has been cleared for farmland. Ninety percent of the soils are pre-Cambrian. The soils (5%) found in the marshy areas between the hills are alluvial. Areas with rich volcanic soils (5%) are characterized by high population densities. Streams, rivers and lakes are well distributed throughout the country. The rainfall is bimodal, with rainy seasons occurring between February and May and October and December. Total annual precipitation is 800 to 2000 mm. Average temperatures range from 16 to 24° C, varying with elevation.

Demographic Aspects

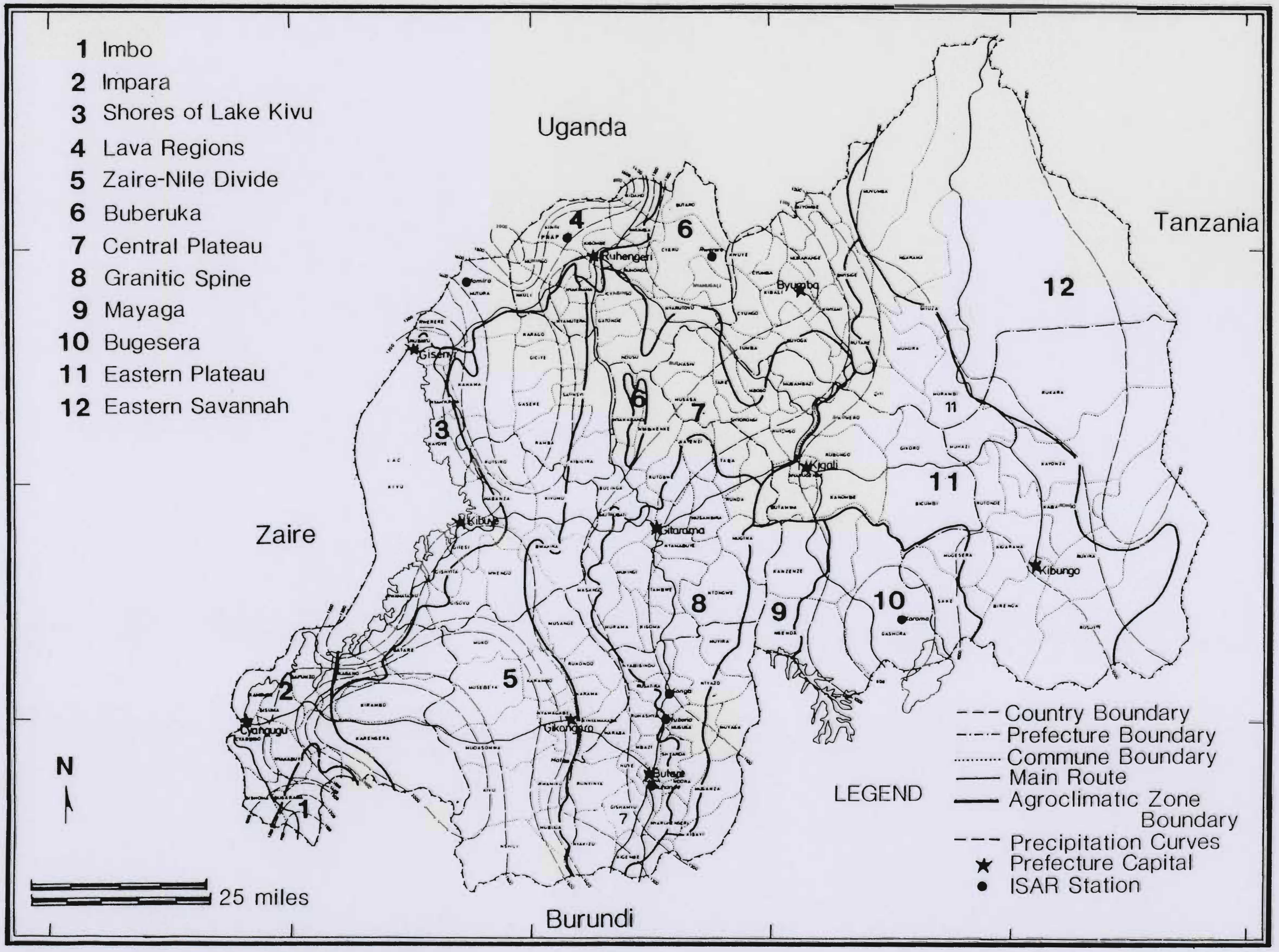
The population was 5.5 million in 1982 with an annual growth rate of 3.5 percent, one of the highest in Africa. The population density of the whole country is 200 inhabitants per square kilometer. Calculated on the basis of arable land area, the density is 400 inhabitants per square kilometer. The population is still largely rural; only 5 percent of the people live in cities. The rural landscape is characterized by scattered homesteads rather than organized villages.

Political Division

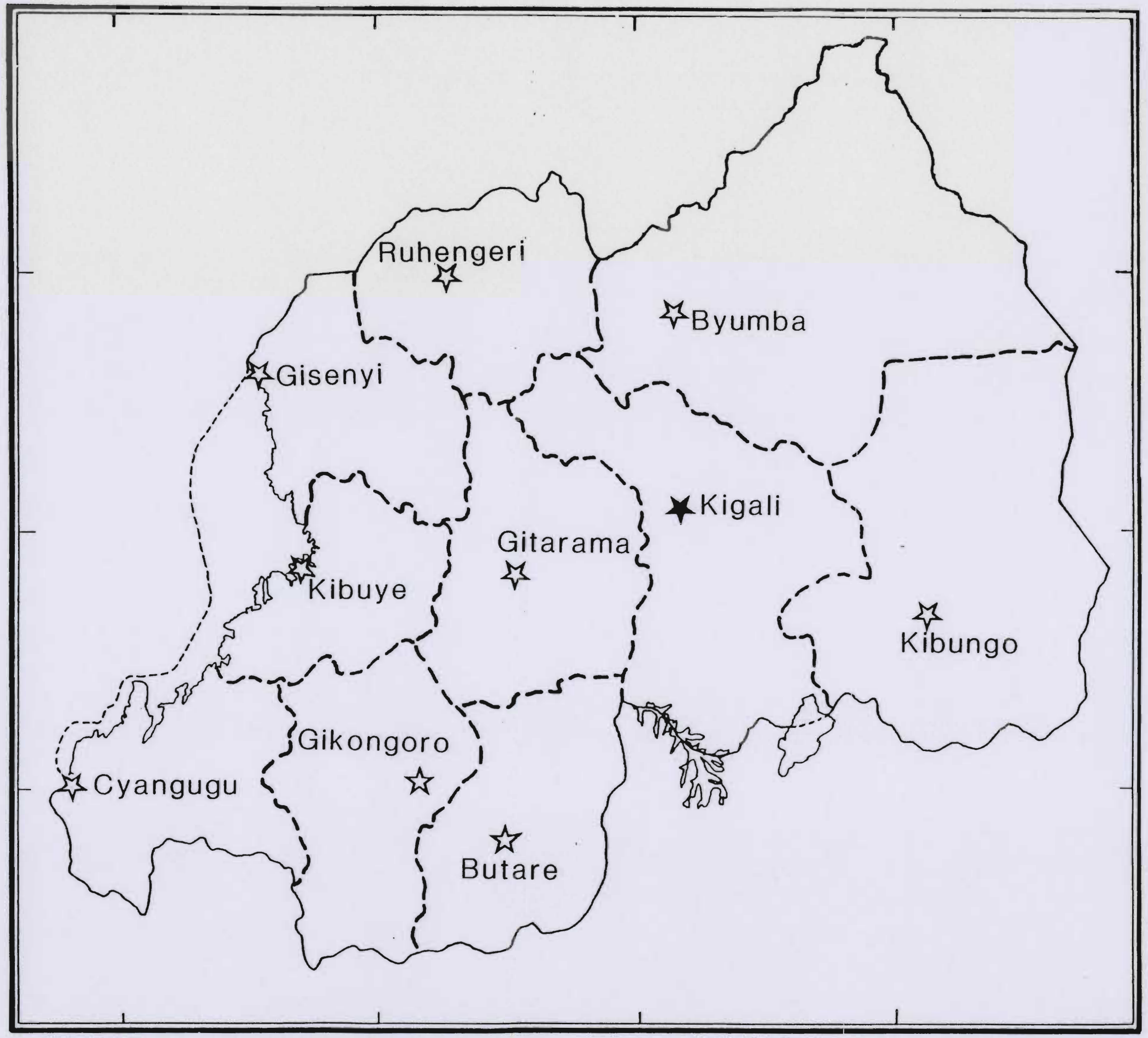
The capital of Rwanda is Kigali. The country is divided into 10 prefectures, each with a center of government. Prefectures are divided into communes, of which there are 143 (Map 2). Communes are divided into sectors. Sectors are divided into cellules which are the smallest political units.

Agriculture in Rwanda

A total of 1,229,600 ha of arable land is available (1980 data). Subsistance farming makes up the greatest part of agricultural effort in the country with each family having about one hectare of non-contiguous small plots to cultivate. Agriculture is characterized by a lack of mechanization, mixed crop and livestock culture and the production of multiple food crops by intercropping. The cropping seasons reflect the rainfall pattern although a third season is possible in the marshy areas. The most important crops, by harvested area (1978-1980), are beans (*Phaseolus vulgaris* L.), banana, sorghum, sweet potato, maize, pea, cassava and Irish potato. Maize, pea and Irish potato are most important at the higher elevations. Soybean, peanut, millet, wheat, rice, taro and yams are also grown, as well as various vegetable crops including tomato, eggplant, cabbage, leek and onion. Fruits grown include papaya,



MAP 1. OUTLINE MAP OF RWANDA



MAP 2. PREFECTURES OF RWANDA

pineapple, avocado and custard apple. The principal industrial crops are coffee, tea and pyrethrum. Cattle, goats, sheep, pigs, chickens and rabbits are produced. The country has been divided into 12 agroclimatic zones, based on elevation, rainfall, soils and types of agricultural production (Map 3).

Agricultural Research

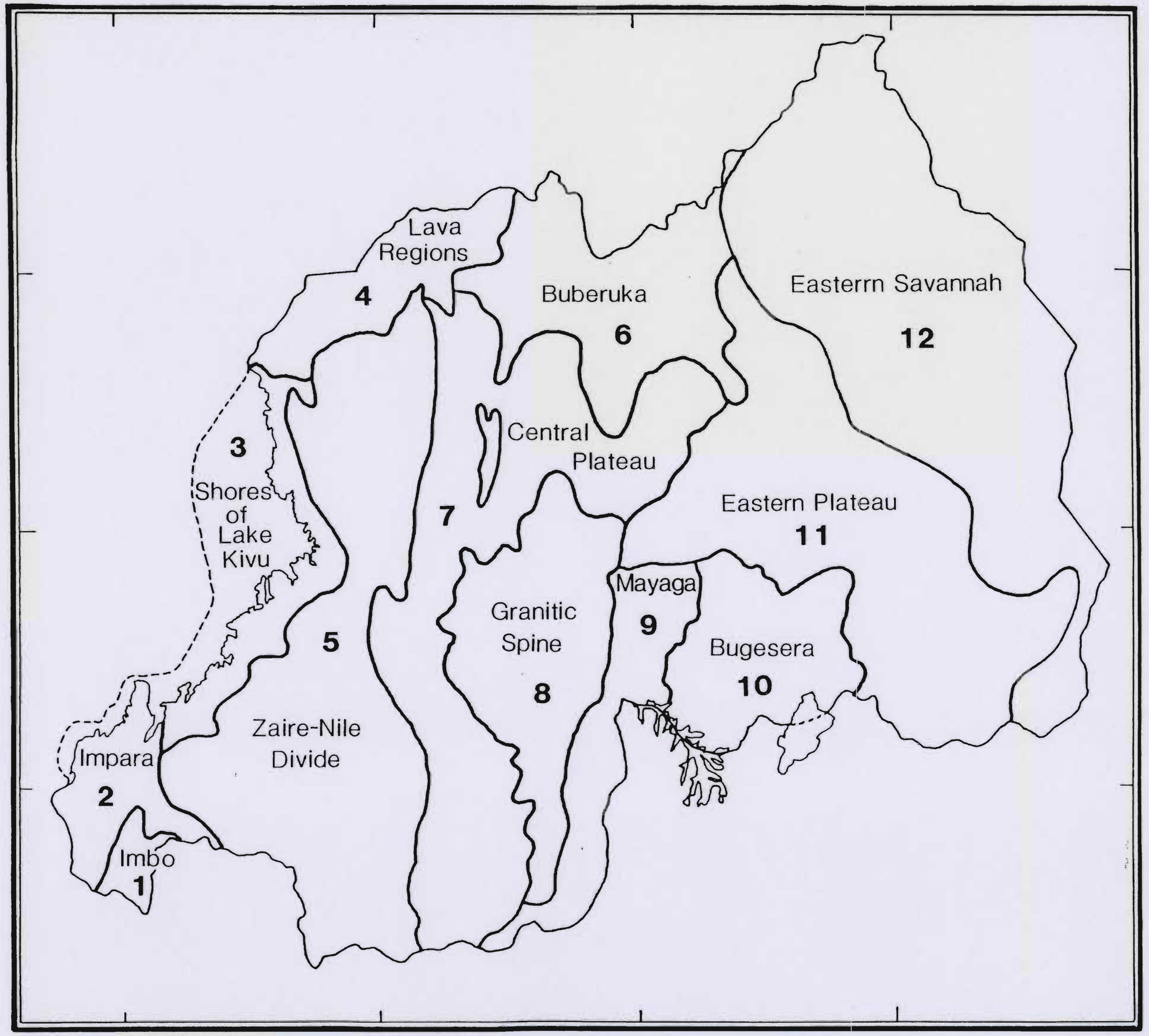
The Institute for Agronomic Sciences of Rwanda (ISAR) has primary responsibility for all agricultural research done in Rwanda. Seven branch stations are distributed in various regions of the country (Map 1). Research is carried out on food and industrial crops, farming systems, forestry and livestock production. At the headquarters in Rubona, laboratory and field space is available for plant breeding, plant protection, soil and plant chemistry and microbiology. Some research is carried out by the Faculty of Agronomy of the National University of Rwanda (UNR). In addition, ISAR scientists collaborate with the Faculty of Agronomy in the training of students,

Bean Production, Storage and Marketing

Beans are the major source of vegetable protein in the Rwandan diet. The estimated consumption of dry beans is 40 kg per person per year (Nyabyenda et al., 1980). In 1984, beans were grown at some time during the year on 98 percent of the 2,100 farms sampled. Beans are usually grown as mixtures of varieties. Yields ranged from 417 kg/ha to 975 kg/ha with a nationwide average of 662 kg/ha, in 1984. The mean yield for the first and most important season, harvested in January, was 760 kg/ha, dropping to 475 kg/ha for the second season. Based on national averages, each family plants 0.35 ha to beans, either as a pure crop or as the primary component in a mixed crop. Only 10 percent of the area used for bean cultivation is devoted to pure crops. On 71 percent of the area, beans are the primary crop and on 18 percent of the area, the secondary crop. The total harvested area of beans is approximately 240,000 ha and the total production in 1984 was 256,306 metric tons.

An estimated 30 percent of total bean production is marketed. Price follows availability and may rise dramatically in a poor year. Excess produce may be sold at harvest or throughout the year when money is needed. The major portion is sold by the farmers themselves at small local markets or by small scale merchants who buy directly from the farmer. Large scale merchants may sell beans in the large markets or to the government warehouses.

Beans are stored by farmers for food and seed, by merchants for later resale, by storage cooperatives and by the government for resale and as food reserves in case of famine. The mature plants are harvested in the field and brought to the house compound (rugo) where they are spread on the ground or hung on hedges or under rafters and allowed to dry. The seed is removed from the pods by threshing the plants with a stick. The chaff is removed from the seed using flat winnowing baskets. Before being put in storage, the seed is hand picked to remove small or poor quality seeds. Mixtures of seed types may be separated by type



MAP 3. AGROCLIMATIC ZONES OF RWANDA

or combined with other mixtures for storage. If an insecticide (commercial or local type) is to be used, it is mixed with the beans just prior to storage.

On farm, beans are most frequently stored in reed baskets lined with a mixture of cow manure and mud. Clay pots, gourds, sacks or oil drums are also used. The storage containers are usually kept in the farmer's house. Beans purchased by merchants may be stored in a similar manner or bagged and stacked in storage rooms built for the purpose, depending on the quantity of beans stored and the expected duration of the storage. Cooperatives usually have hangars or silos designed for the storage of large quantities of grain.

Storage of beans and other commodities by the government is coordinated by the National Granaries of Rwanda (GRENARWA). GRENARWA was organized in 1974 to 1) reduce losses during storage of food products 2) encourage food crop production and stabilize market prices of these commodities and 3) standardize the geographical availability of the products by arranging transport from areas of surplus to areas of deficit. GRENARWA was incorporated with the National Office for Development and Marketing of Food and Livestock Products (OPROVIA) in 1982. The capacity of the OPROVIA/GRENARWA warehouses is 16,000 metric tons. Marketing is handled by the regional OPROVIA stores.

OBJECTIVES OF THE COMPONENT

The objectives of this component are as follows:

1. Verify if resistance to bruchids exists in the varieties of beans (P. vulgaris) grown in Rwanda.
2. Develop a program for screening for resistance in local and introduced varieties with the cooperation of the national bean improvement program of ISAR.
3. Develop an extension and training program which shows the importance of resistance in a system of control of storage insects, in particular the bruchids which attack stored beans.

The following set of hypotheses and subhypotheses was designed to provide information to fulfill the objectives. Each subhypothesis corresponds to an experiment or set of experiments.

Hypothesis I.

Varieties of beans grown in Rwanda show variable resistance to attack by storage insects, notably Acanthoscelides obtectus Say and Zabrotes subfasciatus Boheman.

- A. Resistance to Acanthoscelides obtectus and Zabrotes subfasciatus exists in the seeds of local varieties of dry beans (Phaseolus vulgaris).
- B. Resistance to Acanthoscelides obtectus exists in the ripe pods of local varieties of dry beans (Phaseolus vulgaris).
- C. The relative level of resistance to Acanthoscelides obtectus in a bean variety is independant of relative humidity and temperature in the range of environments typical of Rwandan storage conditions.
- D. Resistance to Acanthoscelides obtectus and Zabrotes subfasciatus is characterized by a reduction in the rate of growth of the insect population on a resistant variety relative to that on a susceptible variety.
- E. The presence of a genotype resistant to Acanthoscelides obtectus or Zabrotes subfasciatus in a mixture of bean varieties infested with that insect affects the level of damage sustained by the mixture as a whole.

Hypothesis II.

The resistance of bean seeds to bruchids can be overcome by the adaptation of the insect to the resistant variety as host (stability of resistance).

GENERAL REVIEW OF THE LITERATURE

1. Origin, Distribution and Host Range of the Insects

The bruchids *Acanthoscelides obtectus* (Bean weevil) and *Zabrotes subfasciatus* (Mexican bean weevil) are the most common insect pests of stored dry beans in Rwanda. Both species originated in South and Central America. *Acanthoscelides obtectus* was introduced into Tanzania in 1913 and was reported in Uganda in 1924 (Lefèvre, 1950). *Zabrotes subfasciatus* was found in Tanzania and southern Zaire around 1915 and has spread rapidly in the last 30-35 years (Taylor, 1981). Distribution and spread of both species is influenced by trade within and between countries. The host range of the two species is under discussion but the primary host for both species is *P. vulgaris* (Johnson, 1981). Secondary hosts of *A. obtectus* may include *Voandezia* and *Sesbania* species (Decelle, 1981, Lefèvre, 1950, Southgate, 1978). In Rwanda, *A. obtectus* is found throughout the country where beans are grown. *Zabrotes subfasciatus* is a problem in the areas of lower elevation (1000-1300 masl) such as the Bugesera region of Kigali prefecture where the temperatures are higher (Butare, pers. comm., 1984, ISAR-Rubona, Rwanda).

2. Biology and Development of the Insects

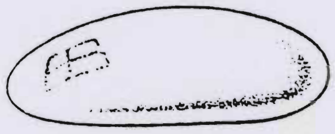
The adults of *A. obtectus* are greybrown with brown patches. They are somewhat triangular in shape and 2.5 to 5.0 mm in length (Davies, 1970). The antennae are club shaped; wider at the outer end than at the point of attachment to the head (Larson and Fisher, 1938, Lefèvre, 1950) (Figure 1). The sexes can be differentiated, with some difficulty, by the shape of the last abdominal segment (CIAT, 1980) (Figure 2). The adults of *Z. subfasciatus* are sexually dimorphic. The females are 1 to 1 1/2 times as large as the males and are black with white patches on the wing covers (Davies, 1972). The males are brownish but can be differentiated from *A. obtectus* by their rounder shape and antennae of equal width (CIAT, 1980) (Figure 3).

In stored beans, the adult females are attracted to the seeds to oviposit. The eggs of *Z. subfasciatus* are whitish and rounded and are attached to the tegument of the seed. *Acanthoscelides obtectus* eggs are elongate and are laid singly or in clumps at the point of contact between seeds. They are not attached to the seed coat (CIAT, 1980). The reported average number of eggs per female varies from 30 to 60 for *A. obtectus* (Lima, 1976, Zaazou, 1948) and 20 to 50 for *Z. subfasciatus* (Carvalho, 1968, Davies, 1972, Golob, 1982, Singh, 1979).

There is some indication that both *Acanthoscelides* and *Zabrotes* adults 'mark' seeds by depositing an unknown substance on the surface during defecation (*Acanthoscelides*) or egg laying (*Zabrotes*) (Szentesi, 1981, Wasserman, 1985). These chemicals prevent aggregation of larvae in seeds by causing larvae (*Acanthoscelides*) or ovipositing females (*Zabrotes*) to avoid the marked seeds.

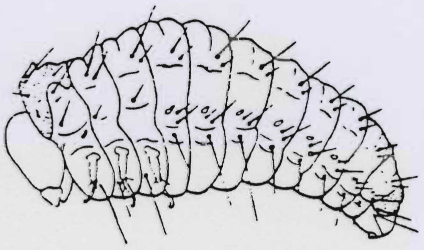
The larvae of both species must bore through the tegument of the seed to reach the cotyledons, upon which they feed. *Zabrotes* larvae bore at the site where the egg is attached while *A.*

Figure 1. Life stages of _Acanthoscelides_ _obtectus_.

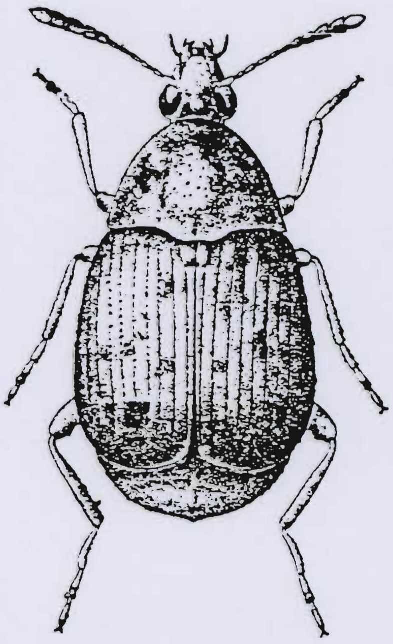


Egg (x60)

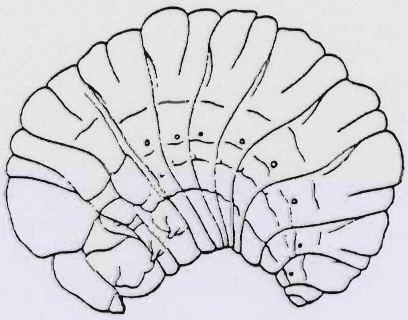
Nymph (x38)



Larva: First instar (x75)



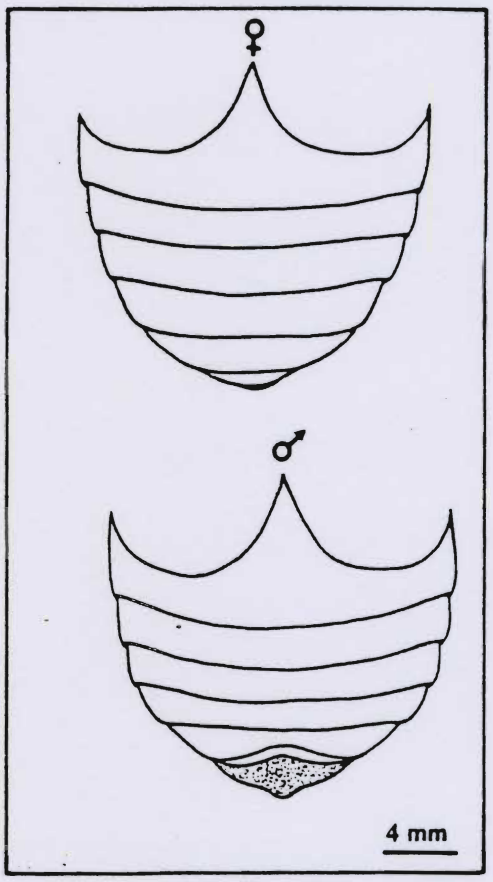
Adult (x15)



Larva: Second Instar (x65)

Lefévre (1950)

Figure 2. Abdominal segments of the adult male and female Acanthoscelides obtectus.



CIAT (1980)

Figure 3. Life stages of Zabrotes subfasciatus

CIAT (1980)

obtectus larvae must search for an appropriate entrance site. Several larvae of *A*. *obtectus* may use the same entrance hole (Lefèvre, 1950). Once the seed coat is pierced, the larvae tunnel parallel to the seed surface, eventually forming a cell or gallery in which to pupate. All four larval instars and the pupal stage are passed within the same seed (Lefèvre, 1950). The length of the developmental period varies with temperature, relative humidity (RH) and the moisture content of the seed (Howe and Currie, 1964, Menusan, 1934, Menusan, 1936). Optimal conditions for *A*. *obtectus* are 27 to 31° C and 70% RH, and for *Z*. *subfasciatus*, 29 to 33° C and 70% RH (Howe, 1965, Scwartz and Galvez, 1980). One seed can support several larvae and may contain larvae of both species.

At the time of pupation, the location of the pupal cell is visible as a round translucent 'window' in the seed coat. The window formed by *Z*. *subfasciatus* can be distinguished from that of *A*. *obtectus* by the dark ring around the circumference (McFarlane and Weaving, 1967). The adult emerges by partially or completely cutting around the window and pushing it out. The emerging adults are sexually mature. Mating and oviposition start one to two days after emergence. Under storage conditions, adults do not feed. Length of adult life and period of oviposition depend on temperature, relative humidity and number of eggs produced (Larson and Fisher, 1938, Zaazou, 1948).

Acanthoscelides can also attack pods in the field. The adults are attracted by olfactory cues and the yellow color of the ripe pods (Jarry, 1981, Pouzat, 1981). The females can find bean plants from a distance of at least 500m even when they are surrounded by other crops (Jarry, 1981). The female bores a hole in the ventral suture between two seeds and oviposits into this opening. The eggs may be laid at one site or several (Larson and Fisher, 1938, Lefèvref 1950). *Zabrotes*, however, is rarely reported to oviposit on beans in the field. On non-dehiscent, undamaged pods, the female must lay her eggs on the outside of the pod. The larvae may penetrate the pod wall but not the seed, and therefore, do not survive (Schoonhoven, 1976). *Zabrotes* females show a greater stimulation to oviposit in the presence of bean seeds than bean pods (Pimbert and Pierre, 1983). The mechanism of attaching the eggs directly to the seeds which interferes with survival on non-dehiscent pods, is an advantage with dehiscent pods. Even if the seed should fall to the ground, the eggs are still attached and the larvae can enter the seed (Pimbert and Pierre, 1983).

The initiation of insect dispersal between stocks or from stores to the field is currently under study. Pouzat (1981) notes that dispersal is related to climatic factors, particularly temperature and light. Shinoda (1984) found that the emigration of *Callosobruchus* *chinensis* was dependant on the age of the insect, presence of bean seeds and density of insects. These factors may also affect the bruchids discussed here. The ecology of the insects in the field is also not well understood. Adults may consume dew or nectar but feeding has never been observed in the field (Larson and Fisher, 1938, Leroi, 1981). Lefèvre (1950), working in Zaire, suggested that dropped bean seeds and the seeds of Sesbania sesban provide a site for multiplication of A. obtectus outside of storage.

3. Importance of Insect-caused Losses in Storage

Reported values for losses due to bruchid damage are highly variable due to differences in methods of investigation and measurement. However, loss, as percent of seeds damaged, can be as high as 60% after 6 months (Oliveira and Sudo, 1979) or 95% after 14 months (Lefèvre, 1953). In general, however, losses due to insects in farm level storage are low (less than 5% of seeds damaged) (Dunkel et al., 1986, Durnez and Dejaegher, 1980, Morris 1979). Several factors influencing the lack of damage on-farm are the short period of storage, use of control measures and rapid action if an infestation begins. However, in Burundi, fear of losses due to insects is one reason why farmers sell their excess production as rapidly as possible (ISABU, 1982). The flood of beans on the market early in the season and the resulting lack of beans at the end of the season is one cause of the price fluctuations common in Burundi and Rwanda (ISABU, 1982, Nyabyenda et al., 1980).

The potential for damage in cooperatives and warehouses is greater. Beans put into storage are frequently already infested and monitoring of bulk stocks is difficult and consequently not routine. Storage periods are longer and proper sanitation of the storage structure is more difficult, providing a constant source of reinfestation.

4. Control of Bruchids

Many methods, chemical, physical and cultural, have been used to control A. obtectus and Z. subfasciatus in storage. Plants toxic or deterrent to bruchids such as Capsicum sp. (pepper) can be mixed with the stocks (Golob and Webley, 1980, Lambert, 1985, Lathrop, 1946, Munyemana, 1986). Vegetable oils mixed with the seed asphyxiate larvae (Van Rheenen et al., 1983). Inert dusts, such as clays or ashes, or bean chaff, may be mixed with the seeds to dessicate the insects and/or make it more difficult for them to locate the seeds (Durnez, 1979, Edje, 1984, Lefèvre, 1950, Schoonhoven, 1976). Proper sanitation of storage containers and structures to remove sources of infestation can aid in control. Temperatures and relative humidities above or below the optimal range restrict reproduction and development of the insects. Chemical insecticides, including DDT, pyrethrum, Malathion^R, Actellic^R and Phostoxin^R, have been used against bruchids for many years. Resistance to certain chemicals has already been found in some bruchid populations (Evans, 1985). In the field, intercropping (Huignard, 1979) and early harvest and threshing (Huignard 1979, Labeyrie, 1957) can reduce the damage caused by A. obtectus. Several insect predators, parasites and parasitoids which have potential for biological control of these bruchids have been reported in the field and in storage (Kistler, 1985, Lefèvre, 1950).

In Rwanda, the most common products used by farmers for control of A. obtectus and Z. subfasciatus are ashes, powdered clay and commercial insecticides. Durnez (1979) surveyed farmers in Butare prefecture in 1979 and found that 11 percent used chemical insecticides, 20 percent used ash and 30 percent used clay. In 1985, Dunkel et al. (1986) found that 85 percent of the farmers surveyed used some sort of insect control, with the majority using chemical insecticides. Chemical controls used in the cooperative and government warehouses include Actellic^R (pirimiphos methyl) powder as a residual insecticide and Phostoxin^R (phosphine gas) as a fumigant. In some situations, the prophylactic use of chemical products on all beans for storage is common.

Previous Research on Host Plant Resistance to Bruchids

Genetic resistance of the host plant to damage by an insect species has been studied for many insect-plant interactions. In general, host plant resistance reduces damage by reducing the feeding, reproduction or rate of development of the insect on the plant or by killing the insect with toxic plant compounds. Tolerance; the ability of a plant to support a population of insects with no loss in economic yield, is another form of host plant resistance. Some advantages of host plant resistance include low cost, fewer adverse side effects and residues in the environment, less danger to the user and the potential for long term reliability. Host plant resistance may not be found at a level sufficient for complete control of an insect pest but even low levels can be used to complement or reduce the use of other methods of control.

Host plant resistance to bruchids may occur in many ways. The ripe pods may have surface characteristics or thick walls that prevent the females from laying eggs. The presence of deterrent chemicals on the pods would also prevent oviposition. Seed coats may be too smooth or hard to allow larvae to penetrate. The cotyledons may contain antibiotic compounds or digestion inhibitors, or be of low nutritive value for the larvae. The timing of seed production may be synchronous with the maximum density of parasites or predators or be asynchronous with the maximum density of adult bruchids (Horber, 1978). It is also possible that several factors may act in concert to produce the resistance or that varieties differ in causal factors.

Much of the initial work on resistance in leguminous crops to bruchids was done on Callosobruchus maculatus (Cowpea weevil) on cowpea (Vigna unguiculata). Resistant lines were identified in breeding studies (Fitzner, 1985, Messina and Renwick, 1985, Nwanze and Horber, 1976, Redden and McGuire, 1983, Singh et al., 1985). The results of biochemical studies suggested that higher levels of trypsin inhibitors confer resistance against C. maculatus (Gatehouse et al., 1979, Gatehouse and Boulter, 1983). The specificity of the host range of various bruchids attacking legumes was determined to be related to the ability of a particular species to digest the starches and polysaccharides present in its host plant and not those in other potential host species (Applebaum and Guez, 1972, Applebaum et al., 1970).

Varietal resistance to A. obtectus and Z. subfasciatus has been identified in P. vulgaris germplasm (Pabon et al., 1976, Schoonhoven and Cardona, 1982). Larson and Fisher (1938) also reported differences in preference in a mixture of varieties. Although differences in susceptibility were found in cultivated varieties, higher levels of resistance were measured in wild P. vulgaris accessions (Schoonhoven et al., 1983). Most research has centered on resistance in the seeds themselves. However, resistance has also been identified in pods of cultivated (Cottier, 1948) and non-cultivated (Schoonhoven et al., 1983) P. vulgaris lines. The biochemical basis of resistance to A. obtectus in certain varieties is reported to be affected by levels of arcelin in the seed (Harmsen et al., 1987) and calcium oxalate crystals in the seed coat (Lukando, 1978).

Several studies have been carried out in East Africa. Davies (1962) in Uganda, found that several local varieties differed by 20% in number of seeds damaged by bruchids after six months of storage. Durnez and Gatsinzi (1983) in Rwanda, found the variety Wulma to be more resistant than other tested varieties to attack by A. obtectus. Lefèvre (1950) determined that neither seed coat color nor thickness explained the resistance of Wulma. Pere (pers. comm., 1984, Nat'l Hort. Res. Sta., Thika, Kenya) in Kenya initiated a study on the factors involved in resistance, including: thickness of the seed coat, water content of the seed coat, crude protein and fat content of the seed, seed size and seed coat color and texture (roughness).

GENERAL MATERIALS AND METHODS

All the experiments described in this report were carried out in the Storage Insects Laboratory at ISAR, Rubona, with the exception of that for Hypothesis I C as noted in the corresponding section. Ambient temperatures and relative humidities, in the laboratory, ranged from 20-25° C and from 65-70% RH. The natural photoperiod, for experiments done in the lab itself, was approximately 12 hours light:12 hours dark throughout the year. The equipment used for the experiments included a heated wooden cabinet which served as an incubator, two Blue M (Model # 1680-200) incubators and one LabLine (Model # 3550-1) incubator. The cabinet maintained a temperature of 33° C at the top level and 30° C at the lower level with a relative humidity of 40-45% RH. The incubators were set at 28° C, which they maintained ± 2° C, when closed. As the incubators were of small volume, opening the door caused a rapid change in temperature. Also, the power supply to the laboratory was not always reliable. Changes in temperature in the incubators when the electricity was off could be minimized but not avoided. The relative humidity in the Blue M incubators was 60-65%. The relative humidity in the LabLine incubator was an unacceptably low 25% and consequently this incubator was used as infrequently as possible. None of the incubators had light sources. Consequently the photoperiod was 24 hours of dark with light breaks when the incubators were opened. The wooden cabinet incubator was heated by means of light bulbs. The interior was not completely shielded from the bulbs so the photoperiod was 24 hours of low light with light breaks when it was opened. Experiments were carried out in disposable plastic petri dishes (100 x 15 mm), in 500ml Mason jars with nylon screening in the lids to allow air exchange, or in 9 dram glass vials with loosely attached screw caps.

The bean varieties used for each test will be described in the corresponding materials and methods section. All seed for these tests was produced in the multiplication fields of the component 'Survey of Bean Varieties Cultivated in Rwanda' of the GRENARWA II Research Project, at ISAR, Rubona. Line identification numbers used in these tests are those given in the Final Report of the same component and the Catalogue of Bean Varieties Grown in Rwanda (Lamb and Hardman, 1986). Seed of the variety Rubona 5, an ISAR introduction, was provided by ISAR. Rubona 5 was used as a standard control as it was reported to be susceptible to bruchid damage. One locally purchased mixture and a mixture produced in the ISAR fields were used for rearing the insects for the experiments. All seed stocks were stored in tightly covered plastic buckets (Mironko Plastics Industries, Rwanda) in the laboratory.

The initial collections of insects were made from the ISAR seed storage facilities at Rubona (A. obtectus) and Karama (Z. subfasciatus) in November 1984 and the GRENARWA warehouse at Kicukiro (both species) in January 1985. A later collection of 'wild' insects was made in December 1985 to prevent the development of a 'lab' colony which differed in behavior from natural bruchid populations.

Rearing of bruchids was carried out in the wooden cabinet. Two to three hundred adults of one species were added to approximately 250 g of beans in a 1 liter Mason jar with a mesh lid. Each jar was labelled with the name of the species and the date of infestation. Z. subfasciatus was grown at 33° C and A. obtectus at 30° C, by using the upper shelves of the cabinet for the Zabrotes cultures and the lower levels for Acanthoscelides. Approximately 28 days after infestation, the next generation of adults would begin to emerge and climb the sides of the jar. Adult insects were collected by sieving, using sieves of U.S. Standard No. 10 and No. 20 mesh size (2.00 and 0.85mm openings respectively). The insects were collected from the sieves by means of an insect aspirator. These adults were used to infest new mass cultures or the various experiments. Adults were collected from each culture twice, in general, unless the need for adults was high. To maintain the mass culture at a high level of fitness, adults were transferred to new beans each generation.

Z. subfasciatus was used in the adult stage for all experiments because the larvae cannot crawl to find and infest seeds. For A. obtectus, newly hatched larvae were used to infest most experiments. To produce larvae, 50-100 adults, 1-2 days after emergence, were placed with 5-10 seeds in a petri dish and allowed to oviposit for 3 days in the incubator at 28° C. The adults and seeds were then removed and the eggs returned to the incubator for 4-5 days until they began to hatch. Newly hatched larvae were transferred to test containers with a small (1/4 inch) paint brush.

To prevent the buildup of an free-living population in the lab, spilled seed and escaped insects were removed as rapidly as possible. All seeds and insects used for mass rearing or experiments were frozen for at least 1 week before they were discarded. All equipment used for collecting, transfering and handling insects was washed with hot soapy water after each use to remove any live adults and to reduce the chance of introducing mite populations into experimental containers. Petri dishes were discarded after use.

RESULTS

A. Hypothesis I A - Resistance to *Acanthoscelides obtectus* and *Zabrotes subfasciatus* exists in the seeds of local varieties of dry beans (*Phaseolus vulgaris*).

Varieties differing in genetic resistance to the bruchids *Acanthoscelides obtectus* and *Zabrotes subfasciatus* have been identified in dry beans (*Phaseolus vulgaris*). Sosa et al. (1982?) found differences in the number of seeds damaged by *A. obtectus* in a free choice test of nine bean varieties. Pabon et al. (1976) determined that the potential population increase of *Z. subfasciatus* differed significantly among seventeen varieties. In a test of over 4000 accessions of beans, Schoonhoven and Cardona (1982) measured significant differences for five parameters of resistance to *Zabrotes*. Much higher levels of host plant resistance to both insects were found in non-cultivated wild accessions of *Phaseolus* (Schoonhoven et al., 1983).

In Rwanda, Durnez and Gatsinzi (1983) tested five ISAR-released cultivars of dry beans for resistance to *A. obtectus* and found differences in the percent of seed damaged. The present study was initiated to determine if locally grown varieties, traditional or introduced, also vary in level of resistance to *A. obtectus* and *Z. subfasciatus* and if this resistance is of a sufficient level to be used for control of these insects in stored beans.

1. Materials and methods:

The seed lines used in this study were collected and multiplied by the component 'Survey of Bean Varieties Cultivated in Rwanda' of this project. After harvest and threshing, the seed was stored in covered plastic buckets at ambient temperature in the Storage Insect Laboratory at ISAR, Rubona. Insects were maintained and collected as described in the preceding section of this report. All tests were conducted in an incubator at 28^o C and 60% RH.

Separate experiments were set up to screen for resistance to each of the bruchid species. An experiment consisted of five replicates of 49 lines and Rubona 5 (R5) as a standard check. R5 was previously determined to be intermediate in resistance. The methods developed for screening tests were based on previously published reports (DeLuca, 1968, Schoonhoven and Cardona, 1982, Thiery, 1984), personal communication (Dobie, 1985, Tropical Development and Research Institute, Everett, 1984, International Institute for Tropical Agriculture) and availability of materials. Fifteen undamaged, well-filled seeds of a seed type were placed in a plastic petri dish (100x15mm). Two pairs of one to two day old *Z. subfasciatus* adults or ten newly hatched larvae of *A. obtectus* were added to the dish. Each dish was labelled with the name of the insect species, the seed line identification number and the date of infestation. The lines and replicates were randomized before being infested. After infestation, the dishes were taped together in stacks of ten and the stacks were placed at random in the controlled environment chamber. Three weeks after the dishes were infested, they were examined daily for presence of adults. When adults began to appear, the number of adults emerging was recorded every two days for approximately two weeks.

The experiments using A. obtectus were run April 30 - June 4 (Test 1), June 24 - July 31 (Test 2), September 23 - November 4 (Test 3), November 5 - December 11 (Test 4) and November 20- December 30, 1985 (Test 5). The tests with Z. subfasciatus were done March 15 - April 22 (Test 1), April 29 - June 4 (Test 2), June 24 - July 31 (Test 3), August 13 - September 21 (Test 4), September 10 - October 21 (Test 5) and October 29 - December 9, 1985 (Test 6). All experiments were analyzed as completely randomized designs. Mean comparisons were made using the Student Newman Keuls test at a significance level of 5% (Steel and Torrie, 1980).

Results and discussion:

The results of the tests of resistance to A. obtectus are given in Table 1. The varieties were rated on the basis of mean number of adults emerging from the infested seeds. Data were insufficient or results non-significant for Tests 2, 3 and 4 so only Tests 1 and 5 are reported. In Test 5, no adults emerged from the seed of 18 of the varieties tested. These lines are not included in the list of `least susceptible' lines as it is unclear what caused the lack of emergence. The results of the tests of resistance to Z. subfasciatus are given in Table 2. In these tests, the varieties were rated on the basis of mean days to emergence of adults. Only Tests 1, 3, 4 and 5 are included. In total, 118 lines were screened for resistance to A. obtectus and 155 lines for resistance to Z. subfasciatus in the tests reported here.

None of the lines were significantly different from R5 which was intermediate in all tests. The results are presented as lines differing significantly from the most or least susceptible variety in each test. Although the extreme varieties (most and least susceptible) vary from test to test, this measurement does give an indication of the relative resistance of the varieties screened. The range of resistance values for each test reported is given in Tables 3 and 4. The values and the ranges vary greatly from test to test in part because of the lines used in each test but primarily because of the great variability within and between tests.

Variability in results was a major problem in this study. An effect of location of the experimental units in the controlled environment chamber was noted. The stacks of petri dishes were randomized in location to prevent confounding of location with seed line. However, such location effects increase the variation within lines. To examine test to test variability, one set of 49 varieties was screened twice for resistance to Z. subfasciatus. The results of the second test were non-significant but the lack of agreement of results is suggested by the fact that the most susceptible line in Test 1 was the least susceptible in Test 2. However, the results of this comparison did illustrate two

Table 1. Local bean varieties differing significantly (Student Newman Keuls 5%) from the extreme varieties in tests of resistance to _Acanthoscelides_ _obtectus_.

	Lines significantly different from extreme						
Test 1. (ANOVA **)[1]							
Most susceptible (135, 138)	16	42	112	126	150	157	269
Least susceptible (16, 126, 269)	114	135	138				
Test 5. (ANOVA *)							
Most susceptible (243)	28	43	71	107	109	121	128
	170	176	178	198	219	281	
Least susceptible (many zeros)	243						

[1] Level of significance from ANOVA
* 5%
** 1%

Table 2. Local bean varieties differing significantly (Student Newman Keuls 5%) from the extreme varieties in tests of resistance to *Zabrotes subfasciatus*.

	Lines significantly different from extreme							
Test 1. (ANOVA **)[1]								
Most susceptible (269)	33	44	53	68	86	211		
Least susceptible (53)	5	19	39	112	212	247	269	
Test 3. (ANOVA *)								
Most susceptible (158)	23							
Least susceptible (23)	5	15	20	31	40	97	115	158
	160	190	214	238				
Test 4. (ANOVA **)								
Most susceptible (117)	21	118	260					
Least susceptible (21)	2	31	40	117	196	231		
Test 5. (ANOVA *)								
Most susceptible (29)	70	246	CP4b					
Least susceptible (CP4b)	29	38	45	103	125	131	251	

[1] Level of significance from ANOVA
* 5%
** 1%

Table 3. Range in mean number of emerged adults of *Acanthoscelides obtectus* for the most and least susceptible local bean varieties in Tests 1 and 5.

	Test 1	Test 5
Most susceptible varieties	5.8 - 6.6	2.4
Least susceptible varieties	0.4 - 1.2	0.2

Table 4. Range in mean days to emergence of adult *Zabrotes subfasciatus* for the most and least susceptible local bean varieties in Tests 1, 3, 4 and 5.

	Test 1	Test 3	Test 4	Test 5
Most susceptible varieties	25.0-25.7	28.6-29.7	29.5-29.9	26.5-27.5
Least susceptible varieties	26.7-27.4	32.3	32.3-32.5	29.2-30.2

concerns that must be considered. The standard screening test must be modified to reduce variation in results from test to test or each variety must be tested in several screening tests to ensure more accurate results. In addition, the variety chosen as the standard check must be as consistent as possible in its reaction to screening. When possible, the standard should be highly susceptible. The difficulties caused by the use of an intermediate standard such as R5 have already been noted.

Another problem is inherent in the method used for measuring resistance to A. obtectus. Emergence of a large number of adults indicates suitability of the seed for development of the insect, i.e. susceptibility. However, a score of zero or a low percent emergence could be a result of the death of the larvae due to insuitability of the seed or some other factor, such as environment, age of the larvae, larval diseases, etc.. In other words, low scores could be an indication of high resistance or could be `escapes'. Including low values due to escapes in the calculated means skews them falsely toward a classification of `resistant'. Upon retesting, such lines are more likely to change in classification than lines rated as susceptible. One case where a rating of susceptibility could be false is if seeds with damaged seed coats were used. This would allow free access of the larvae to the cotyledons, bypassing resistance factors involving the seed coat.

Schoonhoven and Cardona (1982) measured five different parameters in their evaluation of resistance. For this study, data collected on number of eggs laid or larvae used and number of adults emerging every two days would have allowed the calculation of total number of adults emerged, mean days to emergence and percent emergence. However, it was difficult to accurately count the eggs of Zabrotes, either immediately after they were laid or later when they became opaque. Percent emergence values based on numbers of eggs counted for the first tests were as high as 164%. Because of the differences in seed coat color and pattern in the lines used, we could not assume equal inaccuracy in egg counts across lines. This made it impossible to calculate percent emergence for Z. subfasciatus. Differences in number of adults emerging for Z. subfasciatus were nonsignificant, due to large variations between replicates in number of eggs laid. For A. obtectus, the mean days to emergence were calculated for those seed lines with non-zero results for at least three of the five replicates. Differences in mean days to emergence were nonsignificant, with a range in means of only 2.7 days.

Dry weight of emerged adults was also considered as a possible measure of resistance. For one set of lines for each insect species, the dry weight of the adults from each petri dish was measured. Weights were taken after freezing the adults removed from the seeds and then letting them thaw and dry at room temperature. The results were nonsignificant for Z. subfasciatus. For A. obtectus, the range of means was 1.4 to 2.1 mg per adult, significant at the 5% level. Mean dry weight of adults for lines classed as most or least susceptible showed no association of resistance with adult dry weight.

Schoonhoven et al. (1983) found a correlation of small seed size with resistance to bruchids. The percent of the seed lines in each resistance class by seed weight is given in Table 5 for both insect species. For the A. obtectus experiments, those lines classed as most susceptible include only large seeded lines. The lines rated least susceptible are predominantly in the small and medium classes, although some large seeded types are present. For the varieties tested with Zabrotes, the overall means and ranges vary little by susceptibility class. However, a large percentage of lines in the least susceptible class are small seeded. For the most susceptible class, medium size seeds predominate.

The association of seed size and resistance is not due entirely to the amount of food available for the larvae. Unlike some of Schoonhoven's wild seed types which weighed less than 10 g/100 seed, few of the lines tested in these experiments weigh less than 20 g/100 seed and all are large enough to support the development of several larvae. The experiments described here are all no-choice experiments, so simple preference of the insect for large seed was not a factor. However, characters other than size of food source or preference may influence the number of larvae developing in a seed. Zabrotes adult females could assess the number of eggs already laid on a seed relative to the seed size and not lay more than a certain number regardless of the lack of other oviposition sites. The time necessary for larval development could be increased by the presence of more than one larva in a seed. A single successful penetration by an Acanthoscelides larva may be used by several larvae (Thiery and Jarry, 1985), concentrating the larvae in a small number of seeds. Competition during insect development in small seeds would also reduce the number of adults emerging.

In addition to determining if significant differences occur between seed lines for resistance to the two bruchid species, it is important to examine the levels of resistance and determine if it is sufficient to be of use as a control measure. The mean number of adults emerged from the lines used in Test 1 for A. obtectus varied from 0.4 to 6.6. If emergence could be reduced 62%, the immediate and long term damage to a stock of bean seed could be greatly reduced. The mean days to emergence of Z. subfasciatus over all varieties, although statistically significant, ranged from only two to four days. This resistance is not sufficient to be useful in controlling the buildup of populations of Z. subfasciatus in stored beans. It appears that, using the methods described here, the potential for resistance of ecomonic value to A. obtectus, but not to Z. subfasciatus, can be found in local bean varieties.

The same varieties were tested for resistance to both bruchid species and a comparison of varieties resistant to each was intended. Seventy-seven of the lines having significant results were tested with both species. Only two lines (112 and

Table 5. Mean, range and distribution of seed weight of local bean varieties tested for resistance to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*.

Class	Mean (g/100	Range seed)	Percentage Small	Medium	Large
Acanthoscelides obtectus					
Most susceptible varieties	44	41-46	--	--	100
Least susceptible varieties	31	15-46	40	45	15
Zabrotes subfasciatus					
Most susceptible varieties	31	18-48	37	50	12
Least susceptible varieties	27	18-42	77	15	8

169) are included in the results for both insects. In both cases, the lines are most susceptible when infested with *Zabrotes* and least susceptible when tested with *Acanthoscelides*. Although the number of lines available for comparison is small, the results suggest that the factors conferring resistance to the two species differ in at least some varieties.

It is interesting to compare the potential for development of resistance between species. *Z. subfasciatus* occurs in a small part of the country, so only those seed lines grown in that area would be under natural selection for resistance to this species, if it differs from resistance to *A. obtectus*. The lines tested in these experiments were collected throughout the country so a preponderance of lines not selected for resistance to *Zabrotes* would be expected to be included. *A. obtectus* is found throughout Rwanda, although it is less of a problem in the regions of highest elevation. Therefore, all the seed lines tested would have been exposed to this insect and could have been selected for resistance. Natural selection for undamaged seeds may be weak, as damaged seeds will germinate and grow, although they are more susceptible to attack by seed rotting organisms. Selection for resistance by farmers could be conscious or unconscious. If damage caused by *A. obtectus* is a major cause of loss or if certain varieties in a mixture are very obviously susceptible, selection against damaged seeds or susceptible varieties would be very strong. If the susceptibility of a variety to bruchid damage is only one of many factors used in selecting seed for planting or if only undamaged, well-filled beans are used for seed, the selection for resistance would be less strong. Information from the Varietal Survey (Lamb and Hardman, 1986) suggests that resistance to bruchids per se is not a character of major importance to the farmers in selecting which varieties are grown in Rwanda.

Conclusions and recommendations:

1. Significant differences in the mean number of emerged adults of *A. obtectus* and mean days to emergence for *Z. subfasciatus* were found in seed lines of beans collected in Rwanda. The resistance to *A. obtectus* is of a usable level and could be included in a program for control of insect damage to beans in storage.

2. The lines with the greatest resistance to *A. obtectus* were 16, 28, 42, 43, 71, 107, 109, 112, 121, 126, 128, 150, 157, 170, 176, 178, 198, 219, 269 and 281. Greatest resistance to *Z. subfasciatus* was measured in lines 21, 23, 33, 44, 53, 68, 70, 86, 118, 211, 246, 260 and CP4b.

3. A trend towards an association of seed size with resistance to both bruchid species was indicated by these results. Large seeded bean varieties were more frequently classed as susceptible than resistant.

4. Improvement of the methodology of screening to ensure the lowest possible variability between replicates in experiments and

between experiments is crucial. Methodology may be dependent on availability of seed, containers and space but within these constraints, the most accurate method must be determined.

a. Varying the number of seed of a standard variety and the number of insects in a set of screening tests allows the calculation of a coefficient of variation for each combination. The lowest coefficient indicates the best combination. The volume and size of the container may affect the success with which the larvae or adults find the seed. Therefore, the seed number/insect number tests should be carried out in a standard sized container.

b. The chosen method should be tested on a set of varieties reported to differ in resistance level. It is essential that the screening method allow a clear differentiation of susceptible and resistant lines.

c. The standard variety should be examined in a series of tests to determine the variability in results to be expected. A resistant as well as a susceptible standard should be used in all tests.

d. Eggs rather than larvae should be used in tests with A. obtectus as they are less likely to be damaged during handling. All eggs must be of known age and separate tests of percent hatch must be carried out for each source and age of eggs.

e. Methods for accurately counting the eggs of Zabrotes should be investigated.

f. Variability due to location in controlled environment chambers should be measured. The use of randomization and blocking in experimental designs should be used to reduce location effects.

g. Several parameters of resistance should be measured for each line because resistance may be the result of multiple factors, not all of which are well identified.

h. The use of a multi-part test as described in Schoonhoven and Cardona (1982) should be considered. (Numbers in parentheses refer to quantities used in the report.) A large number of lines (4000) is tested using a small number of replicates (1) and few parameters (1) are measured in the initial phase. Those lines scored as susceptible are not retested. Subsequent tests use a larger number of replicates (3) and measured parameters (5). After each phase, the susceptible lines are discarded. Schoonhoven (1976) states that at least three generations of a seed line must be screened to ensure a classification of resistance.

i. All lines tested in this experiment should be retested with the improved methods to ensure that the reported results are accurate.

5. Once varieties of high resistance and susceptibility have been identified, further experiments are possible.

a. The mechanisms of resistance can be examined.

b. Field tests of the economic value of the resistance are needed to determine what levels of resistance are necessary to control the insects in storage on farms or in warehouses.

c. Breeding for increased resistance is possible.

B. Hypothesis I B - Resistance to Acanthoscelides obtectus exists in the ripe pods of local varieties of dry beans (Phaseolus vulgaris).

Several species of bruchids are known to attack pods of leguminous crops in the field before harvest, including Callosobruchus maculatus on cowpea (Vigna unguiculata) (Fitzner et al., 1985, Messina, 1984), Callosobruchus chinensis on mungbean (Vigna radiata) (Talekar and Lin, 1981) and Zabrotes subfasciatus and Acanthoscelides obtectus on common bean (Phaseolus vulgaris) (Jarry and Chacon, 1983, Labeyrie, 1957, Labeyrie and Maison, 1954, Pimbert and Pierre, 1983). Of these, only A. obtectus females bore holes into the pod and deposit the eggs on the seeds rather than relying on the larvae to bore through the pod wall to reach the seed or requiring dehiscent or damaged pods as a site to lay eggs. This behavioral trait results in reduced mortality of eggs and pre-penetration larvae and greater potential for damage to seeds still in the field. Acanthoscelides is the most important bruchid pest of beans in the field in Rwanda and newly harvested seeds are an important source of infestation for storage.

Varietal differences in susceptibility to bruchid oviposition on pods have been found for C. maculatus and C. chinensis on their respective hosts (Fitzner et al., 1985, Messina, 1984, Talekar and Lin, 1981). The results of laboratory studies on varietal differences in the resistance of ripe pods of several Rwandan bean varieties to oviposition by A. obtectus are described in the following report.

Materials and methods:

Pods of the ten varieties of beans used in this experiment were collected from a seed increase field at ISAR, Rubona at the yellow/mature-but-not-dry stage. The varieties were chosen to cover a range of seed types and pod colors (Table 6). Experiments were carried out at the ambient temperature and relative humidity of the laboratory and natural daylength (12/12 hour light/dark) in 500ml Mason jars with nylon mesh lids. Five pods were used per jar (1 jar = 1 replicate) and 6 replicates of each variety were used. Rubona 5 (R5) was included as a standard check variety. The jars were arranged in a completely randomized design.

Each jar was infested with 5 pairs A. obtectus adults, 1-2 days after emergence, on 28-29 May, 1986. The insects were selected from the experimental colony maintained at ISAR, Rubona. After one week, on 5 June, 1986, the insects were removed and the number of eggs laid per pod was counted. An analysis of variance was calculated for the number of pods used for oviposition and the mean number of eggs per pod. Means were compared with the Student Newman Keuls test (Steel and Torrie, 1980).

Results and discussion:

The number of pods used for oviposition by the insects differed significantly by variety (Table 7). The mean values ranged from 1 pod for variety 184 to 4 pods for variety 7 and the

Table 6. Local varieties used in the test of resistance of bean pods to oviposition by *Acanthoscelides obtectus*.

Variety	Pod color	Pod maturity
6	green	early
7	purple	intermediate
11	green	early
30	pink	late
36	green	late
159	speckled	early
184	speckled	early
232	pink	late
275	speckled	intermediate
R5	green	early

Table 7. Mean number of pods used for oviposition by *Acanthoscelides obtectus* by variety. (ANOVA significant at 1%)

Variety	Mean number of pods	SNK (1%)[1]	
184	1.5	│	
6	1.8	│	
11	2.3	│	│
36	2.3	│	│
232	2.3	│	│
30	2.7	│	│
275	2.8	│	│
159	3.0	│	│
R5	3.7		│
7	3.7		│

[1] Varieties connected by a line are not significantly different. SNK 1% = Student Newman Keuls test at a significance level of 1%

susceptible control R5. The number of eggs laid per pod also varied significantly (Table 8). The ranking of varieties in this case was reversed compared to the previous table with the lowest mean number of eggs for variety 7 and the highest for variety 184. The total number of eggs per replicate over all varieties was not significantly different. There was no significant difference in the number of pods used for oviposition or the number of eggs laid per pod by the date of infestation or by the location of the jar on the bench.

There was an inverse relationship between the number of pods used for oviposition and the number of eggs laid per pod over all varieties (Table 9). Although a high number of eggs per pod seems an inaccurate expectation for less suitable varieties, there is a behavioral basis to the observation. Acanthoscelides females will oviposit into holes in the pod bored by other females if they cannot bore an opening themselves. Therefore, in an unsuitable variety, any holes which are bored will be used by several females and the number of eggs per hole will be very high.

The experiment described in this report was a 'no-choice' test, i.e. each variety was presented to the insects separately. In Rwandan field situations, however, mixtures are grown and preference for variety could be a factor. Preliminary results of laboratory tests with mixtures of varieties of pods suggest that preference does occur. The preference is reflected in a marked differentiation of varieties suitable and unsuitable for oviposition.

Several factors have been suggested to affect pod suitability for bruchid oviposition. Messina (1984) suggests that smoothness of the pod surface may affect oviposition. Labeyrie and Maison (1954) and Pimbert and Pierre (1983) have studied pod maturity as a factor in the oviposition preference of A. obtectus. Information available on pod color and maturity (Table 6) for the varieties used in this test do not lend support for either characteristic as a factor in determining the site of oviposition.

Suitability of pods for oviposition is not necessarily related to susceptibility of seed to damage by larvae. For this reason, survival to adult emergence may be reduced in a variety having pods susceptible to oviposition. The varieties tested for suitability for oviposition were also tested for resistance to seed damage by A. obtectus in section I A. None of the varieties used in the tests of pod suitability were ranked as either highly susceptible or highly resistant in the tests of seed resistance. Another factor which may affect adult emergence is larval competition. Where large numbers of eggs are laid in a single pod, as in the pods of unsuitable varieties in this test, the number of larvae searching for development sites may exceed the number of larvae which can successfully develop in those seeds.

Resistance in the field is of less importance to the development of an insect population in storage than is resistance in the dry seed. Resistance to oviposition in the field reduces one source of the initial population of insects in the stock. However, the final size of the insect population is determined by

Table 8. Mean number of eggs laid per pod by *Acanthoscelides obtectus* by variety.
(ANOVA significant at 1%)

Variety	Mean number of eggs/pod	SNK (1%)[1]
7	28.2	│
275	34.7	│
R5	40.8	│ │
159	47.3	│ │
232	47.7	│ │
36	49.7	│ │
30	54.5	│ │
11	60.5	│ │
6	61.2	│ │
184	76.3	│

[1] Varieties connected by a line are not significantly different.
SNK 1% = Student Newman Keuls test at a significance level of 1%

Table 9. Mean number and range of eggs/pod by number of pods used for oviposition by *Acanthoscelides obtectus* over all varieties of beans.

Number of pods used for oviposition	*n*	Mean number of eggs/pod	Range of number of eggs/pod
1	10	80 ± 8	50-125
2	18	49 ± 6	10-100
3	23	43 ± 3	15-75
4	12	37 ± 3	26-50

maintenance of the stocks, environment and length of storage as well as the initial population size. Resistance of the seed to attack will affect all generations of the insect, not the initial generation alone, and acts to slow the development of the insect population.

The sylvatic reservoir and source of adult insects ovipositing in pods in the field has not been firmly established. In Rwanda, bean plants are harvested whole, when the pods are yellow but not dry, and the final drying of the plants and pods occurs in the area around the house. This allows bruchids from the storage area to move easily into the drying beans. Labeyrie (1957) measured significant increases in attack of pods by A. obtectus with an increase in the length of time between maturity and harvest and harvest and threshing. These peak stages for attack occur in proximity to a potential source of insects (stocks or storage areas already infested). Thus, the presence of a `wild' population is not necessary for contamination of newly harvested beans.

Conclusions and recommendations:

1. Resistance or suitability of P. vulgaris pods to oviposition by A. obtectus females differs with variety and can be measured as number of pods used for oviposition. In this test, varieties 6 and 184 were the most resistant.

2. Pod resistance to A. obtectus reduces the initial insect infestation but seems to have no effect on subsequent development of the insect population in stored beans. Suitability of pods for oviposition may not be related to suitability of seeds for larval development.

3. Possibilities for future research:

a. Measurement of survival to adult emergence in addition to number of pods used for oviposition. Compare varieties with i) nonresistant pods with resistant seeds, ii) nonresistant seeds with resistant pods, iii) nonresistant seeds and pods and iv) resistant seeds and pods.

b. Timing of insect use of pods in field and near house after harvest in view of modifying cultural practices of harvest and drying.

c. Field tests of resistance compared to laboratory results.

d. Further tests of insect preference for oviposition site in the field and laboratory.

C. Hypothesis I C - The relative level of resistance to *Acanthoscelides obtectus* of a bean variety is independant of relative humidity and temperature in the range of environments typical of Rwandan storage conditions.

Acanthoscelides obtectus can develop and reproduce under a wide diversity of environmental conditions. Shepard (194?) reported survival within the ranges of 17 to 34° C and 20 to 98% relative humidity (RH). The optimum conditions, which result in the most rapid population increase, are reported to be 27 to 31° C and 50 to 75% RH (Howe, 1965, Lefevre, 1950, Schwartz and Galvez, 1980, Shepard, 1947). Varying the temperature and/or RH away from the optimum affects the behavior and development of the insect. Menusan (1934) found that the days to emergence of adults decreased with increasing RH from 10 to 90% and increased with decreasing temperature from 31 to 17° C. Zaazou (1948) reported an increase in number of eggs laid as temperature was increased from 15 to 25° C and as RH increased from 30 to 75%. Perttunen (1972) found that *A. obtectus* adults showed a consistent preference for lower RH's in a two choice test unless they had been previously dessicated.

Most tests of resistance are carried out under optimum temperature and RH conditions to allow accurate identification of resistant varieties. However, measurements of resistance under varying or non-optimal environmental conditions are rarely reported. The temperatures at which beans are stored in Rwanda vary with elevation and type of storage container and building, e.g. basket or metal drum, thatch roofed house, concrete silo or metal roofed warehouse. Relative humidity in storage varies with the season and with initial grain moisture content. Thus, to be a useful component of a control program, the level of genetic resistance must be consistent over a wide range of conditions. In this study, a set of varieties of known resistance at optimal conditions was infested with *A. obtectus* under a range of environments typical of Rwandan storage conditions.

Materials and methods:

Three RHs, 33, 54 and 69%, were chosen to simulate the RH range of Rwanda and to correspond to possible moisture content values of beans put into storage. The RHs were created and maintained using saturated salt solutions in Nalgene plastic dessicators (Table 10). The RH levels were not monitored within the dessicators. Saturated salt solutions produce relative humidities within a range about the reported value. In addition, temperature affects the water activity of the salt, influencing the RH produced. Still it is expected that the variation in RH values for a given salt is smaller than the variation of the values between salts. Temperatures were not regulated but were approximated by conducting the test in three GRENARWA warehouses where beans are stored; one in each of the major elevation zones. Actual temperatures and ambient RH values of the three locations were measured over the period of the test (Table 11). Although the Kibungo warehouse is at a lower elevation than the one at

Table 10. Relative humidities used in testing the environmental stability of resistance in beans to *Acanthoscelides obtectus*.

Relative humidity (%)	Moisture content of the beans corresponding to the RH[1] (%)	Salt used to maintain %RH
33	9	MgCl
54	12	$Mg(NO_3)_2$
69	15	KI

[1] (Edmister, pers. comm., 1985, OPROVIA, Kigali, Rwanda)

Table 11. Locations in Rwanda used in testing the environmental stability of resistance in beans to Acanthoscelides obtectus.

Location	Elevation zone	Elevation (m)	Temperature over period of test Min (°C)	Max (°C)	Mean (°C)	Mean RH over period of test (%)
Kora	High	2430	12	22	17	62
Nyanza	Medium	1760	20	28	24	51
Kibungo	Low	1675	18	25	21	50

Nyanza, the average temperature is lower. The Nyanza warehouse is metal roofed and the internal temperatures were higher than expected. In each warehouse, the dessicators for the experiment were placed adjacent to the hygrothermographs used to monitor the environmental factors. At Kora and Kibungo the dessicators were placed on cardboard over the cement floor and at Nyanza, they were placed on a table. Because temperature varied with time over the period of the test, results are discussed in terms of `location' rather than `temperature'.

Seven varieties were chosen, based on the results of preliminary varietal screening tests, to include both resistant and susceptible varieties (Table 12). Rubona 5 was used as the standard check. Until the beginning of the experiment, the seeds of all varieties were stored in covered plastic buckets at the ambient temperature of the laboratory. Fifteen sound seed of a variety in a 9 dram screw-top glass vial made up one replicate and each variety was replicated five times within each RH/location treatment. The insects used in this study were supplied from the mass culture of A. obtectus described previously. Eggs were collected and allowed to hatch in an incubator at 28° C and 50% RH before infestations were made. Because the warehouses could not all be visited on the same day, the age of the larvae used varied somewhat between sites. Ten actively moving larvae were selected to infest each vial. Vials were infested in random order. The caps were then replaced loosely and the vials placed in the dessicators at random.

The experiment was designed as a split split plot with locations as main plots, RHs as subplots and varieties as subsubplots. Neither locations nor RHs within locations were replicated due to lack of equipment. Infestations were made June 6-10, 1985 and the vials examined for adults in July. The numbers of adults which emerged per vial were counted August 7-28, 1985.

Results and discussion:

Counts were made 58, 59 and 62 days after infesting at Kibungo, Kora and Nyanza respectively. The number of adults per vial ranged from 0 to 10. No insects were recovered in the vials from the Kora warehouse at the initial counting. When these vials were placed in an incubator at 28° C for two weeks, adults did emerge from the seeds. This indicates that the environment at Kora, notably the low temperature, did not kill all of the insects but increased the length of the developmental period. The reduction in the means relative to the other locations may be another result of the low temperatures. These results are supported by Menusan (1934), who found that at a temperature of 17.3° C, the duration of the larval and pupal stages was 78-100 days at RH values between 25-80%. The results from Kora are not included in the following discussion.

The measurement of resistance as affected by environment is complicated by the fact that both plant resistance and insect development are measured in the same units, such as time or number of adults emerged. It is for this reason that farmers, in

Table 12. Local varieties used in testing the environmental stability of resistance in beans to *Acanthoscelides obtectus*.

Variety	Relative resistance class
24	Susceptible
68	Resistant
152	Resistant
191	Intermediate
200	Intermediate
210	Susceptible
248	Resistant
R5	Intermediate

the high elevation regions, when asked which bean varieties were resistant to insect damage in storage, responded that all their varieties were resistant (Lamb and Hardman, 1986). In fact, the lack of damage was due to slow insect development at low temperatures. However, the interaction of plant genotype with environment can be illustrated by the ranking of a set of varieties in a series of environments. If the change in number of emerging adult A. obtectus is due only to the effect of environment on the insect, the means of the varieties may increase or decrease but the ranking of the varieties will remain constant. The ranks of the eight varieties with location and RH are listed in Tables 13 and 14. Separate ANOVAs by location and RH resulted in few significant differences between varieties but the rankings do suggest trends. There is little difference in the ranked order of varieties when tested at Nyanza and Kibungo. However, the rank of certain varieties, notably 68, 152 and 200, changes dramatically when tested at the three different RHs. The patterns of ranks for these three varieties are very different, suggesting different mechanisms for the interaction of genotype and environment.

Lefevre (1950) noted an interaction between temperature and RH on the development and reproduction of A. obtectus. Therefore, in addition to the single factor interactions of variety with location or RH, there is possible a three way interaction of variety, location and RH, as shown in Table 15. The interactions show great variability and are difficult to interpret. In most combinations, variety R5 is ranked low while varieties 24 and 191 are ranked high, with some inconsistencies. Varieties 200, 210 and 248 tend to have intermediate rank values. Varieties 68 and 152 are the most variable in rank.

The results show trends of reaction to temperature and RH (Figures 4 and 5) although differences between means are not significant. In general, more adults emerged in the vials at Nyanza than in those at Kibungo. Other researchers have also reported an increase in number of emerged adults with increasing temperature (Menusan, 1934). However, five of the eight varieties showed a decrease in number of adults emerging with increasing RH, completely opposite to reported changes in emergence with RH (Menusan, 1934). Thirty-three percent RH approaches the minimum RH for A. obtectus (Howe, 1965, Zaazou, 1948) so it is surprising that the greatest emergence was found in that environment. Preliminary tests showed no significant differences in mortality caused by the vapors from the salts used to maintain the RHs. Temperatures at both locations were well within the adequate range for the development of this insect and an interaction of high or low RH with intermediate temperature seems unlikely.

Although the mechanism(s) of resistance to A. obtectus in Rwandan bean varieties have not been determined, the physical or chemical factors reducing insect damage are a potential site for the interaction of temperature and RH with variety. Seed coat hardness or thickness may prevent larvae from entering the seed.

Table 13. Varieties ranked by mean number of <u>Acanthoscelides</u> <u>obtectus</u> adults for test locations, Nyanza and Kibungo. (Ranks listed low to high.)

Nyanza	Kibungo
R5	R5
248	210
210	248
152	152
68	68
200	200
191	191
24	24

Table 14. Varieties ranked by mean number of <u>Acanthoscelides</u> <u>obtectus</u> adults for relative humidities. (Ranks listed low to high.)

33	54	69
R5	152	R5
68	R5	200
210	248	248
248	210	210
152	200	191
191	191	152
24	24	24
200	68	68

Table 15. Varieties ranked by mean number of *Acanthoscelides obtectus* for location by relative humidity interactions. (Ranks listed low to high.)

Nyanza 33	Nyanza 54	Nyanza 69	Kibungo 33	Kibungo 54	Kibungo 69
R5	R5	R5	R5	248	R5
68	152	248	68	152	191
248	210	200	210	210	210
210	248	210	248	24	248
152	200	68	152	R5	200
191	191	152	200	200	24
24	68	191	24	191	152
200	24	24	191	68	68

Figure 4. Mean number of emerged Acanthoscelides obtectus adults by variety and location of test.

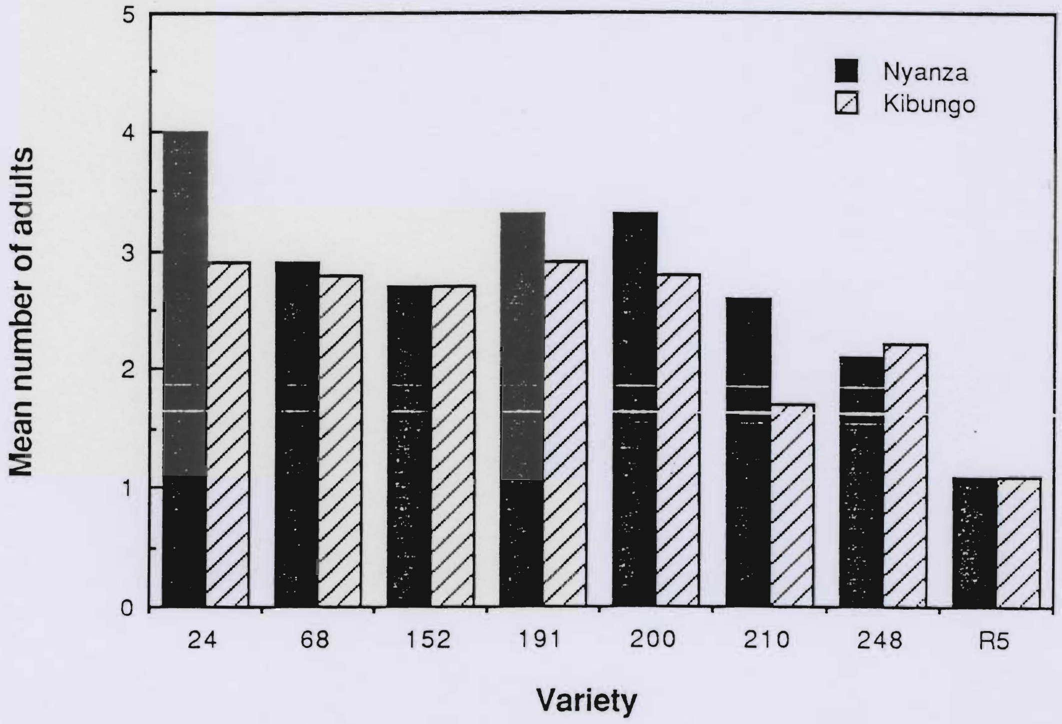
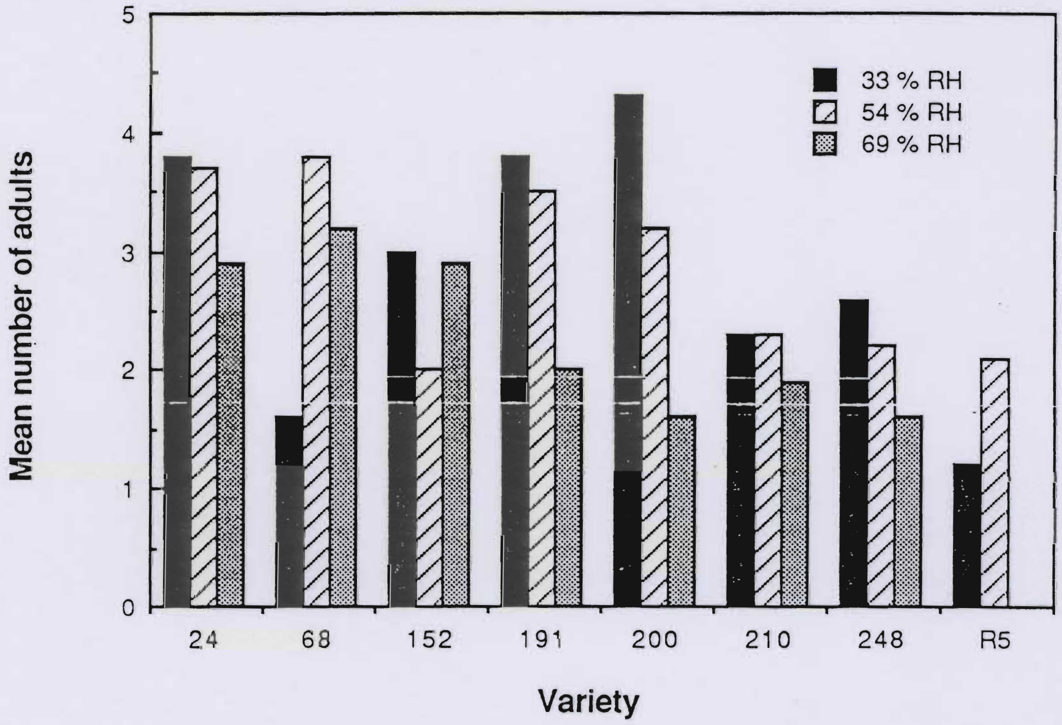


Figure 5. Mean number of emerged <u>Acanthoscelides</u> <u>obtectus</u> adults by variety and relative humidity.



1 Value for R5[1] at 69% RH was zero.

The physical characteristics of the seed coat, and consequently its effect on resistance, are affected by the RH/moisture content and its interaction with temperature. Lipman (in Larson and Fisher, 1938) reports that low RH prevents larvae from entering the seed. Volatile components of particular varieties may act as attractants or deterrents for the adults or larvae. Low temperatures would reduce the chemical activity of these compounds, reducing their effect on insect behavior. Menusan (1934) found that the weight of emerging adults increased as the days to emergence increased under lowered temperaturees. A variety may be nutritionally sufficient for an insect to survive during a short developmental period but lack the food reserves or nutritional balance to sustain an insect during a prolonged development at low temperatures.

Conclusions and recommendations:

1. The results of this experiment suggest that environment affects the expression of resistance to A. *obtectus* in dry beans. Not all varieties are affected equally and the differences may be caused by the effect of temperature and RH on the physical or chemical factors causing the resistance. The variation in resistance with environment is important if varieties are released or recommended as resistant for use in an area of widely variable temperature or RH. Where production practices result in storage of beans with varying moisture contents in structures which may raise the storage temperature above normal temperatures, such information may also be important.

2. This experiment should be repeated to verify the results and allow for further interpretation of these preliminary results. In particular:

a. Temperature and RH should be more closely monitored and controlled, both at the site of the test and within the dessicators.

b. A control at optimum temperature and RH should be included in the experiment. If several measures of resistance are taken, e.g. percent emergence and days to emergence, the appropriate optima for each must be used, as they are not necessarily the same (Howe and Currie, 1964).

c. The seeds used in the tests should be equilibrated to the appropriate temperature and RH before infestation.

d. Sufficient replication should be included to allow statistical tests of all interaction effects. Replication in space is preferred to replication in time.

3. The results from Kora suggest that low temperature, as a method of insect control, is more effective than resistance at higher temperatures. A more accurate comparison would require a test of insect developmental period and percent emergence over several generations at the various locations.

Hypothesis I D - Resistance to Acanthoscelides obtectus and Zabrotes subfasciatus is characterized by a reduction in the rate of growth of the insect population on a resistant variety relative to that on a susceptible variety.

Screening tests to identify varieties resistant to insect damage are usually designed to be rapidly and easily carried out. The measurements of survival or number of progeny are made during a single generation of the insect population. However, beans in storage are in contact with their bruchid pests throughout the development of the population. The pressure on a resistant variety, therefore, changes as the population increases and more seeds are damaged. This experiment was designed to study the longer term effects of resistant varieties on the development of bruchid populations.

Lefèvre (1950) in Zaire and Davies (1962) in Uganda studied the development of Acanthoscelides populations in local mixtures and varieties. In both tests, infested stocks were sampled each month for a year. The authors found differences in insect numbers by variety and by location, but the results were highly variable due to sampling methods. Longstaff (1981) discusses the theoretical basis of population development for insects. He notes that both the environment and factors inherent to the insect, such as response to crowding, affect the number of insects in each generation. Insects produce free water, frass and heat, affecting the microenvironment of the stock. These by-products accumulate with time and number of insects in the population. As insect numbers increase, crowding increases, and with it, interference with or interruption of mating, oviposition and survival. The presence of large numbers of dead insects may or may not affect the population in the same manner as living insects but it is certain to have an effect.

The resistance level of a bean variety is one factor of the microenvironment of the insect. Therefore, it could affect the development of the insect population. The objective of this study is to examine the change in insect number and rate of increase of Acanthoscelides obtectus and Zabrotes subfasciatus populations in a closed environment over a five month period on varieties having different levels of resistance.

Materials and methods:

The varieties used in this experiment are listed in Table 16. Rubona 5 was used as the standard check and a mixture was included for comparison. The resistance of the varieties, as measured by a preliminary screening test, is also indicated. The beans were produced and stored as previously described. The varieties in the mixture were not identified nor was any attempt made to standardize the replicates.

Approximately 200 g. of a variety or mixture were put into mesh-covered 500ml Mason jars. Five replicates of each variety were used. Each jar was infested with five pairs of newly emerged adult A. obtectus or Z. subfasciatus. The jars were

Table 16. Local bean varieties used to test the effect of resistance to bruchids on the development of the insect population.

For *Acanthoscelides obtectus*:

Variety	Resistance[1] rating	Estimated # seeds/jar
16	R	1000
68	R	1000
114	S	450
116	S	500
151	I	500
210	S	650
239	S	550
248	R	600
R5	I	500
Mix	-	-

For *Zabrotes subfasciatus*:

Variety	Resistance[1] rating	Estimated # seeds/jar
5	I	900
9	R	700
16	R	1000
19	S	500
44	R	475
114	S	450
151	I	500
248	I	600
R5	I	500
Mix	-	-

[1] R - resistant
I - intermediate
S - susceptible

arranged in a completely randomized pattern for each species and maintained in an incubator at 28^{o} C. and 60 % R.H.. The number of insects in each jar was counted approximately monthly for five months. Insects were separated from the seeds and smaller material by sieving. After each count, all seeds, live and dead insects and debris were replaced in the jar. The jars were then replaced in the same order in the incubator. The starting date for each count is given in Table 17. As the number of insects increased, the time needed to complete a count of all replicates also increased. The first counts required several days to finish while a period of at least two weeks was necessary to complete the fifth count.

Results and discussion:

The mean numbers of insects collected at each count date for each variety are given in Table 18. Howe (1965) calculated a higher rate of increase for A. obtectus than for Z. subfasciatus under optimum conditions. In this experiment, the number of insects is higher for A. obtectus than Z. subfasciatus only at the first count. Acanthoscelides, being larger, may show the effects of population density and limited environment more rapidly than does Zabrotes. At the first count, however, neither factor would be important enough to affect the population size.

For several replicates of varieties infested with Z. subfasciatus there were no progeny produced and those reps were omitted from all further calculations (varieties 5, 9, and 151 are missing one rep each and variety 44 is missing two). In calculating the increase in insect numbers between counts, all negative differences were set equal to zero. In most cases, the differences were small and were likely to be the result of counting errors. No negative differences occurred before the third counting date. Mites were observed in some jars infested with Z. subfasciatus at the fourth counting. By the fifth counting most jars of both species were infested. Although the species of mites in this test was not identified, Lefèvre (1950) cites Pediculoides ventricosus as a mite parasite of Acanthoscelides obtectus eggs. The effect of egg parasitism, in these tests, would be a decrease in the number of adults produced each generation. Because the infestation started late in the experiment and it was impossible to separate the effects of the mites from the effects of other factors, such as crowding, no adjustment for the presence of the mites was made in the results.

Analyses of variance by count and by variety were computed. For most varieties, differences between counts were significant at the 1 or 5 % level. For varieties 9 and 44, infested with Zabrotes and 210, 248 and the mixture infested with Acanthoscelides, none of the differences between counts were significant. Differences between varieties were significant only for Acanthoscelides at the first count and for Zabrotes at the fourth count. Variability between replicates was high at each count. High variability between measurements has been noted in all other experiments in this report and is due, in part, to the

Table 17. Starting dates for insect counts and corresponding number of weeks from infestation by insect species for the test of insect population development.

Count	Starting dates		No. weeks from infestation	
	Acanthoscelides	Zabrotes	Acanthoscelides	Zabrotes
0	5/06/85	5/06/85	0	0
1	19/07/85	19/07/85	6	6
2	16/08/85	16/08/85	10	10
3	23/09/85	16/09/85	15	14
4	5/11/85	18/10/85	21	18
5	10/12/85	19/11/85	26	22

Table 18. Mean number of insects, dead and alive, at each count date by variety and insect species.

For Acanthoscelides:

	Number of weeks after infestation				
Variety	6	10	15	21	26
16	68	536	938	1252	1365
68	114	571	964	1424	1516
114	142	437	575	860	971
116	148	715	1066	1442	1748
151	154	892	1166	1695	2087
210	129	642	935	1151	1290
239	126	486	610	779	896
248	155	721	1066	1372	1562
R5	105	932	1366	1899	2296
Mix	101	437	673	885	909
	(19)[1]	(195)	(321)	(411)	(458)

For Zabrotes:

5	49	726	1612	2463	3358
9	57	697	2013	3037	3514
16	76	1076	1573	2897	3871
19	44	564	1382	2266	2879
44	47	530	1247	2040	2750
114	52	680	1976	2776	3413
151	62	634	1736	2556	3333
248	54	871	1772	2614	3385
R5	78	879	2042	2751	3390
Mix	69	750	1351	2269	3144
	(14)	(157)	(254)	(206)	(225)

[1] Number in parentheses is the standard error of the mean for the count date.

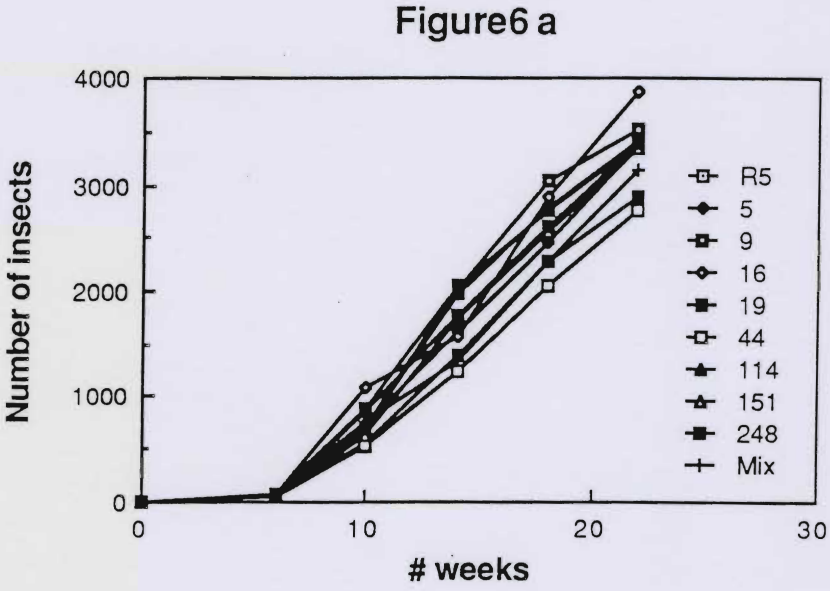
natural variability in both the insects and the seeds. Other factors also affect the variability of the results. Repeated handling of the samples resulted in the deterioration of the dry dead insects, such that they could no longer be counted. It may also affect the reproduction and survival of living insects. Also, the dates given for each count are starting dates only. In the later counts, the time between the scoring of the first and last replicate may be enough to affect the variability of the results.

Significant deviations from expected ratios due to location effects were measured. Chi square values were calculated for 1) top vs. bottom shelf, 2) front of shelf vs. back, and 3) right side of shelf vs. left, for each count and species. Of the 50 possible combinations, nineteen were significant, in particular, front vs. back for Acanthoscelides and top vs. bottom for Zabrotes. This variation is likely to be due to temperature differences in the chambers.

Although differences between varieties at each of the counting periods are not significant, trends in the data can be illustrated by graphs. Longstaff (1981) notes that populations of insects grow exponentially in an unlimited environment. As the environment becomes limiting, however, the rate of population growth decreases. Thus, plots of number of insects against time are sigmoid in shape. The initial lag phase of the curve indicates the time necessary to produce a population sufficiently large to grow exponentially. The plateauing of the curve is caused by factors limiting population growth, as discussed earlier. Compared to that of a susceptible variety, the curve of a resistant variety could show 1) a longer lag period due to a slower increase to the critical population size or to a higher critical population size, 2) a lower angle for the slope of the exponential phase due to fewer progeny being produced or to an increase in the time for development of the progeny, and/or 3) a lower point at which the curve plateaus due to environmental limits characteristic of the variety, such as seed size. The evaluation of which type of change is most effective in relation to insect damage to a crop is dependent upon the time necessary for population increase in relation to crop phenology and the amount of insect damage necessary to result in economic loss. In the case of short term storage of dry beans, a longer lag period, lower exponential growth or more rapid plateauing would result in less damage to the stock. For long term storage, however, a lower limit at which the curve plateaus, which corresponds to a lower total number of insects in the stock, would be the most useful change.

Figures 6 a-j show the development of Zabrotes on the varieties used in this experiment. All the varieties are included in Figure 6a while the remaining graphs show a single variety compared to the standard variety Rubona 5. These graphs suggest that the point at which the number of insects no longer increases has not yet been reached for any of the varieties. The point at which exponential growth starts is apparently the same

Figure 6. Development of *Zabrotes subfasciatus* populations on nine local varieties and one mixture of beans (*Phaseolus vulgaris*).



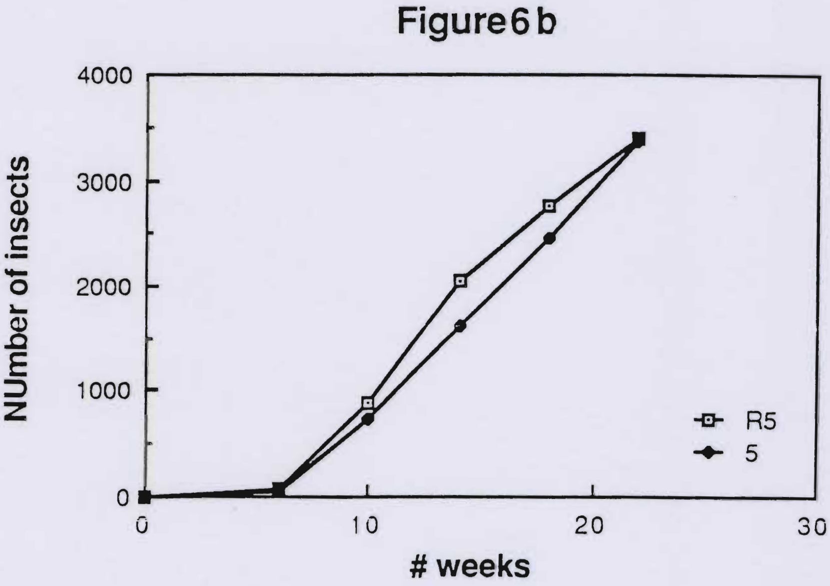


Figure 6c

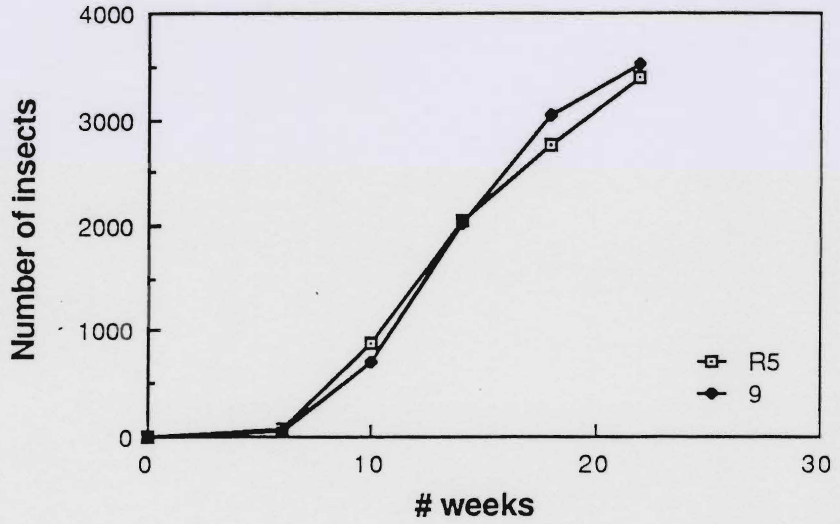


Figure 6d

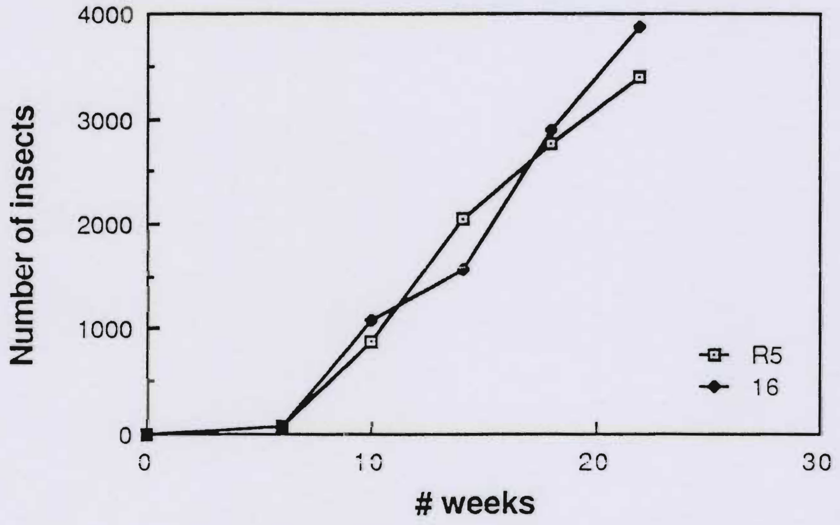


Figure6 e

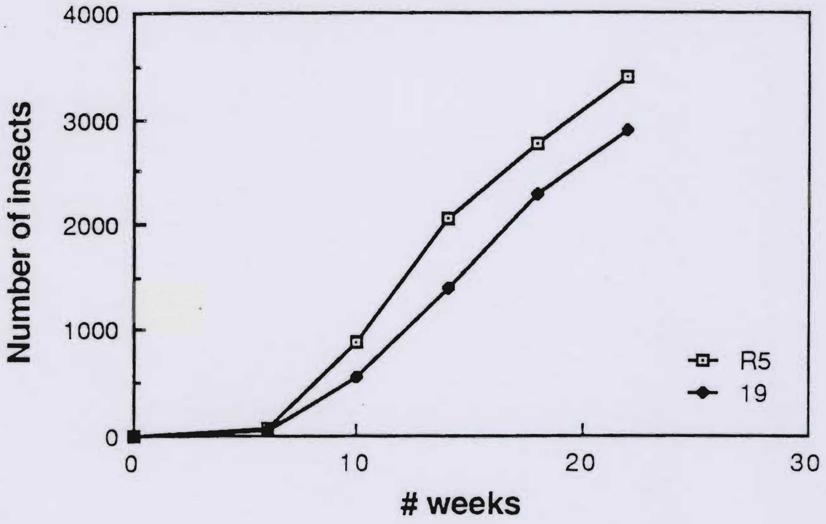


Figure6 f

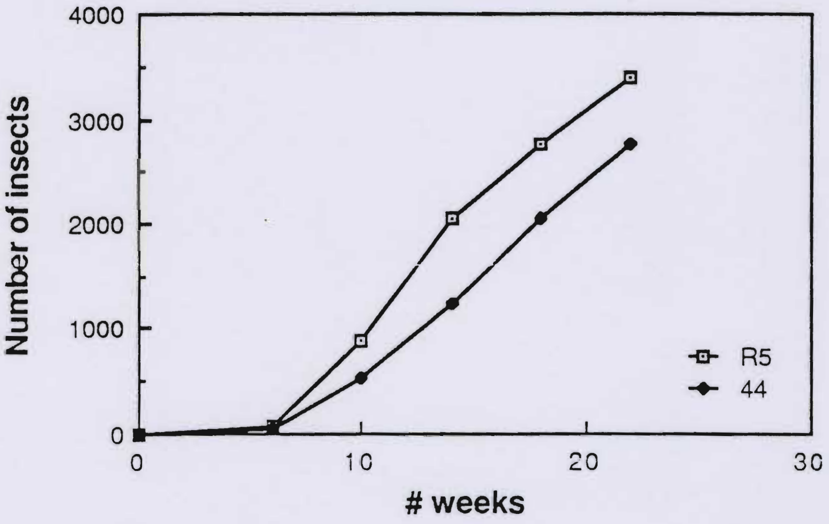


Figure 6 g

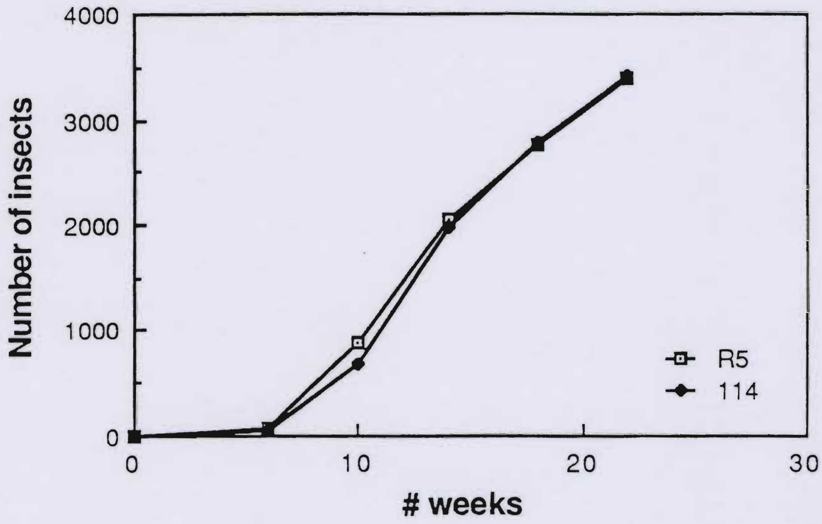


Figure 6 h

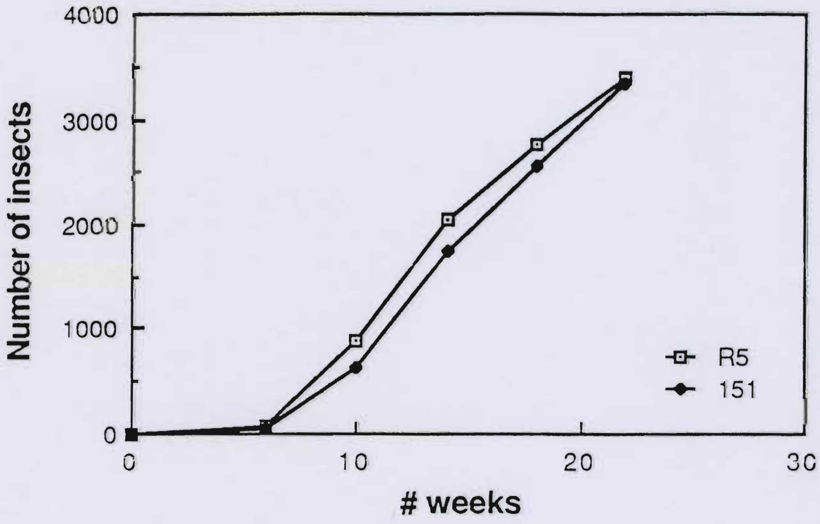


Figure6 i

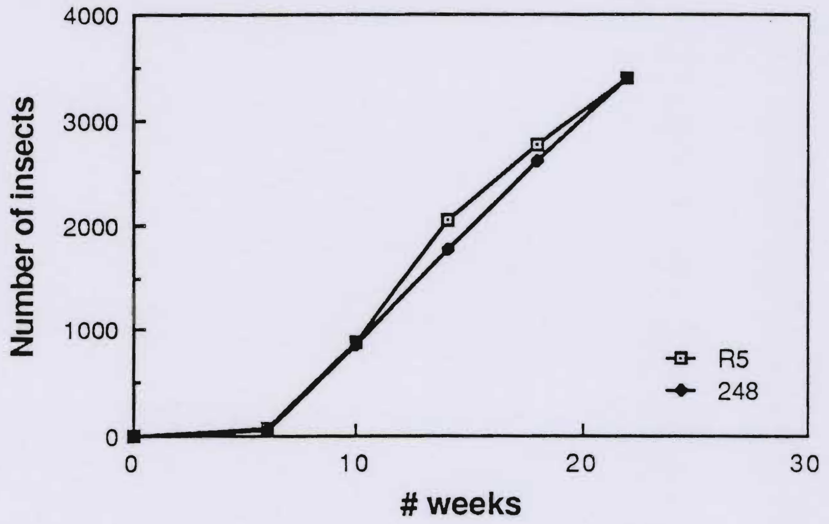
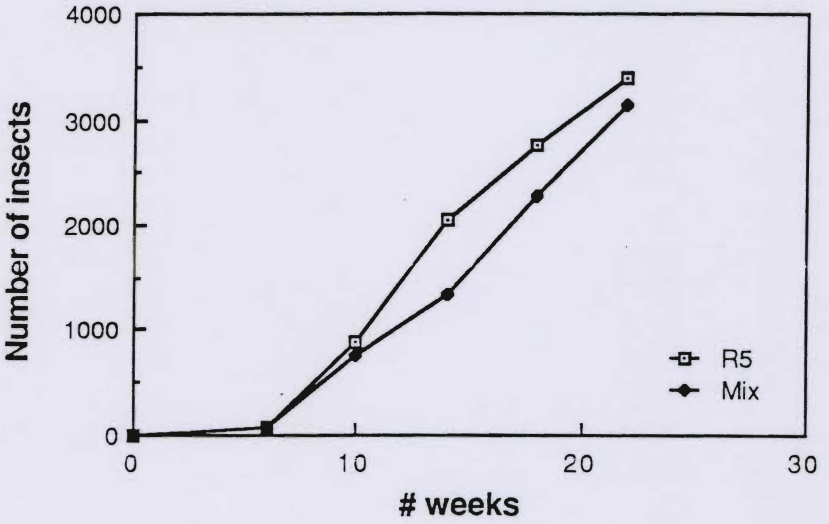


Figure6 j



for all varieties but this may be an effect of the scale of the graphs.

Three groups of varieties can be identified from the graphs. The curves of varieties 5, 9, 114, 151 and 248 (Figs. 6b, 6c, 6g, 6h, and 6i) are very similar to the curve of R5. These varieties are intermediate in resistance. Susceptible variety 16 (Fig. 6d) and resistant varieties 19 and 44 (Figs. 6e, 6f) have curves, in general, higher and lower respectively, than R5. The curve of the mixture suggests that it is fairly resistant. Only half of the varieties tested (5, 44, 151 and 248) are given the same resistance rating as in the preliminary screening trial (Table 16).

In Figures 7a-j, the development of Acanthoscelides on the nine test varieties and the mixture is shown. The curves of some varieties show a lower angle for the exponential portion and a distinct plateau. The varieties for which this is most accentuated, 114 and 239 (Figs. 7d, 7h), are scored as resistant. The others, varieties 16, 68, 116, 210 and 248 (Figs. 7b, 7c, 7e, 7g and 7i), are intermediate in resistance. The curve of variety 151 (Fig. 7f) is very similar to that of R5. Thus, variety 151 is scored as susceptible. Again, the mixture shows resistance. None of the varieties tested have the same score as in the preliminary screening test. This discrepancy may be the result of differences in short and long term reaction to insect attack but may also be caused by the use of `relative' resistance scores, i.e. relative to R5 in this experiment.

In comparison to graphs of total numbers of insects, graphs of the rate of increase are not sigmoid. In theory, the rate should increase to a peak and then decrease. At each point, the rate is affected by the number of insects, the number of progeny which they produce and how rapidly the progeny develop. The points of interest on the graph are the height of the peak, when it occurs and the slope of the curve as it increases and decreases. Thus, it is expected that a resistant variety would result in a curve with a lower peak, and/or a later peak. The angle of increase of the curve may be either steeper or flatter, depending on when the rate of population growth starts to increase relative to the time of the peak rate. A steep decrease, i.e. a rapid decline in rate, is also a possible effect of a resistant variety.

The graphs of the rate of development of Zabrotes populations on the test varieties are shown in Figures 8a-j. The rate is measured in number of insects/week and it was calculated for five periods. The graph for R5 peaks at time 3 at about 300 insects/week. Based on the peak rate and when it occurred, varieties 19 and 44 (Figs. 8e, 8f) are resistant (lower and later peaks), varieties 5 and 248 (Figs. 8b, 8i) are between resistant and intermediate (lower peaks), varieties 9, 114 and 151 (Figs. 8c, 8g and 8h) are intermediate and variety 16 (Fig. 8d) is susceptible. The mixture (Fig 8j), having a later and lower peak, is also resistant. These results correspond very well with the results from the graphs of total insect numbers.

Figure 7. Development of Acanthoscelides obtectus populations on nine local varieties and one mixture of beans (Phaseolus vulgaris).

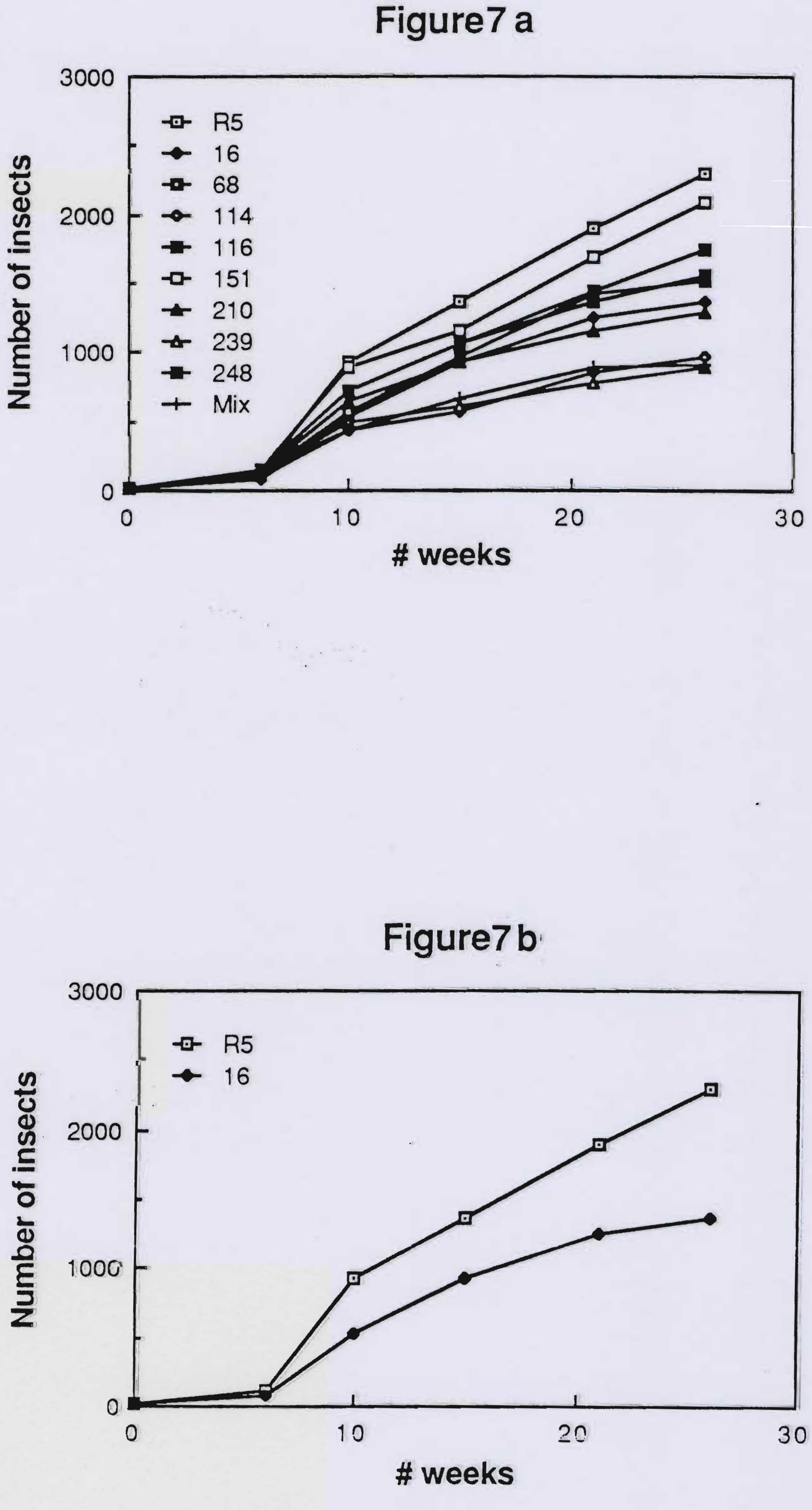


Figure7c

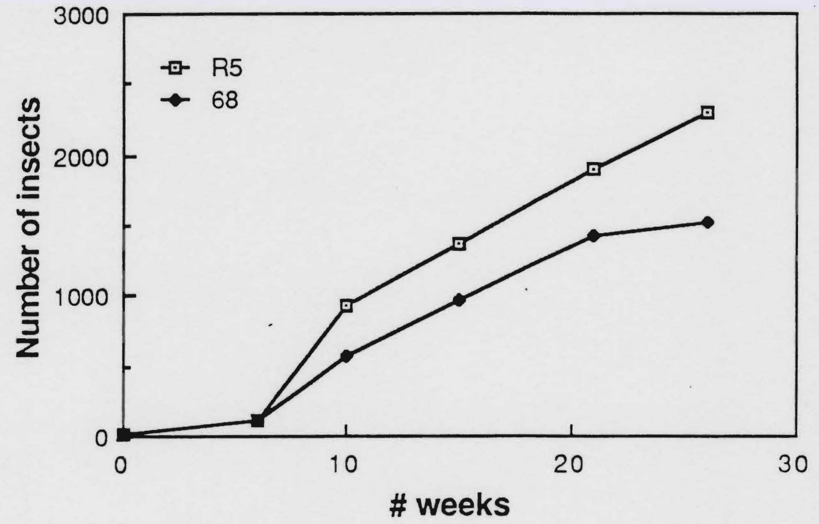


Figure 7d

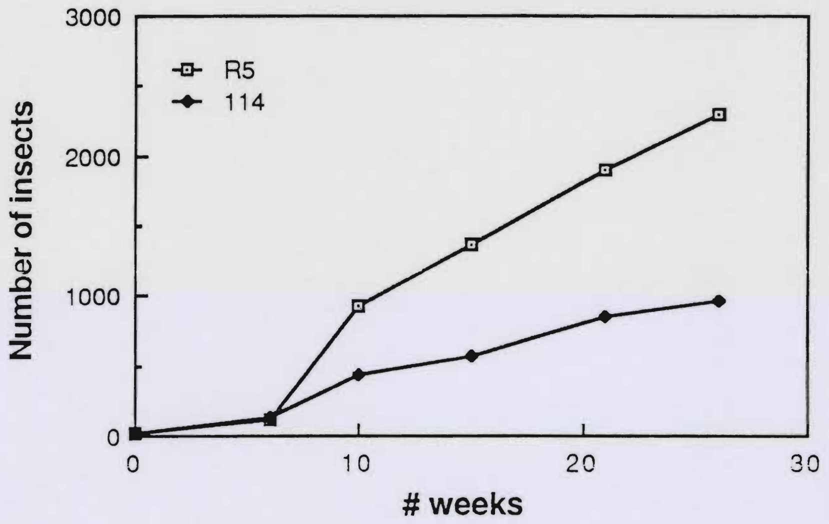


Figure7 e

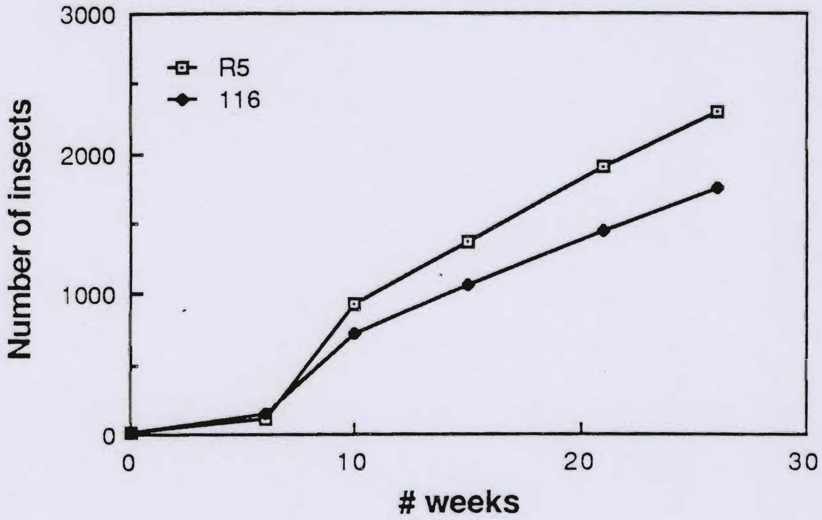


Figure7f

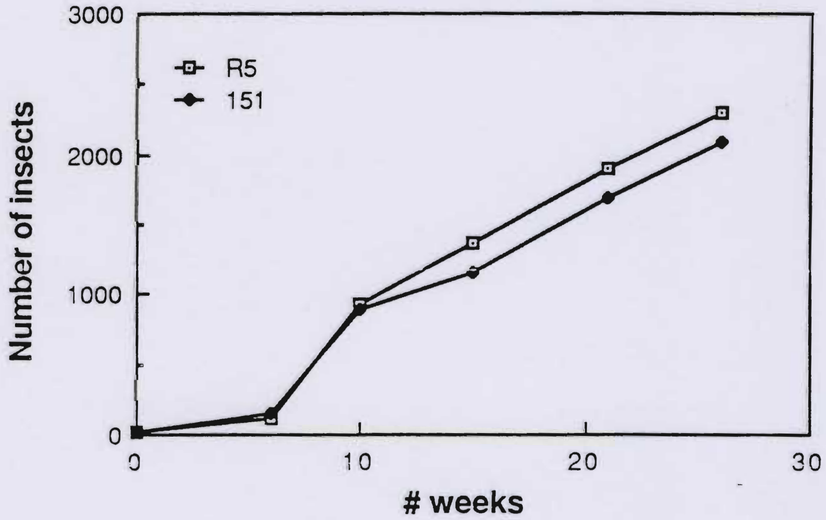


Figure 7g

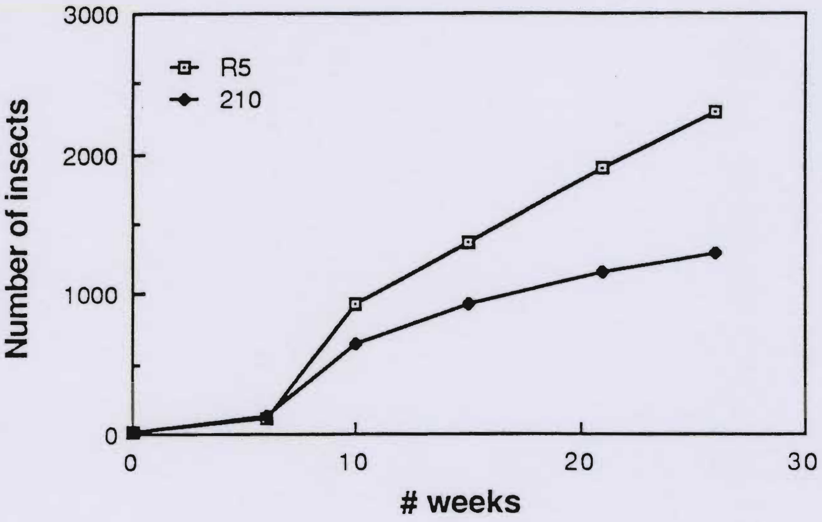


Figure 7h

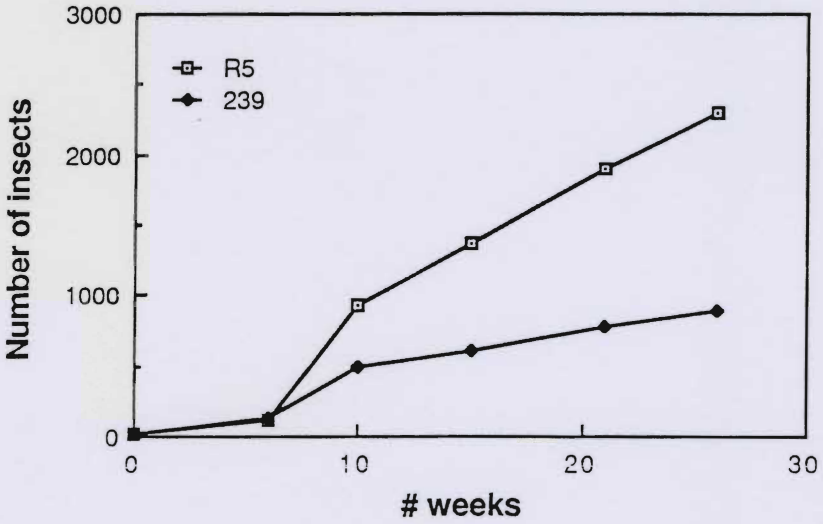


Figure7 i

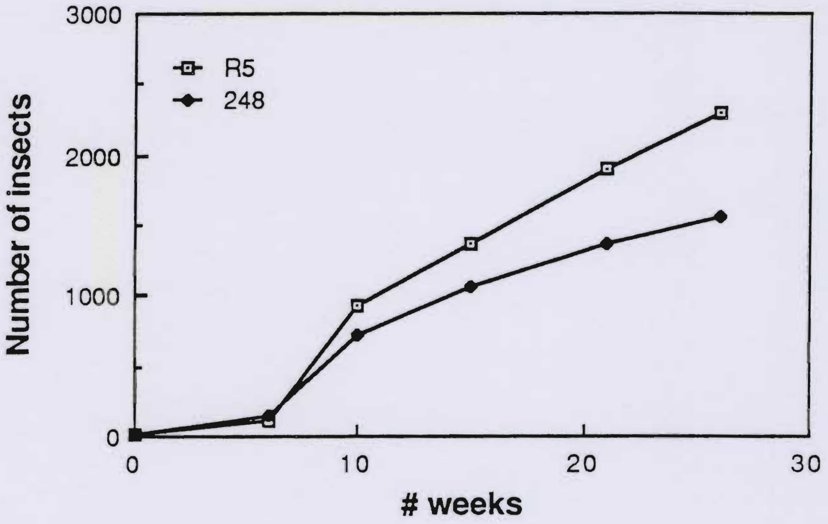


Figure7 j

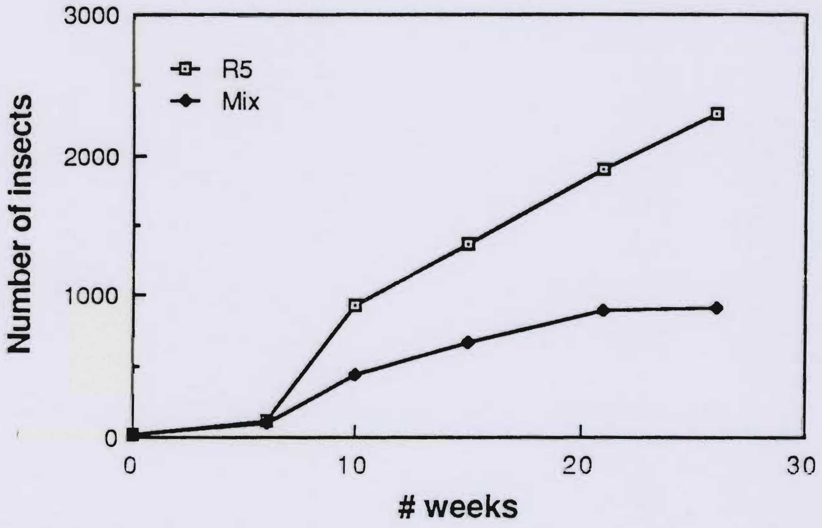


Figure 8. Rate of development of Zabrotes subfasciatus populations on nine local varieties and one mixture of beans (Phaseolus vulgaris).

Figure8 a

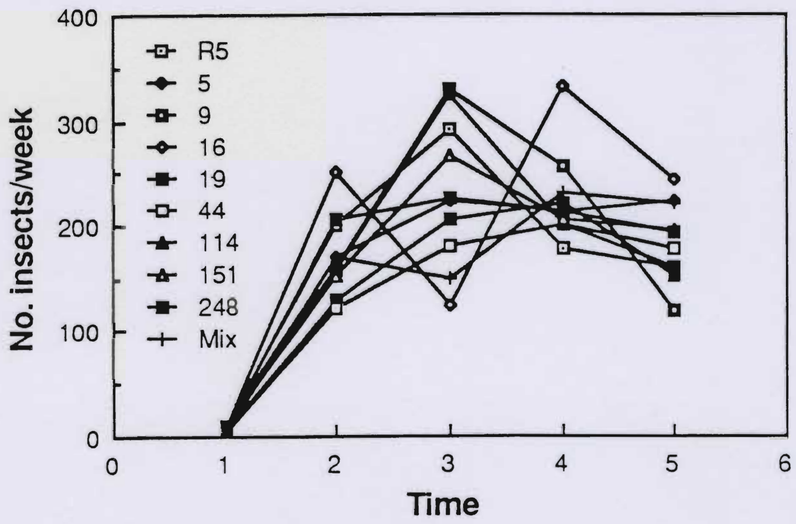


Figure8 b

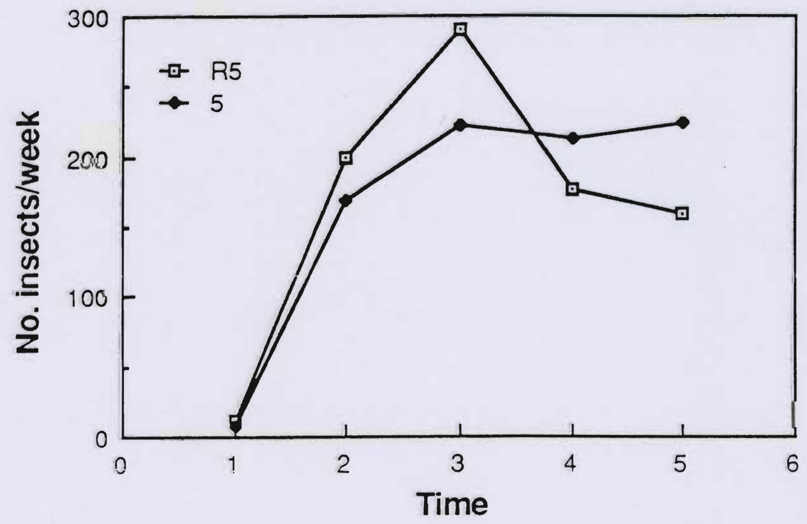


Figure8c

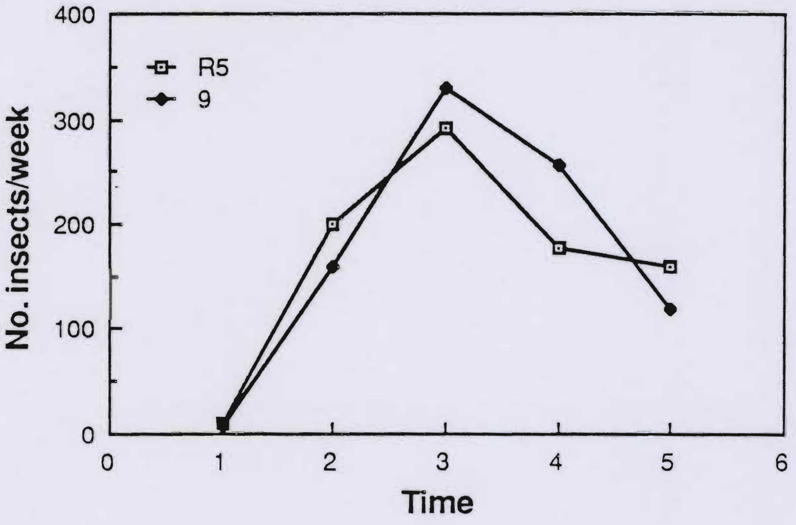


Figure8d

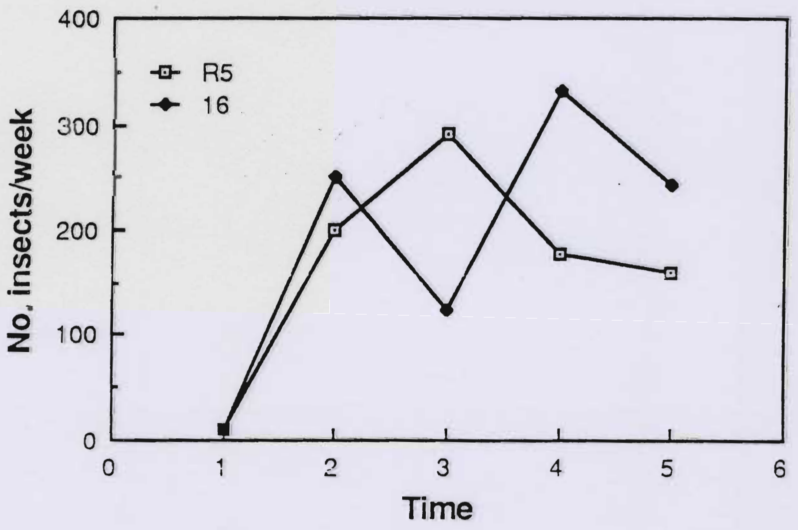


Figure8 e

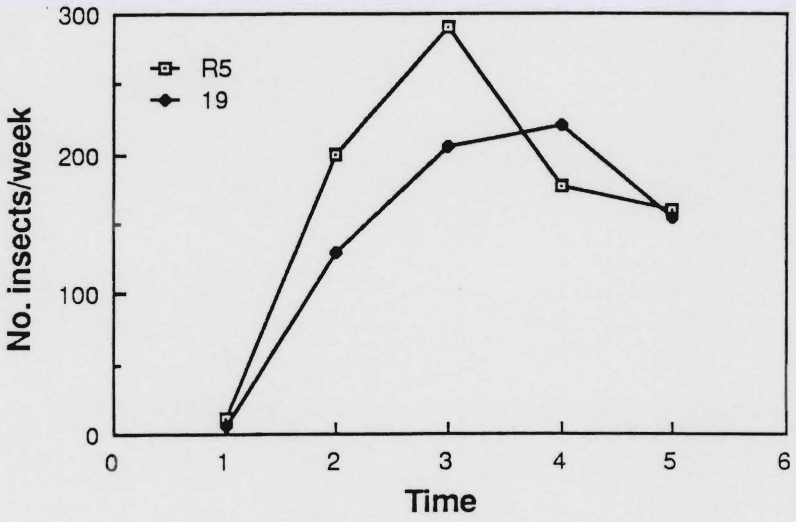


Figure8 f

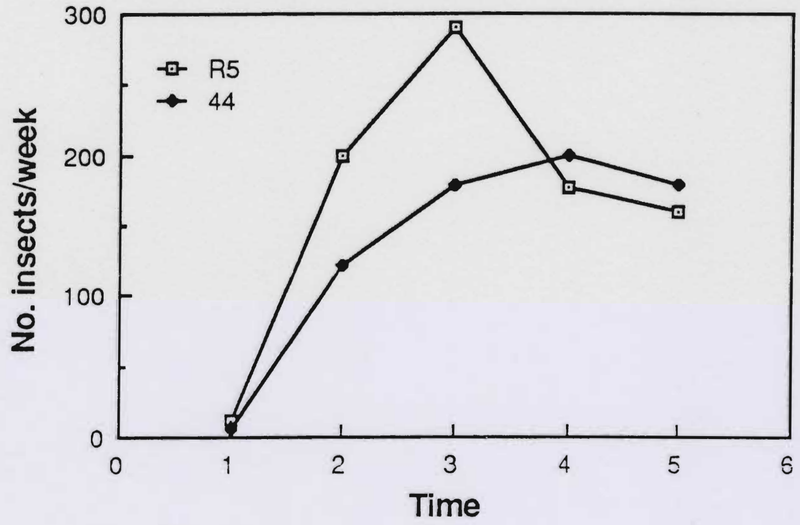


Figure8g

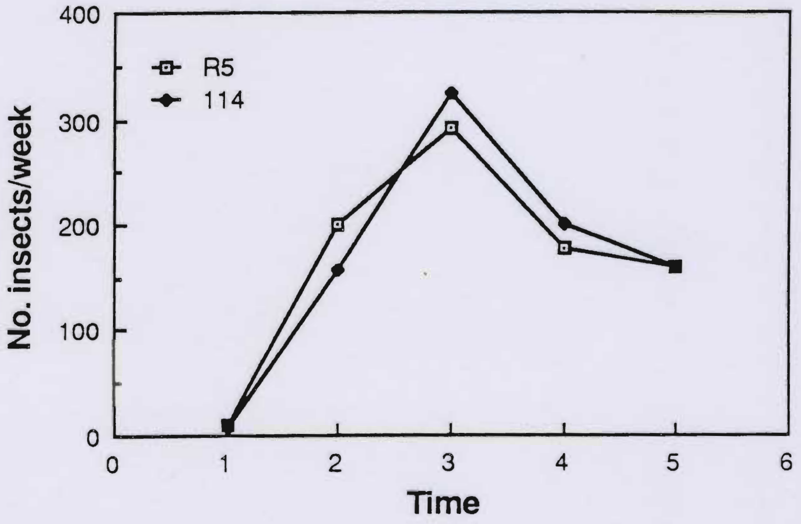


Figure8h

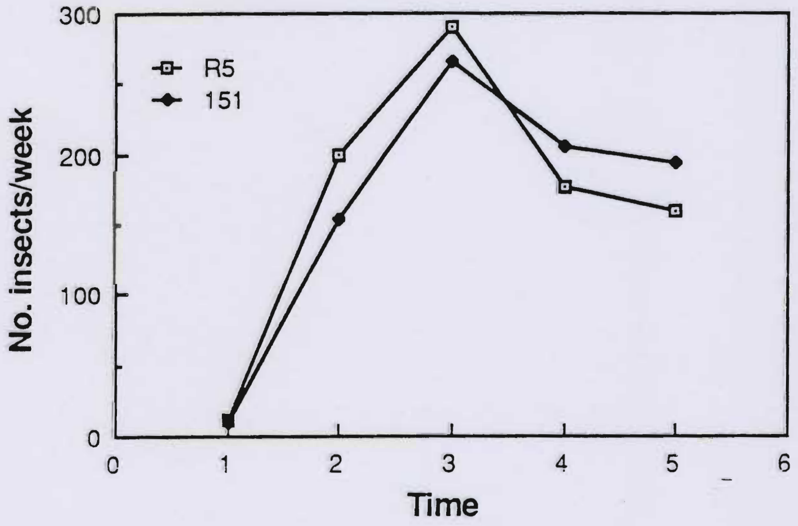


Figure8 i

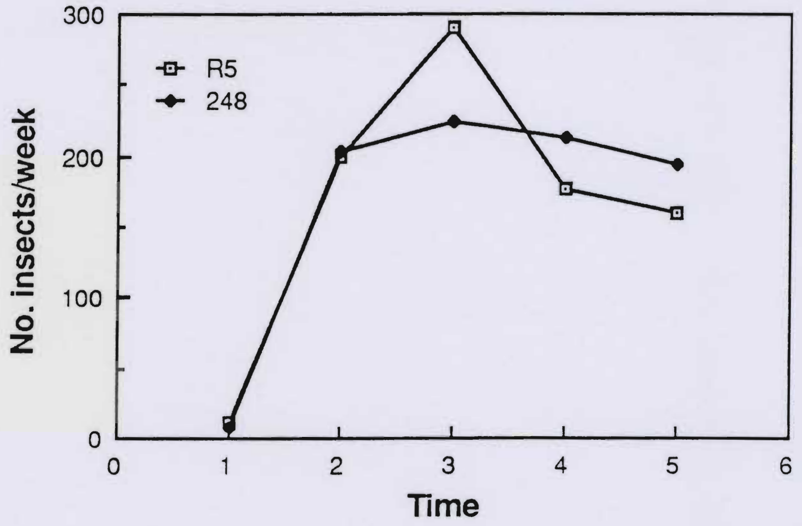
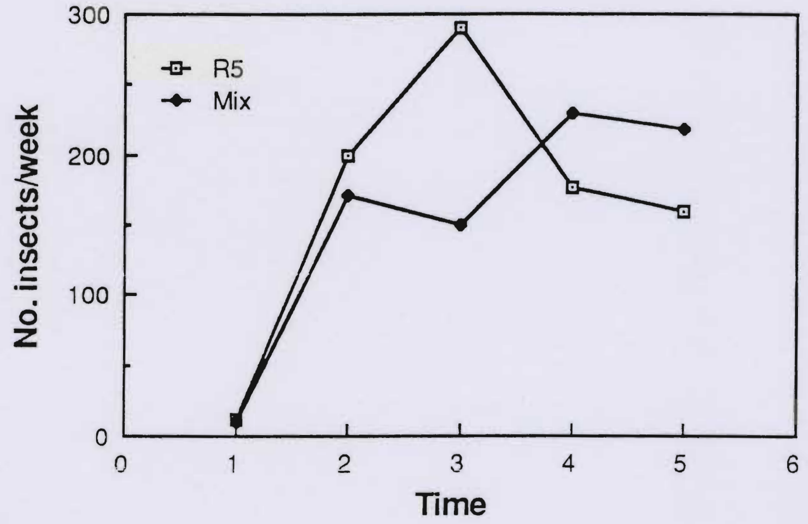


Figure8 j



The graphs of rate of population growth for A. obtectus are given in Figures 9a-j. For all varieties, the peak rate occurred during the second time period, so the designation of level of resistance is based on the height of the peak. Varieties 114 and 239, as well as the mixture (Figs. 9d, 9h, and 9j), are resistant, varieties 16, 68, 116, 210 and 248 (Figs. 9b, 9c, 9e, 9g and 9i) are intermediate and variety 151 (Fig. 9f) is susceptible. As with Zabrotes, the results from the graphs of total numbers of insects and rate of increase are the same.

Zaazou (1948), in his study of the effect of population density on the rate of reproduction, compared the number of beans per bruchid female with the number of new progeny per female. These same values were calculated for the first count of this experiment with Zabrotes and are listed in Table 19. The results for Acanthoscelides are similar. Although this experiment was not designed to measure the effects of density specifically, the results indirectly support those of Zaazou. The number of seeds per female was calculated for each variety using the values for seed weight and the weight of seeds per jar. Comparing the values for varieties 19, 151 and R5, for which there were 100 seeds/female, it can be seen that the reduction in number of progeny per female for variety 19 is not due to lack of space for development alone since fourteen adults emerged from the similarly sized seeds of variety R5. This suggests that factors other than seed size are responsible for the resistance of variety 19.

Conclusions and recommendations:

1. Resistance to bruchids in dry beans can be measured, graphically, by the change in the total number of insects and the rate of increase of the population with time. Using such graphs, the varieties examined in this test were easily divided into three classes; resistant, intermediate and susceptible, relative to the standard variety Rubona 5.

2. Using this method, varieties 19 and 44 are resistant, 5, 9, 114, 151 and 248 are intermediate and 16 is susceptible to damage caused by Zabrotes subfasciatus . Varieties 114 and 239 are resistant, 16, 68, 116, 210 and 248 are intermediate and 151 is susceptible to Acanthoscelides obtectus.

3. Resistance scores for varieties were not the same as those given in short term screening tests.

4. The mixture used in this study was determined to be resistant to both species.

5. Screening tests such as the one described in section IA are necessary for the rapid and easy testing of large numbers of varieties. However, longer term screening tests, such as this one, are more indicative of the actual situation during storage. Therefore, those varieties found to be the most resistant in rapid screening tests should be retested in longer term tests.

Figure 9. Rate of development of <u>Acanthoscelides</u> <u>obtectus</u> populations on nine local varieties and one mixture of beans (<u>Phaseolus</u> <u>vulgaris</u>).

Figure 9 a

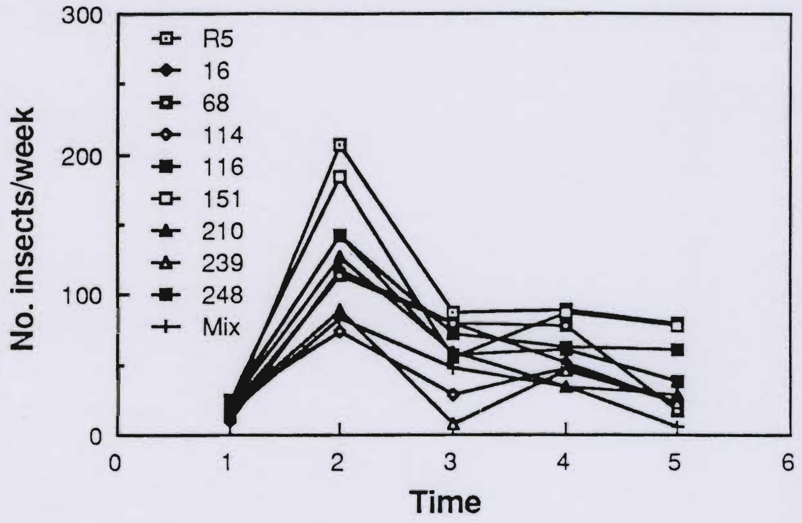


Figure 9 b

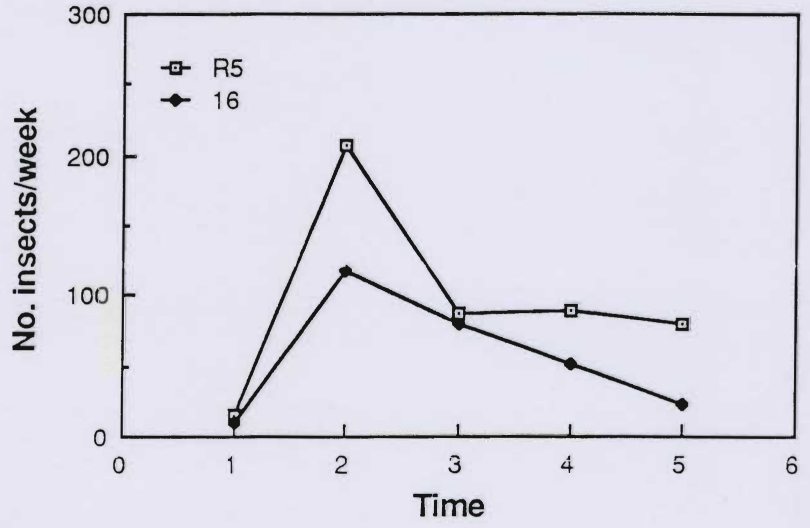


Figure 9c.

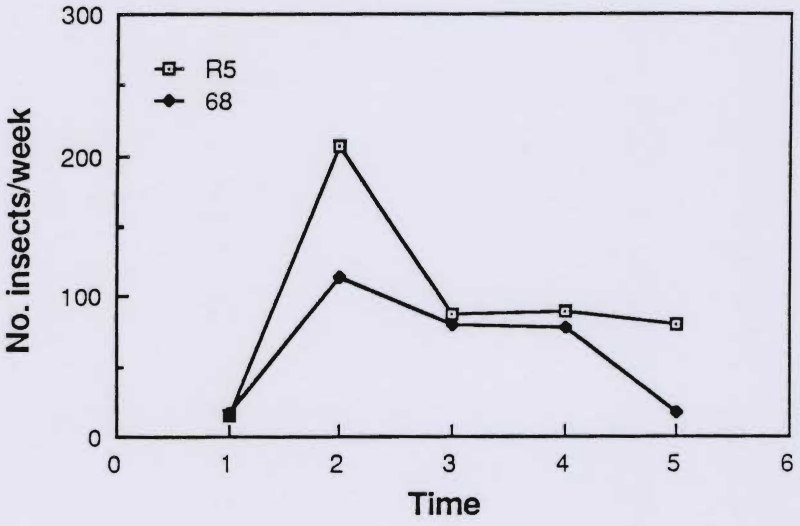


Figure 9 d

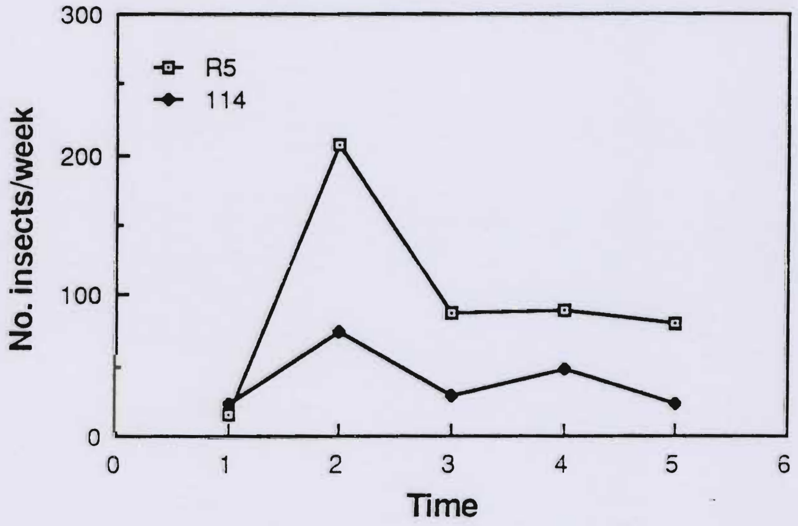


Figure 9 e

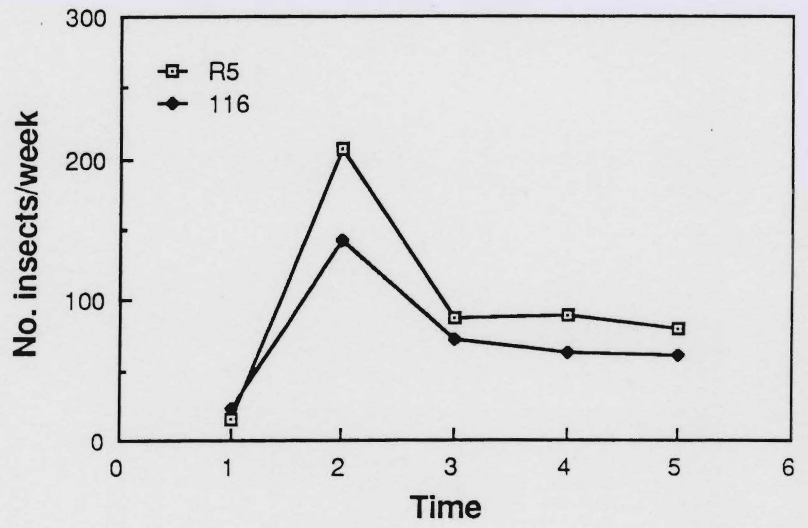


Figure 9 f

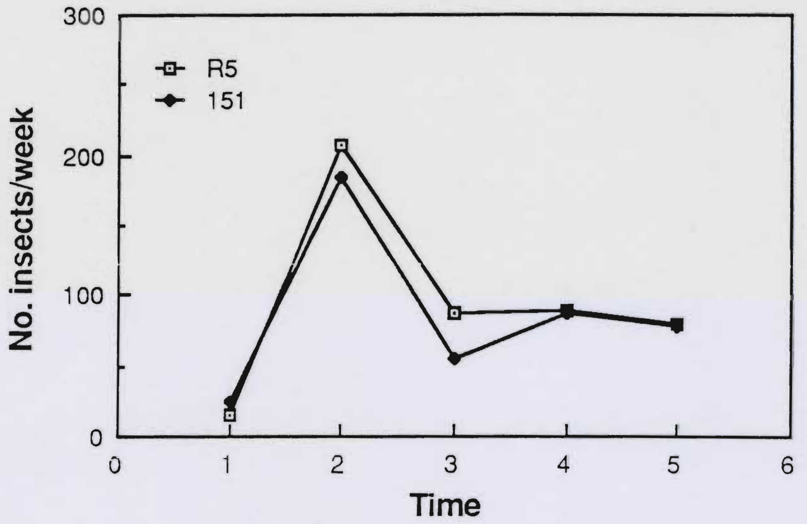


Figure 9g

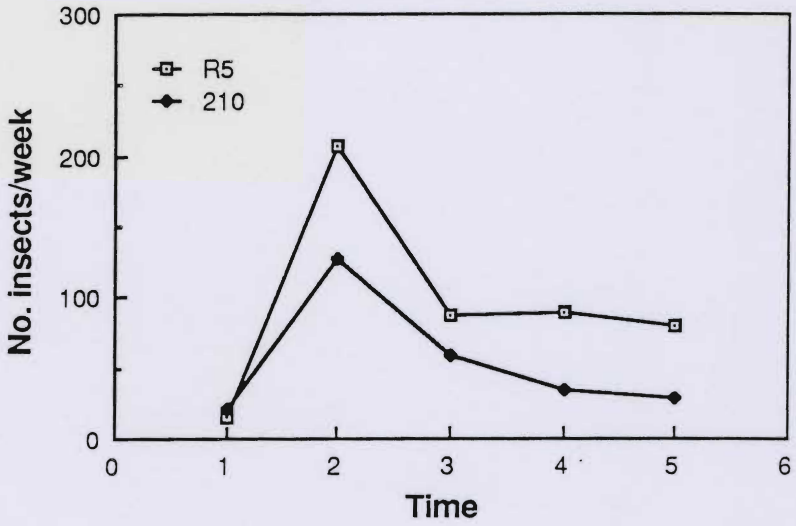


Figure 9h

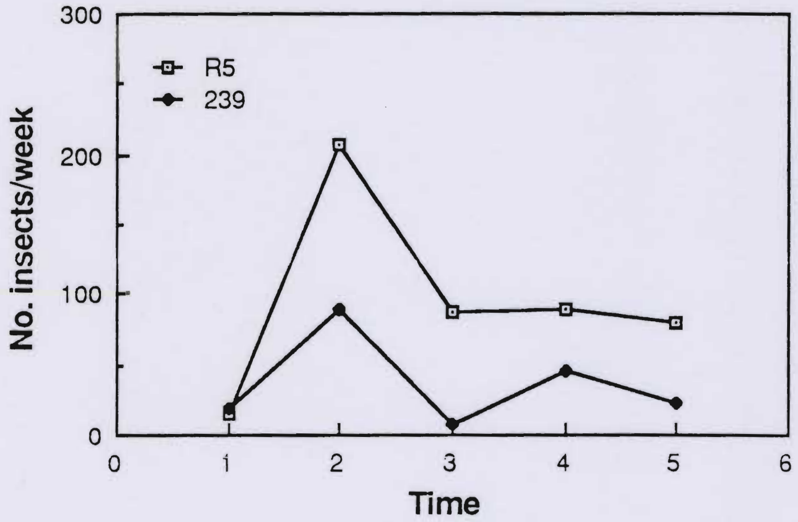


Figure 9 i

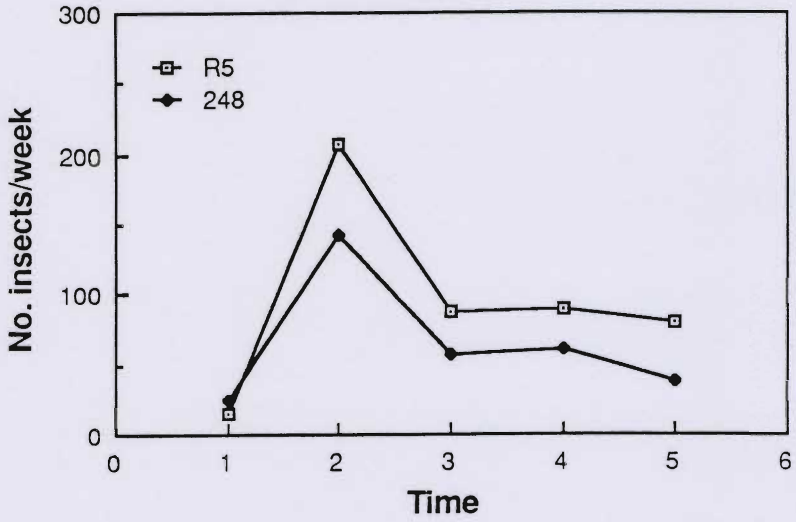


Figure 9 j

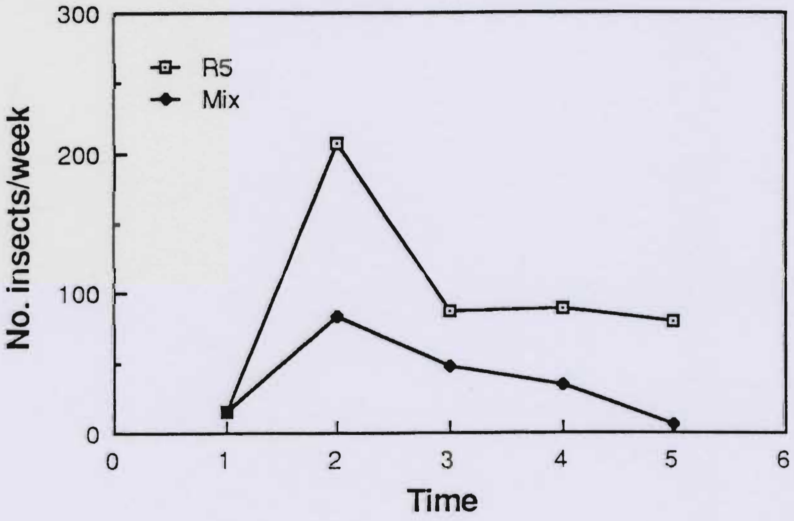


Table 19. Number of beans available for oviposition per female and number of new progeny per female for tests using *Zabrotes*.

Variety	Number of beans/female[1]	Number of progeny/female	Resistance score
5	180	8	I
9	140	9	I
16	200	13	S
19	100	7	R
44	95	7	R
114	90	8	I
151	100	10	I
248	120	9	I
R5	100	14	S

[1] Estimated for each variety by:
(# grams of seed per jar/# grams per 100 seed)/5 females

The results of the long term tests may not only lend support to the results of the rapid screening but also suggest how the resistant varieties sustain less damage and when the damage is most likely to occur.

6. Comparison of the mean number of insects and the estimated number of seeds per jar shows that, under optimum environmental conditions and high infestation rates (50 insects/kg), all or nearly all of the seeds were damaged by the tenth week of the test. Varieties susceptible to either species had a higher percentage of seeds damaged as did all varieties infested with Zabrotes relative to Acanthoscelides. Although these conditions are not likely to occur in storage at any level in Rwanda, they do indicate the potential for bruchid damage to stored beans.

7. In future tests of this sort, some protection from mite infestation would be appropriate. As mites enter the jars by crawling, surrounding the base of each jar with oil or a sticky substance should prevent an infestation. Cleanliness is also important to prevent mites spreading from jar to jar. All equipment should be washed in hot soapy water between counting each jar. If possible, infested cultures should be destroyed.

E. *Hypothesis IE*: The presence of a genotype resistant to *Acanthoscelides obtectus* or *Zabrotes subfasciatus* in a mixture of bean varieties infested with that insect affects the level of damage sustained by the mixture as a whole.

In Rwanda, beans are grown, stored and used as mixtures of varieties. A new variety developed for resistance to bruchids or any other factor will be added to a farmer's current mixtures, probably comprising only a small proportion of the mixture. Therefore, it is of interest to know what effect the mixing of a resistant variety with susceptible varieties has on the amount of damage sustained by the mixture as a whole.

There has been little information published on the effects of a mixture of bean varieties on the damage caused by bruchids. Lefèvre (1950) studied the development of bruchid populations on local mixtures in Zaire but did not discuss damage as it affects individual seed types. Larson and Fisher (1938) infested a mixture of seeds of 22 varieties of *Phaseolus vulgaris* with *A. obtectus* and noted the number of seeds of each type infested and the number of bruchids which emerged. They found that certain varieties were preferred over others and that some varieties were not infested at all. In this study, artificial mixtures of one resistant and one susceptible variety were used to examine the effect of varying the proportion of the resistant variety on damage to bean seeds caused by *Acanthoscelides obtectus* and *Zabrotes subfasciatus*.

Materials and methods:

Two artificial mixtures were created for testing with each of the bruchid species, *A. obtectus* and *Z. subfasciatus*, from varieties determined to be resistant and susceptible in previous screening tests (Table 20). Forty seeds in a plastic petri dish (100x15mm) were used for each treatment. The treatments were 100%, 75%, 50%, 25% and 0% of the resistant variety, corresponding to 40, 30, 20, 10 and 0 seeds, with the rest being made up of the susceptible variety. There were five replicates per treatment.

Thirty larvae of *A. obtectus* or six pairs of adults of *Z. subfasciatus*, left on the seeds for three days, were used to infest each treatment. All of the treatments with *Zabrotes* were infested on 28 July, 1985. The treatments with *Acanthoscelides* were infested 30-31 July, 1985. The dishes were arranged by insect species in a completely randomized design in an incubator at 28° C and 60% RH.

The data collected were number of adults emerging in each dish from 30 August, 1985 to 11 September, 1985, the number of damaged seeds of each variety and the number of exit holes in the damaged seeds of each variety.

Results and discussion:

The analyses of variance for number of adults emerging and total number of damaged seeds show that differences by percent of the resistant variety are significant for Mixture 1 for *A.*

Table 20. Local bean varieties used in the artificial mixtures for testing with _Acanthoscelides obtectus_ and _Zabrotes subfasciatus_.

Mixture	Varieties Susceptible	 Resistant
For _Acanthoscelides_:		
1	137	68
2	135	126
For _Zabrotes_:		
1	19	9
2	24	57

obtectus and Mixture 2 for Z. subfasciatus (Table 21). Comparing the percent of each type of seeds damaged for Mixtures 1 and 2 for Acanthoscelides, it appears that variety 68 is more resistant than variety 126 while varieties 137 and 135 are similar in susceptibility. With the same comparison for Zabrotes, the difference between resistant varieties 9 and 53 is greater than the difference between the susceptible varieties 19 and 24, which are quite similar. This suggests that the strength or level of the resistance is important in the choice of a resistant variety to be used in a mixture. In all cases, however, the number of adults which emerged and the percent damaged seeds increased with a decrease in percent of the resistant variety.

The mean separations for number of adults emerged and percent seed damaged, were calculated using the Student Newman Keuls method (Steel and Torrie, 1980) for those mixtures with significant F values (Table 22). The results suggest that for Acanthoscelides Mixture 1, amounts of the resistant variety of less than 75% of the mixture do not result in significantly lower damage than 0% of the resistant variety. For Zabrotes Mixture 2, however, a mixture of any composition results in more damage than 100% resistant and less damage than 100% susceptible seeds. As mentioned previously, these values are dependent on the level of resistance of the resistant variety used. For the varieties listed here, a high percent of resistant seeds is necessary in a mixture to protect it from damage by A. obtectus while even a low proportion of a resistant variety will protect the mixture from damage by Z. subfasciatus.

The percent of resistant and susceptible seeds damaged and the number of exit holes per damaged seed were compared for each mixture. For all treatments, seeds of the susceptible variety were more frequently damaged than resistant seeds even when the proportion of susceptible seeds in the mixture was low (Table 23). The observed number of resistant seeds damaged was compared to the number expected to be damaged if insects infested and emerged from seeds at random. Chi square values were calculated. The results showed that the expected number was significantly higher than the observed number for Acanthoscelides Mixture 1 (p=0.19, 0.05 and 0.21 for %R=75, 50 and 25) and Zabrotes Mixture 2 (p=0.29, 0.06 and 0.54 for %R=75, 50 and 25). The same calculations for susceptible seeds showed that the expected number of damaged seeds is significantly lower than that observed, except for the treatment 25% R for Acanthoscelides Mixture 1 (p=0.27, 0.25 and 0.82 for %R=75, 50 and 25) and Zabrotes Mixture 2 (p=0.06, 0.10 and 0.88 for %R=75, 50 and 25). Also, the number of exit holes per damaged seed, although less dramatically different, was higher for the susceptible varieties (Table 23). The values given in Table 23 are for Acanthoscelides Mixture 1 and Zabrotes Mixture 2 but the results for the other mixtures are similar.

These results show that, in a mixture, more susceptible seeds than resistant seeds are damaged and more adults emerge from susceptible seeds than from resistant seeds, no matter what proportion of resistant and susceptible seeds is used. This

Table 21. Results of the analysis of variance for number of adults emerging and percent of the total seeds damaged in mixtures of local beans infested with _Acanthoscelides_ _obtectus_ or _Zabrotes_ _subfasciatus_.

Number of adults emerging		Significance level of F[1]	Standard error
Acanthoscelides	Mixture 1	**	1.10
	Mixture 2	NS	1.91
Zabrotes	Mixture 1	NS	12.76
	Mixture 2	**	6.89
Percent of the total seeds damaged			
Acanthoscelides	Mixture 1	**	1.83
	Mixture 2	NS	2.55
Zabrotes	Mixture 1	NS	12.89
	Mixture 2	**	7.60

[1] NS - not significant
* - 5% significance level
** - 1% significance level

Table 22. Mean separation by Student Newman Keuls test at 5% for the mean number of bruchid adults emerged and the mean percent of the total bean seeds damaged.

Percent of resistant variety	Number of adults emerged	Percent of the total seeds damaged
For *Acanthoscelides* Mixture 1:		
100	0.6	1.8
75	2.0	3.6
50	6.6	11.8
25	6.8	11.8
0	8.2	14.2
	(1.10)[1]	(1.83)
For *Zabrotes* Mixture 2:		
100	3.6	7.0
75	28.4	44.6
50	28.2	47.2
25	25.0	37.2
0	54.8	76.4
	(6.89)	(7.60)

[1] Number in parentheses is the standard error of the mean.

Table 23. Percent of seeds of local bean varieties damaged and number of exit holes per damaged seed for mixtures infested with *Acanthoscelides obtectus* or *Zabrotes subfasciatus*.

Percent of seed resistant variety	Percent of seed damaged		No. holes/damaged	
	S seeds[1]	R seeds	S seeds	R seeds
For *Acanthoscelides*:				
100	-	2	-	1.0
75	10	1	1.3	1.0
50	22	2	1.5	1.0
25	15	0	1.5	-
0	14	-	1.5	-
For *Zabrotes*:				
100	-	7	-	1.3
75	70	36	2.5	1.3
50	66	28	1.8	1.4
25	41	26	1.6	1.4
0	76	-	2.0	-

[1] S - susceptible
R - resistant

could indicate a preference for the susceptible variety by the larvae of Acanthoscelides and the ovipositing females of Zabrotes or a higher survival of larvae in the susceptible seeds. The data presented here are not sufficient to differentiate between these two possible causes. However, the latter hypothesis is supported by the number of damaged seeds and amount of damage per seed when either the resistant or susceptible variety is tested alone (100%R, 0%R=100%S). In many cases where insect preferences exist, a no-choice test will result in higher damage to the resistant variety alone than when the susceptible variety is present. In some cases, the damage to the resistant variety equals that occurring when the susceptible variety is tested alone. In this test, however, the damage in the mixture is always greater than or equal to that for the resistant seed alone. The damage occurring in the resistant variety alone is much less than that occurring in the susceptible variety alone. It is possible that a dual mechanism of preference for the susceptible variety and reduced survival in the resistant variety could exist.

Conclusions and recommendations:

1. The presence of a resistant variety in a mixture of beans infested with bruchids reduces the amount of damage in the mixture as a whole. The amount of damage depends on the proportion of resistant and susceptible seeds and the level of resistance and susceptibility of the varieties in the mixture. Acanthoscelides reacted differently to the proportion of the resistant variety than did Zabrotes in the artificial mixtures tested in this study.

2. Resistant seeds had less damage and were damaged less often (percent of seeds damaged and number of exit holes per damaged seed) than would be expected if the insects infested and emerged from seeds at random. These results suggest a preference by the insect for susceptible seeds and/or reduced survival in resistant seeds.

3. The use of resistant varieties in mixtures to control damage in the mixture as a whole requires further study but the preliminary results are positive. Future research should include:

   a. Tests of artificial mixtures with varieties of known resistance level in various combinations. For example, mixtures of very resistant with very susceptible, intermediate with very susceptible, very resistant with intermediate, etc., varieties could be tested.

   b. Tests of artificial mixtures of greater complexity using varieties of known resistance levels. Increasing the number of varieties will increase the difficulty of the test but the test will more closely resemble actual storage conditions.

c. The use of local mixtures with a resistant variety added in several proportions. Testing a mixture with many component varieties may increase or decrease the proportion of the resistant variety needed to reduce damage, depending on the level of resistance of the other varieties in the mixture.

F. Hypothesis II - The resistance of bean seeds to bruchids can be overcome by the adaptation of the insect to the resistant variety as host (stability of resistance).

One of the proposed advantages of host plant resistance as a method of insect control is that it does not require constant reapplication. However, the interaction between the insect population and the resistant host plant is not static. The gene pool of the insect may contain sufficient variation to allow some individuals to adapt to the factors which make the plant resistant. Changes in the plant population through breeding or natural selection may also alter the reaction of the insect to its host plant. In this context, the stability of the resistance indicates how long the resistance will be effective. A stable resistant plant/insect interaction suggests that 1) the insect and plant populations are not changing relative to each other, or 2) the plant population can adapt to or withstand changes in the insect population which allow it to use the plant as a host. Gould (1979) measured the survivorship and fertility of successive generations of spider mites, Tetranychus urticae, on lima beans, a favorable host, and cucumber, a marginal host, to demonstrate how rapidly an insect population can change to successfully utilize a marginal host. Resistant and susceptible varieties can be considered as marginal and favorable hosts, respectively, and the stability of the resistance tested in a similar fashion. An example of a test of stability is given by Schoonhoven and Cardona (1982). They measured the effect of continuous rearing of Zabrotes subfasciatus on resistant and susceptible accessions of beans on the ability of the insect to cause damage.

To measure stability, a population of insects is reared for several generations on resistant plants. In theory, this exposes the insects to a more intense pressure to adapt than would occur under field conditions. Insects from the altered and original populations are then reared on resistant and susceptible plants. The survival and/or fertility of each population, or the damage it causes, on each plant type is compared to determine if the altered population is more adapted to the resistant plant as a host. This experiment was designed to test the stability of the resistance of Rwandan bean varieties to Acanthoscelides obtectus and Zabrotes subfasciatus.

Materials and methods:

Four varieties of beans, including the standard Rubona 5 (R5), were tested with each bruchid species. The varieties and their resistance ratings from a preliminary screening test are given in Table 24. Each replicate consisted of fifteen seeds of a variety in a plastic petri dish (100x15mm). Adult insects, 1-2 days after emergence, were placed in each petri dish and left on the seeds for three days. The number of pairs used per dish is discussed in the following section. The infested dishes were arranged in a completely randomized fashion by species in the incubator and were maintained at 28^{o} C and 60% RH.

Table 24. Varieties used to test the stability of resistance to *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in local beans.

	Variety	Resistance rating
For *A. obtectus*	68	R
	151	I
	137	S
	R5	
For *Z. subfasciatus*	9	R
	248	I
	19	S
	R5	

Four subpopulations of each insect species were created by rearing the insects continuously on each of the test varieties. Adult progeny from each generation were used to infest new seeds of the test variety to produce the next generation. After 5-6 generations, each variety was to be infested with insects from each subpopulation to determine if adaptation to that variety had occurred. However, due to difficulties in producing sufficient progeny in the initial test (infested 19-20 June, 1985), a second test was infested (28 July, 1985) with three rather than two pairs of adults. Adequate progeny of Z. subfasciatus were produced to continue the test through three generations before evaluating the adaptation of the progeny. A third test was started using five pairs of A. obtectus per replicate but, again, insufficient adults were produced to infest the next set of dishes. Thus, the subpopulations of A. obtectus were not evaluated.

The number of progeny adults emerging on each scoring date was recorded for each bean variety and insect generation during the development of the subpopulations. The same data were collected for the evaluation of each subpopulation.

Results and discussion:

In general, the number of adult progeny produced on the susceptible variety was higher than the number produced on the resistant variety for each test and insect generation (Table 25). This supports the results of the preliminary screening tests. The number of progeny from the intermediate line was more variable, in some cases nearly equal to the number of progeny from the susceptible line, in other cases closer to that of the resistant line. The values for Rubona 5 are most similar to those of the resistant line. In order to more clearly illustrate the trends, only the results from the resistant and susceptible lines will be discussed here.

In theory, the infestation of a resistant host with insects reared on a susceptible host should result in lower survivorship or number of progeny than the infestation of a susceptible host with the same insects. If adaptation to the resistant host occurs when insects are reared on resistant plants, one would expect to find a higher number of survivors or progeny of the adapted subpopulation than of the unadapted population when both are used to infest resistant plants. For example, Gould (1979) found higher survivorship on cucumber, the marginal host, by mites reared on cucumber for eleven weeks than by mites reared on beans. Adapted insects may be able to produce as many progeny on resistant plants as unadapted insects produce on susceptible plants. The results of this experiment show that when insects reared on resistant plants are used to infest resistant or susceptible plants, the number of adult progeny resulting is much lower than when insects reared on susceptible plants infest the same varieties (Table 26). This suggests that, after three generations on the resistant variety, the insects have not adapted to be able to utilize it successfully as a host. The low number of adults which emerged from the susceptible variety when it was infested with insects from the resistant variety suggests

Table 25. Mean number of adult progeny collected from each generation of the tests of stability of the resistance of local beans to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*.

Test	Generation	Variety	Acanthoscelides	Zabrotes
1	1	R	23	15
		S	54	58
	2	R	10	150
		S	53	230
2	1	R	22	377
		S	109	266
	2	R	-	360
		S	-	530
	3	R	-	204
		S	-	349

Table 26. Mean number of adult progeny produced by two subpopulations of _Zabrotes_ _subfasciatus_ used to infest a resistant and susceptible variety of local beans.

	Variety	
Subpopulation	Resistant	Susceptible
Reared on resistant host	19	12
Reared on susceptible host	42	62

that the overall fitness of those insects was very low. This is another indication that the insects have not adapted to the resistant variety.

Two factors may affect the results reported here. Only three replicates of each insect subpopulation/variety combination were evaluated. The number of adult progeny collected from each replicate was very variable, resulting in non-statistical differences between varieties or insect populations. Due to difficulties in establishing insect populations on resistant seeds, only three generations of insects were reared on the resistant line before the subpopulations were evaluated. Schoonhoven and Cardona (1982) tested Zabrotes after five generations on the accessions and found similar results. In Gould (1979), the minimum number of generations was approximately twelve and the maximum, approximately fifty. The resistance to Z. subfasciatus in beans is not high and consequently, the selection pressure for adaptation is not strong. It is likely that many more than three generations are necessary to detect a change in the insect population toward adaptation to a resistant host.

Conclusions and recommendations:

1. An evaluation of the number of adult progeny of Zabrotes subfasciatus emerging from a resistant and a susceptible variety which were infested with insects reared for three generations on the same resistant and susceptible varieties, suggests that no adaptation by the insect to the resistant variety as a host occurred. These results are consistent with a stable resistance reaction. However, their accuracy is affected by the variability of the data, the small number of replicates used and the small number of generations of insects reared on each variety.

2. The fitness of the subpopulation of insects reared on the resistant variety, as measured by the number of adult progeny emerging, is much lower than that of the insects maintained on the susceptible variety.

3. Further research on the stability of the resistance to bruchids in Rwandan beans is of lower priority than more fundamental research. When varieties with higher levels of resistance are developed and widely used, the issue of stability may be more important. However, the difficulties involved in this type of research may limit its success.

MAJOR CONTRIBUTIONS

1. A rapid method for screening bean varieties for plant resistance to bruchids was developed and tested. Over one hundred local varieties were tested for the level of resistance to each insect species. The results show that several of the local varieties have resistance to bruchids. The highest levels of resistance to A. obtectus were sufficient to provide short term control of bruchids. The resistance to Z. subfasciatus, in the varieties tested, was too low to be used as the sole factor in avoiding or reducing insect damage. Plant resistance to one bruchid species is not always associated with resistance to the other. (Hypothesis 1A)

2. Seed size was associated with resistance. Large seeded varieties tended to be susceptible. However, other tests indicated that seed size was not the only factor involved in resistance to bruchids. (Hypotheses 1A, 1D)

3. Ripe pods of five bean varieties showed differences in attractiveness for oviposition and in number of eggs laid per pod by A. obtectus. An inverse relationship between the number of pods used for oviposition and the number of eggs laid per pod was noted. This resistance to oviposition was not strongly associated with the level of resistance to seed damage in the varieties tested. (Hypothesis 1B)

4. A set of eight varieties were tested at three GRENARWA warehouses, which differ in elevation and average temperature. Three different relative humidities were maintained with saturated salt solutions. The environment did not affect the resistance of all the varieties in the same manner, so the relative ranking of varieties changed with location and relative humidity. (Hypothesis 1C)

5. Low temperature was an effective method of insect control. A longer larval development period and a reduced number of adults emerging were measured in infested seed kept at the Kora warehouse (mean temperature 17° C). (Hypothesis 1C)

6. Varietal resistance of bean varieties to bruchids can be measured by the change in number of insects or rate of population growth over time. Using the results of a five month test of ten varieties, a resistant, intermediate and susceptible group could be distinguished for each insect species. The relative resistance scores from this test were not the same as those from the rapid screening test of the same varieties. (Hypothesis 1D)

7. The presence of a resistant variety in an artificial mixture of two varieties reduced the amount of damage caused by either bruchid species in the mixture as a whole. As the proportion of the resistant variety in the mixture increased, the amount of damage decreased. Seeds of the resistant variety were less frequently damaged than would be expected if larvae chose seeds

in the mixture at random. This suggests that preference exists for site of oviposition by *Zabrotes* or penetration by larvae of *Acanthoscelides*. (Hypothesis 1E)

8. With infestation levels of 20 females/kg and optimum environmental conditions, a total weight loss of 39% and number of damaged seeds of 87% occurred in 6 months of storage. The greatest increase in damage occurred during the second month of the test. This indicates that control decisions must be made as soon as an infestation is detected. (Appendix 1)

9. A test of the screening method was made by varying the number of seeds and insects. The most effective combination was 75 seeds with 75 larvae of *Acanthoscelides* or 8 pairs of *Zabrotes* adults in a 100x15mm petri dish under optimum conditions. (Appendix 2)

10. A laboratory, equipped for research on storage insects, was set up at ISAR, Rubona.

11. Training in identification and handling of the bruchid species and experimental design and execution was provided for the Rwandan laboratory personnel. In addition, a second year Agronomy student from the National University of Rwanda did a three month internship with this project component.

12. An incubator was designed and built from locally available materials for insect rearing and experiments requiring controlled temperature.

13. The personnel of this component worked in cooperation with the Alternative Storage Methods component of this project, the Entomology section at ISAR and the International Center of Tropical Agriculture (CIAT), Colombia for several studies.

14. Research reports were presented at a regional bean conference in Burundi, the Journées des Etudes on beans at ISAR, Rubona, and an exit meeting at OPROVIA. A paper was presented at the North Central Branch of the Entomology Society meetings in Iowa.

RECOMMENDATIONS

A. Research recommendations:

1. Refine the screening methodology to reduce variability and improve reliability. Possible factors to change include the use of eggs rather than larvae of *A. obtectus*, a method of accurately counting the eggs of *Z. subfasciatus*, the use of smaller containers or a larger number of seeds to fill the containers and increasing the number of replicates of each variety tested.

2. Determine the importance of insect infestations originating in the field versus those originating from old stocks or poorly cleaned storage containers to determine if further research on pod resistance to bruchids is warranted. However, varietal resistance in pods should not be studied in place of varietal resistance in seeds as pod resistance affects only the initial infestation and not the damage caused by later generations of insects.

3. Test varieties in long term (at least six month) screening tests to better evaluate resistance under actual storage conditions. These tests should be carried out under optimum and actual environments. Test the correlation of long term and rapid screening tests.

4. Test local mixtures with the addition of an increasing proportion of a resistant variety for insect damage. The mixtures should be standardized for number of seed of each type and data collected for damage to each of those seed types. Test the resistance in mixtures in long term as well as short term tests.

5. Determine the effect of low temperatures on the development of bruchid life stages and populations. Some factors to be considered are the length of time for emergence and the survival rate for each generation. Determine how level of damage changes with time of storage under cool conditions.

6. Research on the level of damage which constitutes an economic loss, i.e. discarding of damaged seed or a lower selling price, is necessary to determine what level of insect control is appropriate. In order to reduce the likelihood of the insects developing resistance to the insecticides used, the chemicals should be used only when necessary to reduce populations below the economic injury level.

7. Identify the factors which cause resistance in those varieties determined to be resistant, in order to increase the efficiency of the screening methodology. It is important to note any associations of resistance factors with undesirable characteristics, such as tough seed coats or bad taste.

B. Extension recommendations:

1. The potential for damage to stored beans caused by the bruchids *A. obtectus* and *Z. subfasciatus* is greater than is indicated by the reported losses. The actual amount of damage is related to environmental conditions, the resistance level of the varieties in the mixture and the intensity of the initial infestation. In large-scale, long term storage, however, it is possible that environment and infestation level can reach near optimum levels with heavy losses resulting.

2. Varietal resistance in local varieties, though not of a sufficient level alone, could be included in an integrated system of bruchid control in storage. The use of resistant varieties may allow the use of fewer insecticide treatments or less insecticide per treatment. Periodic monitoring is necessary to ensure that the resistance has not broken down.

3. The use of a resistant variety in a mixture to reduce the damage in the mixture as a whole suggests that not all varieties need have resistance to bruchids to control losses due to insects. However, the proportion of a resistant variety necessary to result in adequate control may be high. Including several varieties with resistance in the mixture may be more acceptable to consumers.

4. Tests under optimum conditions and high infestation levels suggest that heavy losses can occur very rapidly. Therefore, the most effective time to treat an infestation is as soon as it is detected. Extensive monitoring is necessary to detect insect infestations as early as possible and to reduce the need for prophylactic chemical treatment of stores.

5. Naturally occurring low temperature increases the development time of bruchids. In the regions of high elevation, these temperatures offer an inexpensive alternative to chemical insect control.

6. The evaluation of resistance to storage insects in beans should continue to be an integral part of the national programs for bean breeding and varietal improvement. The development of varieties which are resistant to insect attack will allow for a more complete system of integrated control of these insects.

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APPENDICES

Appendix 1: Measurement of losses due to *Acanthoscelides obtectus* in a local mixture of dry beans (*Phaseolus vulgaris*) under controlled conditions.

The potential for damage to stored beans caused by bruchids is often mentioned but few authors cite accurate estimates of losses. Schoonhoven (1976) discussed a farm and warehouse survey in Colombia where losses were estimated at 7% after short storage periods (type of damage, weight or number of seeds, was not specified). Lefèvre (1950) tested local and introduced varieties of beans at two sites in Zaire and found that the percent of damaged seeds ranged from 2 to 92 after 4 to 6 months of storage. Burke and Pfost (1976) note a 10% infestation rate for beans in Rwandan markets six months after harvest. In the surveys of Durnez and Dejaegher (1980) and Dunkel et al. (1986), loss in weight of beans stored on farm was measured by repeated sampling and low levels of insect damage were found.

The methods used to measure and report damage levels vary. Adams and Schulten (1978) mention three; weight loss, percent damaged seed and the use of an index to convert percent damage figures to weight loss. Weight loss alone may not accurately reflect losses to the consumer while percent damaged seed does not always reflect the level of damage incurred. In this study, weight loss and number of seed damaged over a six month period were measured to examine losses due to *Acanthoscelides obtectus* in beans under controlled environmental conditions.

Materials and methods:

A locally available mixture of beans, standardized by removal of the varieties occuring with the lowest abundance, was used for the experiment (Table 1). Ten replicates of 100g each of the mixture, in 500ml Mason jars, were infested November 20, 1985 with two pairs of adults, 1-2 days after emergence, from a laboratory colony of *A. obtectus*. The number of seeds of each variety in each replicate was counted before infestation. The test jars, arranged in a completely randomized design, were maintained at 28° C and 50% RH in a wooden incubator in the Storage Insect Laboratory at ISAR, Rubona. Each month, for a six month period, the number and weight of beans which had 1) multiple exit holes, 2) a single exit hole and 3) no damage were measured for each variety. All seed, insects and debris were remixed in the jars after the data were collected each month.

Results and discussion:

The mean total weight loss of the test samples over the six month period was 39% (SE 3.33). Eighty-seven percent of the seeds were damaged (SE 3.89) with 81% (SE 4.98) having multiple exit holes. Table 2 shows the weight lost and number of seeds damaged per month, as a percent of the original weight and number of seeds. The greatest change occurred during the second month of the infestation. It is important to note that the second month of this experimental infestation is equivalent to the

Table 1. Rwandan bean varieties included in the standard mixture for the test of loss measurement.

Variety	Seed type description	Weight of 100 seeds
1	long oval yellow green with black hilum ring	37
2	round oval yellow with black hilum ring	32
3	round oval pink	39
4	long flat purple	47
5	long flat cream mottled with purple	40
6	round oval yellow brown	36
7	long flat cream zebra-striped with black	40
8	long flat cream flecked with purple	40
9	round flat brown	23

Table 2. Mean loss in weight and mean number of bean seeds damaged per month of infestation with _Acanthoscelides obtectus_.

	Number of months after infestation					
	1	2	3	4	5	6
Weight loss per month (%)	0.9 (0.39)[1]	16.7 (1.05)	9.4 (1.33)	4.3 (0.91)	5.2 (1.30)	3.3 (0.86)
No. of seeds damaged per month (%)	13 (1.96)	57 (3.32)	7 (2.58)	4 (0.80)	4 (1.15)	2 (0.80)

[1] Numbers in parentheses are the standard errors of the mean.

second month of storage after harvest only if the infestation coming from the field is very high and conditions are optimal for the insect. The number of seeds damaged reaches a limit before the amount of damage stops increasing because an increase in the number of exit holes in a seed does not change the classification of the seed as damaged. However, weight loss can increase until the bean seed does not offer enough substance to feed or protect a larva. It does not appear that the insects ceased to reproduce and feed after six months as the amount of damage continued to increase even in such a limited resource as 100 g of beans.

These losses occurred under high infestation levels (2 females to 100 g of beans) and optimum environmental conditions. Similar levels would not be common under on-farm conditions. However, in cooperative bulk storage and warehouses, high temperatures can occur, heavily infested beans are often mixed with clean stocks and monitoring of infestation levels is difficult. Under these conditions, losses of this magnitude are possible.

Loss to consumers is not always measured on the basis of actual weight loss as damaged seeds may be rejected for consumption and discarded. Dunkel et al. (1986) found that Rwandan consumers differentiated seeds having one exit hole as consumable from those having several as not consumable. In this test, if the seeds with multiple exit holes are discarded, 81% (SE 4.93) of the weight of the infested lot is lost after six months. When beans are plentiful, even those seeds with one hole may be rejected, resulting in a loss of 87% (SE 3.820) of the original sample weight.

Conclusions and recommendations:

1. The mean weight loss due to Acanthoscelides obtectus after six months of storage was 39%. Eighty seven percent of the seeds were damaged by the insect. These losses were measured under optimal environmental conditions and a high level of infestation but the potential for similar levels of damage exists in poorly managed bulk storage facilities in Rwanda.

2. The greatest change in weight loss and number of damaged seeds occurred during the second month of the test. This does not reflect the actual conditions in local storage but suggests that the time of maximum loss occurs relatively quickly after the infestation has begun. Thus, management and marketing decisions must be made as quickly as possible when an infestation of Acanthoscelides is found.

3. The perception of loss, whether it is measured on a weight or seed number basis, may vary between producer, consumer, merchant and researcher. It is important to consider such differences in definition and to ensure that measurement and discussion of losses in storage caused by insects are on an appropriate basis.

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Appendix 2: Studies on a screening methodology for genetic resistance to bruchids in local beans.

The method used in screening plants or plant parts for genetic resistance to insects is the most important factor in identifying such resistance. The process should be as simple and rapid as possible but must allow the differentiation of resistant and susceptible types. Consistancy and repeatability of results are essential.

The screening methods reported for tests of legume seed resistance to bruchid damage vary considerably (Dobie, pers. comm., 1985, TDRI, Slough, England; Everett, pers. comm, 1983, IITA, Ibadan, Nigeria; Schoonhoven and Cardona, 1982). Schoonhoven and Cardona (1982) used coefficients of variance to evaluate a methodology to test beans for resistance to _Zabrotes subfasciatus_. In this experiment, a similar procedure was used to compare treatment combinations of four numbers of seeds with four levels of insect infestation for screening for resistance to _Acanthoscelides obtectus_ and _Zabrotes subfasciatus_.

Materials and methods:

Two varieties, one resistant and one susceptible, were tested with each insect species. The line resistant to _Acanthoscelides_ was 248 and 137 was its susceptible counterpart. Line 9 was resistant to _Zabrotes_ and line 19 was susceptible. All seeds used were produced by the Varietal Survey component of this project and were stored in sealed plastic buckets in the Storage Insects Laboratory at ISAR-Rubona. The insects were produced as a part of the laboratory culture in the same facility. Pairs of adult _Zabrotes_, 1-2 days after emergence, or newly hatched _Acanthoscelides_ larvae were used in this experiment.

The treatments were number of seed and number of insects. Ten, twenty-five, fifty or seventy-five seed were infested with 10, 25, 50 or 75 _Acanthoscelides_ larvae or 2, 4, 6 or 8 pairs of _Zabrotes_ adults. All tests were done in plastic petri dishes (100 x 15mm) and five replicates of each treatment combination were used. The _Zabrotes_ test was infested 18-21 June, 1985 and the test with _Acanthoscelides_, 25-30 June, 1985. The dishes were arranged in random order by insect species and maintained at 30° C and 45% RH in the wooden incubator.

Emerged adults were collected 14-28 July, 1985 for _Zabrotes_ and 23 July - 2 August, 1985 for _Acanthoscelides_. The number of adults emerging on each collection date was recorded. The days to emergence and total adults emerged were calculated for each replicate. The mean and the coefficient of variance (CV) were computed for each treatment and each measurement. Means for the resistant and susceptible lines were compared by the Student Newman Keuls test (SNK) at a significance level of 1%.

Results and discussion:

In theory, the combination of number of insects and number of seeds which results in the lowest calculated CV should produce the most accurate and reliable results in a screening test. The

CV values for the four varieties are given in Tables 1 and 2 for the criterion `total adults emerged'. The range of values for mean days to emergence (28-30 for Zabrotes, 25-31 for Acanthoscelides) was so narrow that the CV values vary little (0.02-0.05 for Zabrotes, 0.02-0.08 for Acanthoscelides). Therefore only the results for total adults emerged will be discussed here. For Acanthoscelides, in general, the CV decreases with increasing number of larvae and number of seeds. For both varieties, the lowest CV is for the 75 seeds-75 larvae treatment. The data for Zabrotes suggest that an increase in the number of larvae results in a decrease in the CV. The result of a change in the number of seeds is less clear. The lowest calculated values are for the 25 seeds-6 pairs of adults combination for variety 9 and, for line 19, 10 seeds-8 pairs. Acanthoscelides larvae must crawl to find seeds to infest. A higher number of seeds, filling the petri dish more completely, would make finding the seeds easier for the larvae. This would prevent the decreased fitness or increased mortality of larvae caused by their search. The choice of a seed to be infested by Zabrotes is made by the adult female. While an increase in the number of seeds may make it easier for a female to find a suitable oviposition site, it will have less effect, if any, on the survival of the larvae. The effect of a change in number of insects is less obvious. As the number of insects used increases, the number of progeny which do not survive is not proportional to that increase but less. Thus, the values for total adults are more similar, rep to rep, and the CV is smaller.

In addition to reliable and accurate results, a screening test must allow the differentiation of resistant and susceptible lines. For Acanthoscelides, the difference in number of adults emerged between the resistant and susceptible varieties was significant at 1% only for those treatment combinations with 75 larvae (Table 3). The number of seeds did not seem to affect the differentiation of the resistance levels. In previous tests, the level of resistance to Zabrotes measured in Rwandan beans was not as complete as the level of resistance to Acanthoscelides. In this test that the mean number of adults emerged from the susceptible variety was greater than that from the resistant variety in only 62% of the treatment combinations with Zabrotes compared to 93% for Acanthoscelides. Consequently, the results for Zabrotes (Table 4) do not strongly support any treatment combination as the most effective.

Although it was not one of the specific objectives of this experiment, the results allow some discussion of the ecology of the insects. Table 5 shows the change in the percent emergence of the Acanthoscelides larvae or the progeny per female of the Zabrotes adults by number of seed or insects. The increase in percent emergence of Acanthoscelides with an increase in number of seeds is explained by the ease with which the larvae find the seed, as discussed in a previous section. However, the percent of larvae emerging as adults decreases, in general, as the number of larvae used increases, even though the number of adults

Table 1. Coefficients of variation for total adults emerged in sixteen treatment combinations for the test of screening methodology for the resistance of local beans to *Acanthoscelides obtectus*.

No. of seeds	No. of larvae	Variety 137	Variety 248
10	10	1.37	-[1]
	25	1.38	-
	50	0.92	1.86
	75	0.56	2.24
25	10	0.61	1.73
	25	0.35	1.69
	50	0.56	1.62
	75	0.34	0.80
50	10	0.35	0.87
	25	0.42	0.70
	50	0.41	0.61
	75	0.37	0.95
75	10	0.38	0.64
	25	0.83	0.77
	50	0.52	0.76
	75	0.30	0.46
10	-[2]	1.04	3.10
25	-	0.91	1.10
50	-	0.87	0.82
75	-	0.80	0.86
-	10	0.67	1.23
-	25	0.95	1.21
-	50	0.74	1.35
-	75	0.57	1.15

[1] No adults emerged in these treatments.

[2] CV calculated for one treatment factor over all values of the other.

Table 2. Coefficients of variation for total adults emerged in sixteen treatment combinations for the test of screening methodology for the resistance of local beans to *Zabrotes subfasciatus*.

No. of seeds	No. of pairs of adults	Variety 137	Variety 248
10	2	0.58	0.57
	4	0.69	0.46
	6	0.27	0.37
	8	0.14	0.29
25	2	0.33	0.31
	4	0.22	0.58
	6	0.28	0.16
	8	0.28	0.41
50	2	0.29	0.67
	4	0.30	0.38
	6	0.22	0.61
	8	0.17	0.28
75	2	0.86	0.56
	4	0.26	0.23
	6	0.34	0.24
	8	0.29	0.30
10	- [1]	0.61	0.57
25	-	0.39	0.51
50	-	0.46	0.63
75	-	0.64	0.54
-	2	0.52	0.56
-	4	0.41	0.40
-	6	0.29	0.44
-	8	0.33	0.33

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[1] CV caluclated for one treatment factor over all values of the other.

Table 3. Mean comparison of resistant and susceptible bean varieties by treatment combination for the test of screening methodology for the resistance of local beans to *Acanthoscelides obtectus*.

No. of seeds	No. of larvae	Significance[1]
10	10	NS
	25	NS
	50	NS
	75	**
25	10	NS
	25	NS
	50	NS
	75	**
50	10	NS
	25	NS
	50	*
	75	**
75	10	NS
	25	NS
	50	NS
	75	**

[1] Mean comparison by Student Newman Keuls test.
NS - non-significant
* - significant at 5%
** - significant at 1%

Table 4. Mean comparison of resistant and susceptible bean varieties by treatment combination for the test of screening methodology for the resistance of local beans to _Zabrotes_ _subfasciatus_.

No. of seeds	No. of pairs of adults	Significance
10	2	NS
	4	NS
	6	NS
	8	NS
25	2	NS
	4	NS
	6	NS
	8	NS
50	2	NS
	4	NS
	6	NS
	8	NS
75	2	NS
	4	*
	6	NS
	8	NS

[1] Mean comparison by Student Newman Keuls test.
NS - non-significant
* - significant at 5%
** - significant at 1%

Table 5. The percent of emergence of *Acanthoscelides obtectus* larvae and the progeny per female of *Zabrotes subfasciatus* by treatment and variety.

Percent emergence of *Acanthoscelides* larvae

Treatment	Variety 137	 248
10 seeds	8	1
25	19	7
50	29	16
75	38	25
10 larvae	29	26
25	19	18
50	20	11
75	26	6

Progeny/female of *Zabrotes*

Treatment	Variety 19	 9
10 seeds	12	11
25	15	13
50	18	15
75	14	15
2 pairs adults	19	15
4	13	13
6	13	14
8	13	12

emerging increases. This may be the result of competition within the seeds by larvae which use the same entry hole. The number of progeny per Zabrotes female is quite similar with all treatments. However, there is a trend of the highest number of progeny/female with the fewest pairs of adults and an increase in number of progeny/female with an increase in number of seeds to 50. Both tendencies suggest that less time is spent or more suitable oviposition sites are found when the competition for sites is decreased by reducing the number of females or increasing the number of sites (seeds).

Conclusions and recommendations:

1. The recommended screening methodology for *Acanthoscelides obtectus* is 75 larvae used to infest 75 seeds. The recommended screening methodology for *Zabrotes subfasciatus* is 8 pairs of adults used to infest 75 seeds. Both recommendations are based on the use of the method described for age of insects, test containers and environment.

2. Limitations in resources may affect the number of insects or seeds that are available for use. If the number of seeds is limited, the use of smaller test containers will allow a reduction in the number of seeds necessary while still providing that the seed covers the surface where the insects are placed. If there are insufficient insects, reduce the number of seeds and use the same proportion of insects to seeds as recommended. However, the number of insects per test unit should not be less than 25 *Acanthoscelides* larvae or 4 pairs of *Zabrotes* adults.

3. Two aspects of the methodology should be examined by repeating the experiments described here:

   a. A retest would allow an examination of the repeatability of the results and strengthen the recommendations.

   b. A retest, using varieties differing more widely in their reaction to *Zabrotes* would allow the choice of a method based on differentiation of resistance level.

Literature cited:

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

Appendix 3: Details of construction of the heated wooden cabinet used as an incubator.

This cabinet was designed by Dr. David R. Thompson for use as an insect rearing chamber (Figures 1 a-e). It was constructed of materials available in Kigali, Rwanda. The insulation used was a combination of styrofoam and cork. The chamber was wired for six light bulbs with a switch for each to allow a range of temperatures. The temperature desired, approximately 30^{o} C, was maintained with two 60 watt bulbs. With a combination of three 100 and three 40 watt bulbs the interior temperatures reached +/- 70 ^{o} C. Two mercury bulb thermometers were installed, one at the upper shelf level and one below the lowest shelf level, to allow for recording the temperature. The ambient interior humidity was at 40-45%. Pans of water or salt solutions could be used to increase the RH.

The chamber was well suited for insect rearing. Other modifications are possible for other uses. Lights could be added for a controlled photoperiod. An electric fan could be used to give a constant temperature throughout the chamber.

Figure 1a-e. Views of the wooden cabinet used as an insect rearing chamber.

Figure 1a.

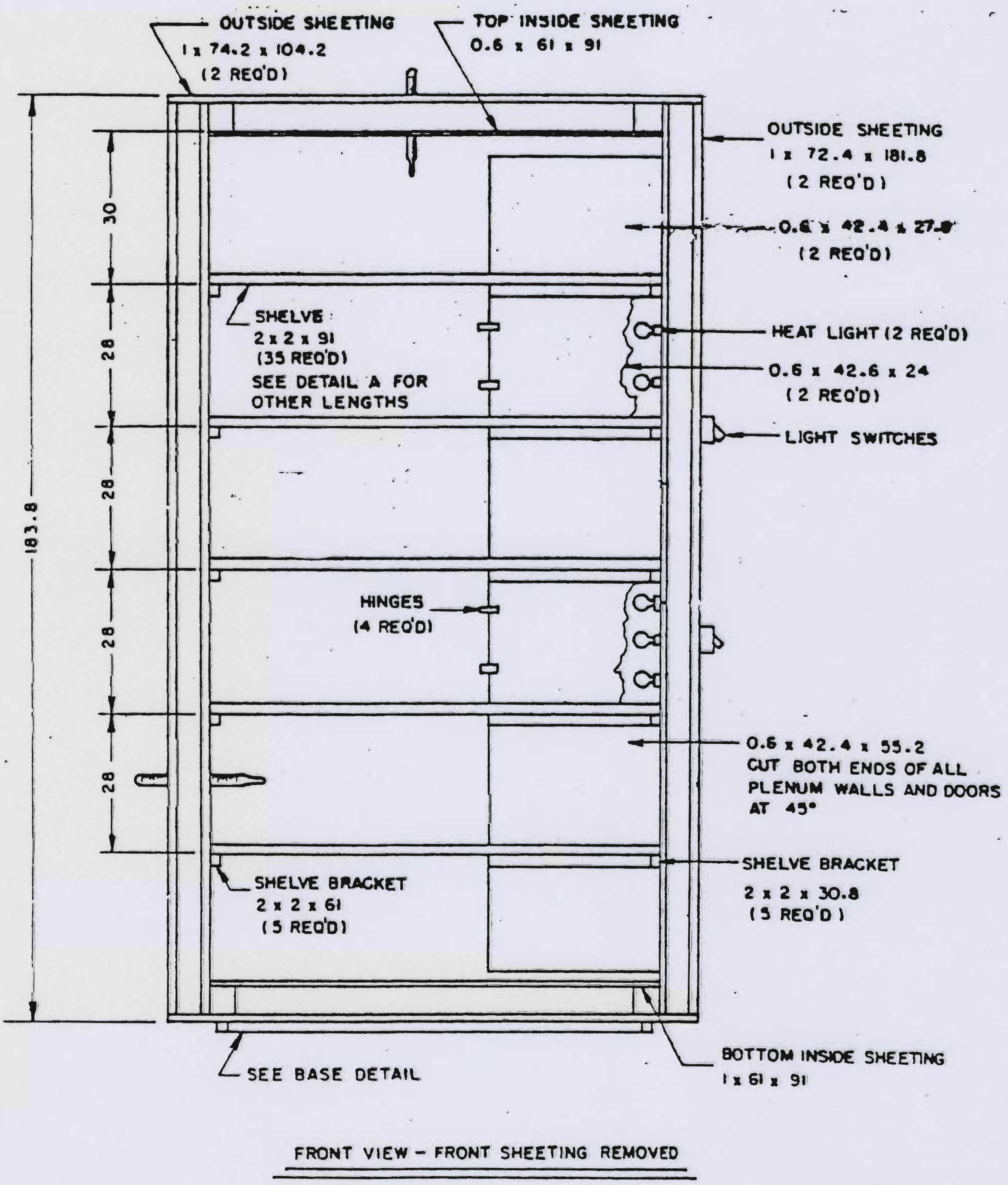


Figure 1b.

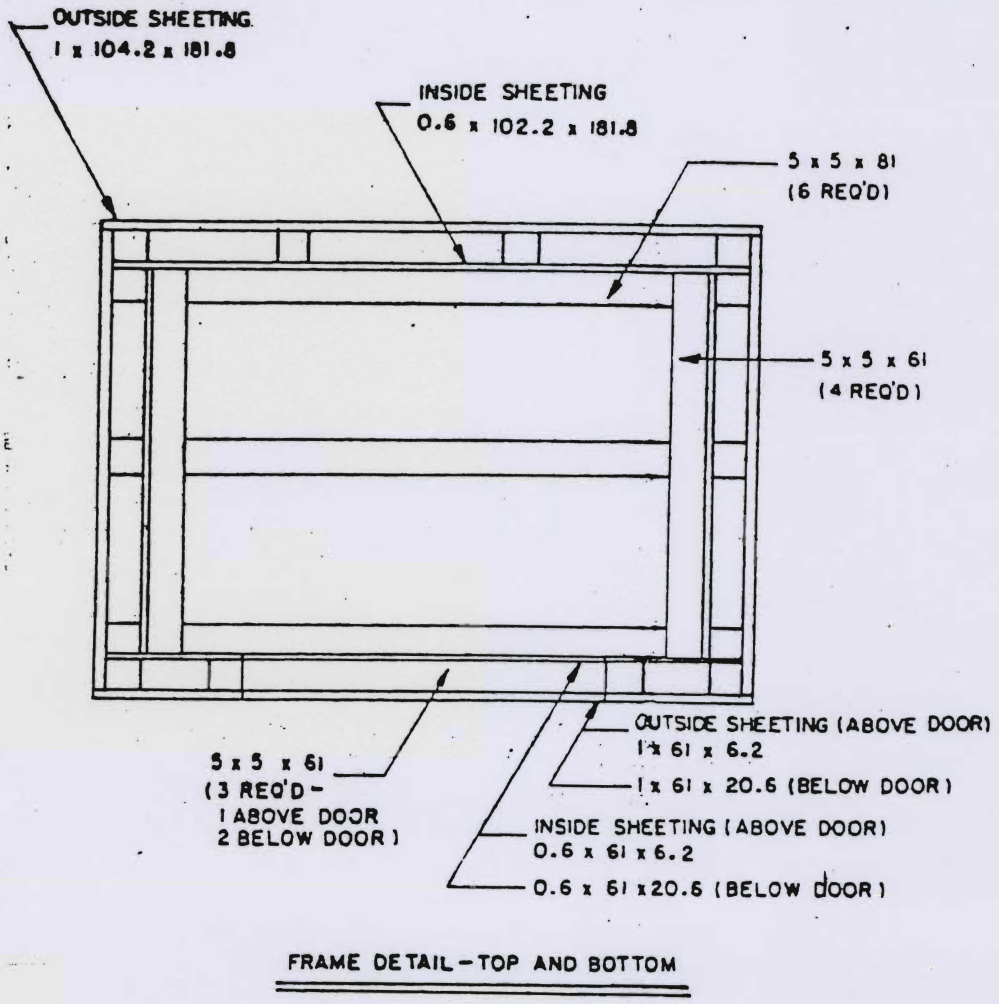


Figure 1c.

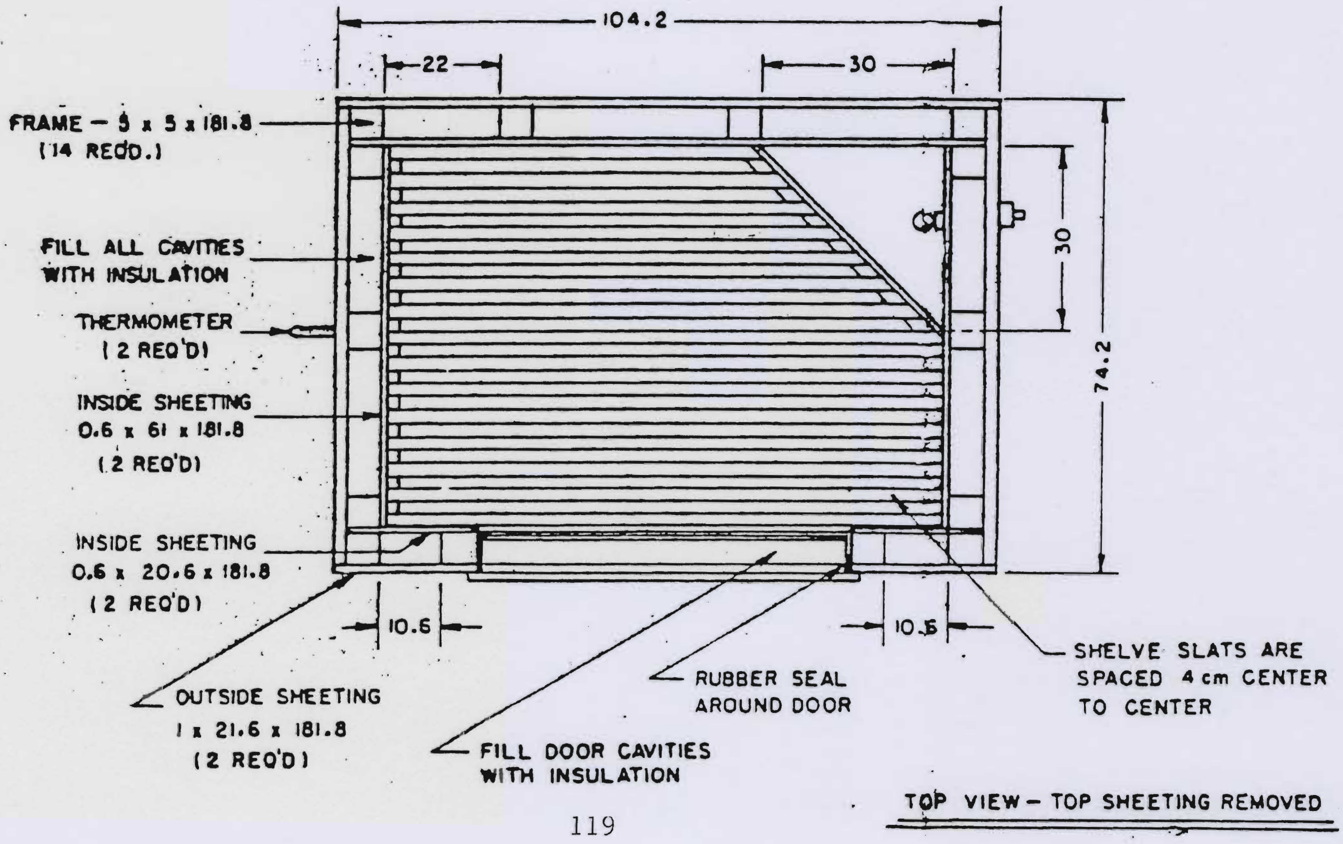
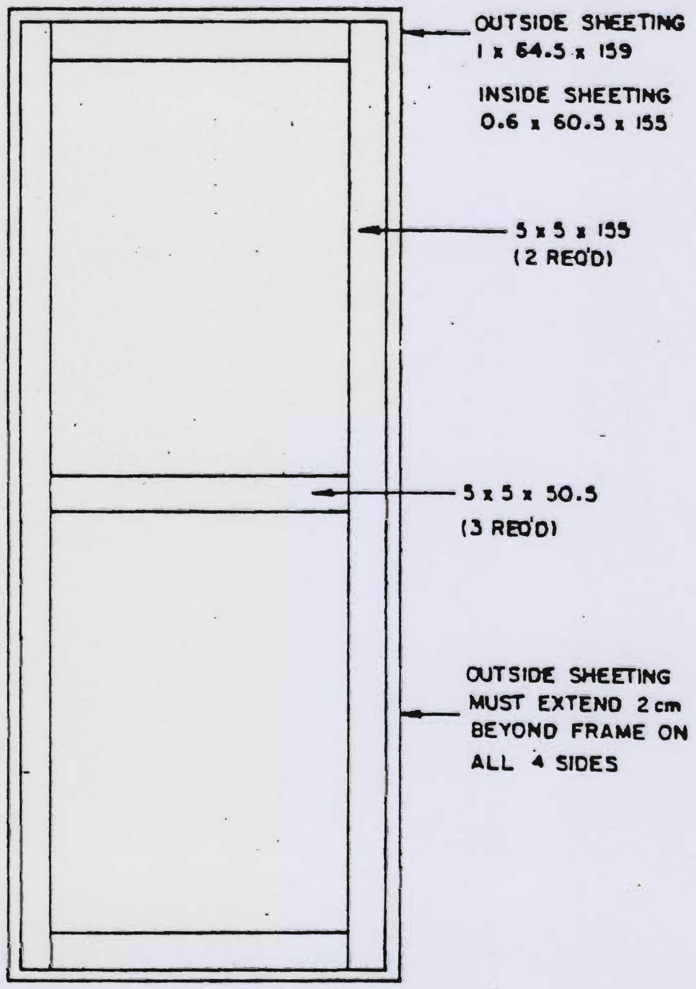
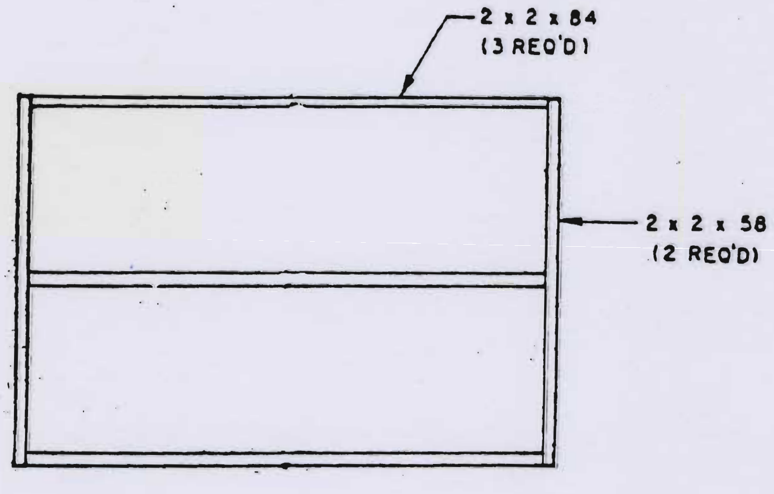


Figure 1d.



DOOR – INSIDE SHEETING REMOVED

Figure 1e.



BASE DETAIL

Appendix 4: List of sources for the equipment purchased for the Storage Insects Laboratory at ISAR.

1. American Scientific Products
   13505 Industrial Park Boulevard
   P.O. Box 41515
   Minneapolis, Minnesota 55441

   a. Labline Incubators (3550-1)[1]
   b. Blue M Incubators (1680-200)
   c. Nalgene Dessicators (D1416-7)
   d. Dessicator Plates (D1416-10)

2. Bioquip Products
   P.O. Box 61
   Santa Monica, California 90406

   a. Insect Collection Equipment

3. Blue M
   Blue Island, Illinois 60406

   a. Blue M Incubators and Equipment

4. Carolina Biological Supply Company
   Burlington, North Carolina 27515

   a. Glass Vials, 9 dram, screw top (71-5147)

5. Curtin Matheson
   2218 University Avenue
   Minneapolis, Minnesota 55414

   a. Transformers, step-down, 2000 watt capacity

6. Fisher Scientific
   6990 Shady Oak Road
   Eden Prairie, Minnesota 55344

   a. Chemical Salts, $MgCl_2$, $Mg(NO_3)_2$, KI

7. Frank E. Fryer Company, Inc.
   7317 Cahill Road
   Edina, Minnesota 55435

   a. Nisho Double View Zoom Stereo Dissecting Microscope

8. National Camera Exchange
   1327 Southeast 4th Street
   Minneapolis, Minnesota 55414

   a. Canon AE-1 Program Camera Body (3504028)
   b. 28mm Tokino Wide angle Lens (8322008)
   c. 50mm Tokino Macro Lens (99838)

9. Sargent Welch Scientific Company
7300 North Linden Avenue
Skokie, Illinois 60077

   a. Refrigerator (S-43651)[1]
   b. Freezer (S-43648-60)

10. Seedburo Equipment Company
1022 West Jackson Boulevard
Chicago, Illinois 60607

   a. Cole Parmer Hygrothermograph (8368-00)
   b. Charts for Cole Parmer Hygrothermograph (8368-20)
   c. Hygrothermograph Pens (8368-75)
   d. Magnifying Lamp
   e. Bulbs for Lamp (B1-1767)
   f. Soil Sieves, U.S. Standard No. 10 and No. 20, and Base Pan (4530, 44535 and 8481)
   g. Sample Pans

11. VWR Scientific
1124 Stinson Boulevard
Minneapolis, Minnesota 55413

   a. Bacharach 7-Day Recorder (35580-031)
   b. Charts for Bacharach Hygrothermograph (35581-034)
   c. Ink for Hygrothermograph (35581-103)

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[1] Number in parentheses is the order number for the item.