

A Study of Bruchid Resistance and Its Inheritance in Malawian Dry Bean Germplasm

By

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The common bean weevil
(*Acanthoscelides obtectus* Say)



The Mexican bean weevil
(*Zabrotes subfasciatus* Boheman)



Bruchid damage inflicted on stored beans

Thesis Abstract

Dry bean (*Phaseolus vulgaris* L.) is economically and nutritionally an important legume, not only in Malawi, but in many parts of Africa and Latin America. Unfortunately, two bruchid species (*Acanthoscelides obtectus* Say, and *Zabrotes subfasciatus* Boheman) are known to cause extensive damage in storage, reducing the economic importance, food value and planting value of the crop. The aim of this study was to: i) ascertain farmers' perceptions of the importance of bruchids as storage pests, and to identify their preferred varietal traits in dry beans; ii) screen Malawian dry bean landraces for effective and adaptable sources of resistance to the two bruchid species; iii) determine the gene action and inheritance of bruchid resistance.

Farmers' perceptions on the importance of the two bruchid species to beans both in the field and in storage were established using a participatory rural appraisal (PRA) in three extension planning areas (EPAs) in Lilongwe agricultural development division (ADD). Results confirmed that the two bruchid species are important storage pests, causing serious storage losses among smallholder farmers. In the absence of any control measures, farmers indicated that more than 50% of their stored beans could be lost to bruchids. Indigenous bruchid control measures are not very effective, making it necessary to search for other control methods. It was also clear from the PRA results that breeders need to consider both agronomic and culinary traits in bean cultivar development. This would enhance uptake of newly developed varieties.

To address the problem of bruchid damage experienced by smallholder farmers, a total of 135 dry bean genotypes, comprising 77 landraces and 58 improved varieties (obtained from collaborating partners) were tested under laboratory infestation (no-choice test methods) and field infestation (free-choice test methods). The objective of this study was to identify effective sources of resistance to the two bruchid species. Results of the study showed that there was a wide variation among the genotypes for resistance to the two bruchid species. Overall results showed that 88% of the genotypes ranged from susceptible to highly susceptible to *Z. subfasciatus* and only 12% of the genotypes were moderately resistant to resistant. Genotype screening for resistance to *A. obtectus* showed that only 12.5% were resistant, whereas 87.5% were moderately to highly susceptible. All of the improved genotypes were 100% susceptible to *A. obtectus* in storage. One landrace, KK35, consistently showed a high level of resistance to both bruchids under laboratory infestation, with results similar to the resistant checks (SMARC 2 and SMARC 4), while another landrace, KK90, displayed stable resistance under both laboratory and field infestation. However, performance of most genotypes was not consistent with field and laboratory screenings, suggesting that mechanisms of bruchid resistance in the field are different from that in the laboratory and field screening should always be used to validate laboratory screening. Resistance in the field was not influenced by morphological traits. The seed coat played a significant role in conferring resistance to both bruchid species in the laboratory, whereas arcelin did not play any significant role in conferring resistance in the landraces.

The inheritance of resistance to *A. obtectus* was studied in a 6 x 6 complete diallel mating design, involving crosses of selected Malawian dry bean landraces. The F1 crosses, their reciprocals, and six parents were infested with seven F1 generation (1 to 3 d old) insects of *A. obtectus* in a laboratory, no-choice test. There were significant

differences among genotypes for general combining ability (GCA) and specific combining ability (SCA). However, SCA accounted for 81% of the sum of squares for the crosses, indicating predominance of the non-additive gene action contributing to bruchid resistance. A chi-square test for a single gene model showed that 5 of the 13 F₂ populations fitted the 1:2:1 segregation ratio of resistant, intermediate and susceptible classes, respectively indicating partial dominance. The eight F₂ populations did not conform to the two gene model of 1:4:6:4:1 segregation ratio of resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible classes, respectively. Average degree of dominance was in the partial dominance range in five F₃ populations, but in general resistance was controlled by over-dominance gene action in the F₂ populations. The additive-dominance model was adequate to explain the variation among genotypes indicating that epistatic effects were not important in controlling the bruchid resistance. The frequency distribution of the 13 F₃ populations for resistance to *A. obtectus* provided evidence for transgressive segregation, suggesting that resistance is conditioned by more than one gene. Reciprocal differences were not significant in the F₂ generation seed; but were significant in four crosses in the F₃ generation seed for adult bruchid emergence, suggesting that maternal effects or cytoplasmic gene effects also played a role in the inheritance of resistance to the common bean weevil.

Through this study, important sources of bruchid resistance in dry bean have been identified in Malawian landraces (KK35, KK90 and KK73). These resistant sources will be used in a breeding programme to develop bruchid resistant bean cultivars, as well as improve resistance in susceptible commercial bean cultivars currently grown by farmers in Malawi.

Declaration by the Candidate

This PhD study was carried out at the African Centre for Crop Improvement (ACCI) in the School of Biochemistry, Genetics, Microbiology and Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg Campus, under the supervision of Professor R. Melis, Dr. J. Derera and Professor M.D. Laing.

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other scientists' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists
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.....
Geoffrey Acrey Duncan Kananji (Candidate)

Declaration by the University Supervisors

This thesis has been submitted for examination with our approval as University of KwaZulu-Natal supervisors

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Professor R. Melis (Supervisor)

Sign Date

Dr. J. Derera (Co-supervisor)

SignDate

Professor M.D. Laing (Co-supervisor)

Dedication

This thesis is dedicated to God the Almighty, who knows the end from the beginning,
and to my parents for seeing this work through.

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I most sincerely appreciate the support of my supervisors, Professor R. Melis, Dr. J. Derera and Professor M. Laing, for making their time available when I needed it most.

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Table of Contents

Thesis Abstract	i
Declaration by the Candidate.....	iii
Declaration by the University Supervisors	iv
Dedication	v
Acknowledgements	vi
Table of Contents.....	viii
List of Abbreviations	xi
Definition of Some Terms Used in the Study	xii
Introduction to Thesis	xiv
1. Importance of dry beans in eastern and southern Africa.....	xiv
2. Bean production statistics for Malawi.....	xiv
3. Status of bean research in Malawi.....	xv
4. Constraints on bean production.....	xvi
5. Bruchids, their importance and control methods in Malawi	xvii
6. Host plant resistance and gene action for bruchid resistance	xvii
7. Justification for the study	xviii
8. Thesis structure	xxi
References.....	xxii
Chapter One	1
Literature Review	1
1.1 Introduction	1
1.2 Origin, gene pools and genetic variation in dry beans.....	1
1.3 Bean genetic diversity in Malawi	2
1.4 The genus Phaseolus	3
1.5 Inter-specific and intra-specific hybridisation in dry beans.....	4
1.6 Bean bruchids and their importance.....	4
1.7 Composition and distribution of the two bruchid species	5
1.8 Ecology and biology of the two bruchid species	5
1.9 Bruchid management and control strategies	6
1.9.1 Cultural and interference control methods.....	7
1.9.2 Chemical control methods.....	8
1.9.3 Biological control methods: Use of parasitoids.....	8
1.9.4 Host plant or varietal resistance	10
1.10 Breeding for bruchid resistance	11
1.11 Bruchid resistance testing methods	12
1.12 Mechanisms of bruchid resistance	13
1.13 Resistance factors to storage pests.....	16
1.14 Role of arcelin in bean bruchid resistance	18
1.15 Factors mediating the expression of resistance and durability of resistance to stored products.....	20
1.16 Gene action and inheritance of bruchid resistance	21

1.17	Mating designs and the usefulness of the diallel.....	24
1.18	Assumptions of the diallel mating design	26
1.19	Summary	27
	References	28
Chapter Two		43
Smallholder Farmers' Perceptions of Dry Bean Damage By Bean Weevils and Varietal Preferences in Malawi and Their Implications for Breeding		43
	Abstract.....	43
2.1	Introduction	44
2.2	Materials and methods	47
2.2.1	Features of the study areas	47
2.2.2	Data collection and analysis.....	49
2.2.3	Indigenous bruchid control methods validation study.....	52
2.3	Results	54
2.3.1	Secondary data	54
2.3.2	Damage caused by bruchids	55
2.3.3	Bruchid control methods used by farmers	56
2.3.4	Appraisal of farmers' bruchid control methods	57
2.3.5	Varieties grown and farmers' preferences	60
2.3.6	Perceptions of bruchid incidence and severity.....	63
2.4	Discussion and conclusion	65
	References	69
Chapter Three.....		73
Resistance of Malawian Dry Bean (<i>Phaseolus Vulgaris</i> L.) Landraces to <i>Acanthoscelides Obtectus</i> Say and <i>Zabrotes Subfasciatus</i> Boheman.....		73
	Abstract.....	73
3.1	Introduction	74
3.2	Materials and methods	77
3.2.1	Bean germplasm collection	77
3.2.2	Bruchid laboratory culture.....	79
3.2.3	Bruchid resistance testing.....	80
3.2.4	Bruchid resistance rating	83
3.2.5	Classification of genotypes for resistance to bruchids.....	84
3.2.6	Testing the resistance mechanism	85
3.3	Results	86
3.3.1	Resistance of bean germplasm to <i>Zabrotes subfasciatus</i> in the laboratory test	86
3.3.2	Resistance of bean germplasm to <i>Acanthoscelides obtectus</i> in the laboratory test	89
3.3.3	Resistance of improved genotypes to <i>Acanthoscelides obtectus</i> in the laboratory test.....	92
3.3.4	Confirmation of genotypes' resistance to <i>Acanthoscelides obtectus</i> under laboratory conditions	94
3.3.5	Resistance of genotypes under field infestation.....	95
3.3.1	Comparison of genotypes' resistance to <i>Acanthoscelides obtectus</i> under laboratory and field infestation.....	98
3.3.2	Relationship between phenotypic traits in the field and adult bruchid emergence in the laboratory	101
3.3.3	Role of seed coat in influencing resistance of bean genotypes to <i>Acanthoscelides obtectus</i> and <i>Zabrotes subfasciatus</i>	103

3.4	Discussion	105
3.4.1	Expression of resistance in landraces to the two bruchid species	105
3.4.2	Bruchid resistance mechanisms: role of arcelin and the seed coat	107
3.4.3	The influence of morpho-physiological traits on bruchid resistance	109
3.4.4	Field resistance of bean genotypes	110
3.5	Conclusion	111
Chapter Four		122
Genetic Analysis of Dry Bean Resistance to the Common Bean Weevil (<i>Acanthoscelides Obtectus</i> Say)		122
Abstract.....		122
4.1	Introduction	123
4.2	Materials And Methods	125
4.2.1	Germplasm	125
4.2.2	Field procedures and diallel mating scheme	125
4.2.3	Resistance testing of the F2 and F3 generation seed.....	126
4.2.4	Parameters used to differentiate bruchid resistance of genotypes	127
4.2.5	Determination of combining ability estimates	128
4.2.6	Segregation analysis	129
4.2.7	Confirmation of the adequacy of the additive-dominance model	130
4.2.8	Estimation of components of variation and genetic parameters	131
4.3	Results	132
4.3.1	Bruchid resistance in the F2 generation seeds	132
4.3.2	Combining ability estimates for adult bruchid emergence in F2 generation	135
4.3.3	Bruchid resistance in the F3 generation seed.....	135
4.3.4	F3 population segregation analysis.....	137
4.3.5	Frequency distribution of bruchid resistance of the F3 genotypes	140
4.3.6	Adequacy of the additive-dominance model	143
4.3.7	Estimation of the components of variation and the genetic parameters.....	143
4.3.8	Relationships among resistance measurements in the F3 generation seed	144
4.4	Discussion	145
4.4.1	Resistance of genotypes	145
4.4.2	Combining ability estimates for bruchid resistance	146
4.4.3	Role of maternal inheritance or the cytoplasmic effects for resistance	148
4.4.4	Transgressive segregation	149
4.4.5	Gene action and the genetic parameters	149
4.5	Summary and conclusions.....	150
References.....		151
Chapter Five		155
General Overview.....		155
5.1	Introduction	155
5.2	Summary of the major findings	155
5.2.1	Smallholder farmers' perceptions of bean bruchid damage and varietal preference	155
5.2.2	Dry bean germplasm screening for sources of bruchid resistance.....	156
5.2.3	Genetic analysis of resistance to <i>A. obtectus</i> of the Malawian dry bean germplasm	157
5.3	Breeding implications	158
5.4	Challenges in breeding for bruchid resistance in dry beans	160
5.5	Directions for future research	161
References.....		162

List of Abbreviations

ABE	Adult bruchid emergence
ADD	Agricultural development division
ARET	Agricultural research and extension trust
ATC	Agricultural trading company
CIAT	Centro internacional de agricultura tropical
CIMMYT	International maize and wheat improvement centre
CRSP	Collaborative research support programme
CTH	Controlled temperature and humidity
DDT	Dichlorodiphenyltrichloethane
DRI	Dobie relative index of susceptibility
DSI	Dobie susceptibility index
EPA	Extension planning area
FEWS	Famine early warning systems
FGW	Final grain weight
GCA	General combining ability
IGW	Initial grain weight
MDP	Mean development period
NARS	National agricultural research systems
PCA	Principal component analysis
PCI	Participatory crop improvement
PHA	Phytohemagglutinin
PPB	Participatory plant breeding
PRA	Participatory rural appraisal
RABE	Relative adult bruchid emergence
RDP	Rural development project
REML	Restricted maximum likelihood
RH	Relative humidity
SCA	Specific combining ability
SPSS	Statistical package for social scientists
D	Additive gene effects
H ₁	Dominance variance 1
H ₂	Dominance variance 2
F	Additive-dominance covariance
E	Environmental variance

Definition of Some Terms Used in the Study

Term	Definition
Arcelin	A seed storage protein that accumulates in the cotyledon of the seed identified in wild bean accessions and confers resistance to dry beans against <i>Zabrotes subfasciatus</i>
Additive gene action	Mean effect of alleles where a heterozygous genotype produces a phenotype that is intermediate between those produced by the two parents
Adult bruchid emergence	F1 progeny that emerge after the development period in the laboratory
Combining ability	A statistical value indicating the capacity of a parent in crosses with other parents to transmit genes for a certain degree of character expression. Good general combining ability (GCA) of a parent signifies the high average performance of its progenies in various crosses, as compared to progenies of other parents in the same test. The "breeding value" of a parent is twice its GCA. Good specific combining (SCA) refers to two parents, which, when crossed together, produce progeny better than expected on the basis of the parental GCA values.
Complete diallel cross	A mating design and subsequent progeny test resulting from the crossing of n parents in all possible combinations including selfs and reciprocals.
Character (trait)	A distinctive but not necessarily invariable feature exhibited by all individuals of a group and capable of being described or measured e.g., color, size, performance. A character of a given individual will have a certain phenotype as determined by the individual's genotype and environment
Decortication	Removal of the seed coat/ seed testa
Epistasis	Action of one gene is modified by one or several genes that assort independently
Free-choice test	Grain is evaluated in a non-restricted environment where insects make their own choice of grain or variety
F1 bruchid generation	1-3 d old bruchid population that is used to infest the grain
F2 generation seed	Seed advanced from the F1 plant populations
F3 generation seed	Seed advanced from the F2 plant populations
Gene pool	The sum total of all the genetic variation in the breeding population of a species and closely related species capable of crossing with the species
Improved varieties	Varieties that were developed and released officially for commercial production
Introgression	The movement of genes from one population into another through hybridization followed by backcrossing. Usually refers to movement of genes from one species to another or among sub-species that have been geographically isolated then brought back together by changes in the species ranges or planting of exotic populations.

Term	Definition
Landraces	Mixture of crop varieties that are farmer-selected and are highly adapted to local environmental conditions
Maternal effects	Genotype of a mother expressed in the phenotype of its offspring unaltered by paternal genetic influence
Maternal inheritance	Aspect of an offspring's genotype inherited solely from the mother
No-choice test	Grain is evaluated in closed ventilated container (glass, jars, bottles) and grain is artificially infested with bruchids
Oviposition	The process of laying eggs by insects
Reciprocal cross	The repetition of a cross where the sexual function of the parents is reversed, i.e., female B x male A is the reciprocal of female A x male B.
SMARC lines	Arcelin-containing lines from a sanilac bean type lacking phaseolin or phytohemagglutinins
Transgressive segregation	The presence of traits or phenotypes that are extreme relative to either of the parental lines

Introduction to Thesis

1. Importance of dry beans in eastern and southern Africa

The dry bean (*Phaseolus vulgaris* L.) is one of the major staple crops in eastern and southern Africa. It is an important source of protein for the poor and combines well with the carbohydrate-dominated traditional food consumed by many. Unlike other legumes, such as groundnut or pigeonpea, the dry bean plant is a source of food throughout the life cycle of the crop as its leaves, green beans and dry beans can be consumed. Dry beans are preferred to other legumes and are widely grown by many farmers because of their short maturity period. This short maturity period is an important agronomic trait because the rainfall pattern has been unpredictable and intermittent in many countries of sub-Saharan Africa. Another reason for the preference for beans to other crops is that farmers are able to harvest something even when adverse conditions prevail. Because beans are relatively resistant to abiotic stress, farmers do not experience a total crop failure, as might be the case with a crop such as maize. A bean plant is also viewed by many farmers as an important soil conditioning crop that can contribute up to 40 kg N ha⁻¹ to the soil (Snapp *et al.*, 1998). As a result of this capacity, the dry bean is an appropriate crop for use in rotation with cereals and also fits into the intercropping systems commonly used by small-scale farmers with limited land. Economically, dry beans are an important source of income (cash) and they play a key role in mitigating rural poverty, which is widespread and severe in many African countries, including Malawi. Because of the advantages of growing dry beans, the Malawi government has a policy aimed at increasing grain legume production for local consumption to improve the nutritional status of rural and urban communities (Malawi Government, 2005).

2. Bean production statistics for Malawi

Dry beans are grown widely in Malawi and adapt well to various climatic and agronomic conditions. Beans are commonly grown by female farmers who usually intercrop them with other food crops such as maize. In the 2003/4 growing season, up to 239,474 ha were under bean production with an average annual production of 109,832 MT (Malawi Government, 2003). Although there has been an upward trend in bean production

globally, bean yield and hectareage figures for Malawi show a fluctuating trend over the years and productivity is still very low (Fig. 1).

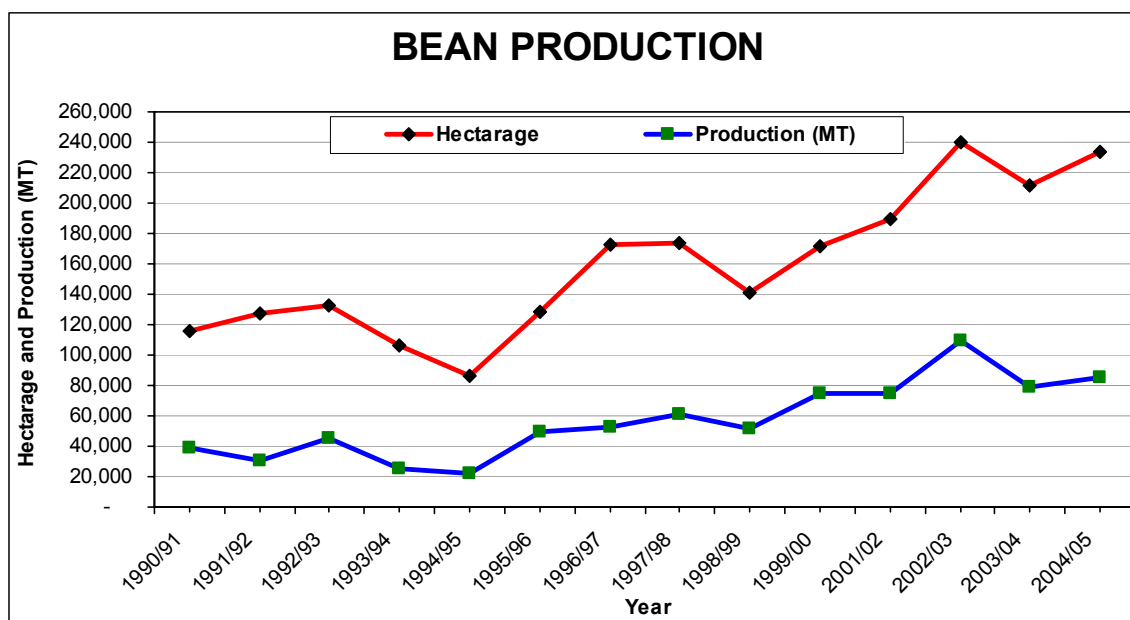


Fig 1: National smallholder bean production statistics for Malawi, 1991-2005

Source: Anonymous, 2005. FEWS, Ministry of Agriculture and Food Security (2005)

3. Status of bean research in Malawi

Considering the nutritional and economic value of beans for many Malawians, there have been concerted research efforts to develop new varieties and production technologies. Over the last 20 years, up to 17 new bean varieties have been developed in collaboration with the Centro Internacional de Agricultura Tropical (CIAT) and released in Malawi for commercial use (Table 1.1).

However, uptake of these varieties for production has not been impressive. Many farmers continue to grow their local landraces. This is because these landraces maintain the unique, preferred characteristics lacking in new improved varieties. Research has often been geared towards improving the yield potential and disease resistance of new varieties. However, not much emphasis has been placed on the acceptability of such new varieties and unique qualities, preferred in the traditional varieties, have been overlooked. In these cases, the “improved”, high yielding varieties have not been accepted by farmers consequently, a participatory rural appraisal (PRA), as a tool to

solicit farmers' views, has become necessary in modern plant breeding in order to identify farmers' varietal preferences. The involvement of farmers in variety development through participatory plant breeding is likely to enhance adoption of newly developed technologies, including new crop varieties (DeVries and Toenniessen, 2001; Bänziger, 2004).

Table 1.1: List of improved dry bean varieties developed and released in Malawi

Year of release	Institution responsible	Number of varieties	Local Names [†]
1980	Bunda College Bean/ Cowpea CRSP project	6	Nasaka (253/1), Sapelekedwa (600/1) Bwenzilawana (373), Kamtsilo (4991/1) Namajengo (336) Kanzama (97/1)
1993	Bunda College Bean/ Cowpea CRSP project	3	Bunda 93 (21-5) Chimbamba (25-2x 8-7) Kalima (PVA 692)
1995	Chitedze Research Station (National Bean Improvement Programme)	6	Mkhalira (A344) Napilira (CAL 143) Maluwa (CAL 113) Kambidzi (A286) Nagaga (A197) Sapatsika (DRK57)
2002	Chitedze Research Station (National Bean Improvement Programme)	2	Kabalabala <i>UBR</i> (92)25 Kholophethe (<i>Sugar</i> 131)
2005	Bunda College Bean/ Cowpea CRSP project	3	BCMV-B2 BCMV-B4 BC-D/O (19)

[†]Breeder's codes are in brackets and/or italicised; **Source:** Saka *et al.* (2006)

4. Constraints on bean production

Factors responsible for low bean yields in most developing countries are grouped into three major categories: climatic (drought, high temperatures), edaphic (poor soil fertility, high aluminium saturation) and biological (diseases and insect pests). Unlike field pests and diseases, damage caused by pests on stored products is completely irreversible. This perpetuates food shortages and reduces cash income for farmers. In Malawi, bean yields under smallholder farmers' conditions continue to be a low 0.45 t ha⁻¹, compared to a yield potential of 2.0-2.5 t ha⁻¹ (Anonymous, 2005). This low yield is further reduced by bruchids in storage.

5. Bruchids, their importance and control methods in Malawi

According to Chirwa (personal communication), up to 200,000 MT of dry beans can be produced in a good season in Malawi. It is estimated that 38% of this production is damaged in storage (Coyne and Hoeschle-Zeledon, 2001) by bruchids (*Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman). This implies that approximately 76,000 MT of beans may be lost to bruchids in storage where control and preventative measures are not in place. The damage to beans assumes considerable significance when viewed in the context of the acute shortage of protein and resultant widespread protein malnutrition, particularly among people living in the rural areas. Securing the harvest against storage pests is necessary because production of most food crops is done only once in a season under a rain-fed system. This one major harvest in the year has to provide for the needs of both the urban and rural areas throughout the year. Smallholder farmers use indigenous methods such as plant botanicals (plant ash, neem and oil) and occasionally pesticides to minimise bruchid damage. Unfortunately, these methods have not been very effective in most cases (Songa and Rono, 1998). No single control method is therefore going to be sufficient to manage insect pests. An integrated approach to insect pest management (biological, chemical, cultural and host plant resistance) must therefore be pursued.

6. Host plant resistance and gene action for bruchid resistance

Host plant resistance is considered the key to integrated pest management as this approach is environmentally friendly, provides cumulative protection against insect pest and is often compatible with other pest management methods (Kogan, 1998). Host plant resistance is the inherent ability of crop plants or varieties to restrict, retard or overcome pest infestations (Kumar, 1984), thereby improving the yield and/or quality of the harvestable crop or stored product. From the farmer's perspective, the use of resistant cultivars may represent one of the simplest and most convenient methods of bruchid control. Three approaches (Painter, 1951) are pursued by plant breeders to develop resistant cultivars: antibiosis, antixenosis (non-preference) and tolerance. The third approach may not be applicable to storage pests because damage, once inflicted, is irreversible. Antibiosis and antixenosis are more appropriate forms of resistance against storage insects. Host plant resistance can be categorised as either vertical or horizontal resistance. Vertical resistance is controlled by various combinations of major genes, which are highly heritable. On the other hand, horizontal resistance is polygenically

inherited and does not involve a gene-for-gene relationship with the insect (Dent, 2000). Understanding the genetics of bruchid resistance and its mode of inheritance is necessary in order to develop an efficient and effective breeding programme.

7. Justification for the study

Although beans are an essential component in the diet of many Malawians, very little research work on breeding for resistance to storage pests has been done so far. However, concerted research efforts by the National Agricultural Research Systems (NARS) and international collaborative partners have led to the development of superior bean varieties, which are currently under commercial production in Malawi. A brief assessment of the impact of these new varieties has shown that household food security and income levels have to some extent improved (Chirwa, 2002). However, many smallholder farmers continue to complain that storage pests are a major post-harvest challenge that needs to be addressed. The bean breeding programmes conducted in Malawi have not focused, to any extent, on identifying bruchid resistant lines and bean entomological studies have concentrated more on field pests than storage pests. This has resulted in the development of superior bean varieties with high levels of resistance to or tolerance of other biotic and abiotic constraints, but more vulnerability to bruchid invasion in storage. In addition, despite the higher yields obtained from improved bean varieties, losses in storage are still high as improved cultivars are highly susceptible and gains made through breeding are not benefiting farmers.

Although two bruchid species (*A. obtectus* and *Z. subfasciatus*) are economically important wherever beans are grown in Malawi, *A. obtectus* is the most important because it was prevalent in all areas. Chipokosa and Nyirenda (1999) reported 98.86% incidence of *A. obtectus* and only 1.4% incidence of *Z. subfasciatus* in Shire Valley and Karonga, suggesting that *A. obtectus* was predominant in these areas, which are non-traditional bean growing areas. However, a participatory rural appraisal (PRA) conducted in the traditional bean growing areas of Lilongwe agricultural development division during 2005, in the current study, showed that *Z. subfasciatus* was also highly prevalent (see Chapter 2). These findings have important implications on the bean breeding strategy in Malawi. It is, therefore, suggested that resistance to both species should be bred in bean varieties for deployment in traditionally bean growing areas in the central region, while those that only have high resistance to *A. obtectus* could be deployed in

the non-traditional bean growing areas. In the current study Malawian germplasm was screened for resistance to both bruchid species (see Chapter 3).

Farmers' perceptions are that damage inflicted on stored beans by bruchids can be severe in the absence of any protective measures. While all other pests that attack beans in the field are rated moderately important, bruchids are rated highly important pests for beans in Malawi (Fig.2). Bruchid damage not only results in quantitative losses, but also in qualitative reduction of the nutritive value because of vitamin loss and deterioration of protein quality. In addition, damaged seeds have reduced germination and seed vigour, thus destroying the value of grain as planting material.

Information available to date shows that most improved varieties grown by farmers succumb to bruchid infestation (Jumbo, 2002). No efforts have been made so far to screen local germplasm to identify effective sources of resistance to bruchid strains found in Malawi. Farmers in dire need to control and/or minimise bruchid damage have resorted to using Dichlorodiphenyltrichloroethane (DDT) because of ignorance and poor extension services. There are recommended grain protectants available to control storage insects e.g. pirimiphos-methyl and malathion. However, their use is limited because they are expensive and sometimes unavailable in remote areas. Indigenous control methods, such as the use of botanicals (wood ash, neem kernels, tobacco leaves and oil) have not been completely effective because the dosage rates and time of application have not been investigated fully or have not reached the farmers due to poor extension services. Research efforts are therefore required to generate appropriate alternative technologies that are easily applied and affordable. Integrated bruchid management, by combining locally available bruchid control methods with the deployment of resistant varieties, would be useful and sustainable for resource-poor farmers (Dent, 2000).

Relative importance of bruchids in bean production areas of sub-Saharan Africa

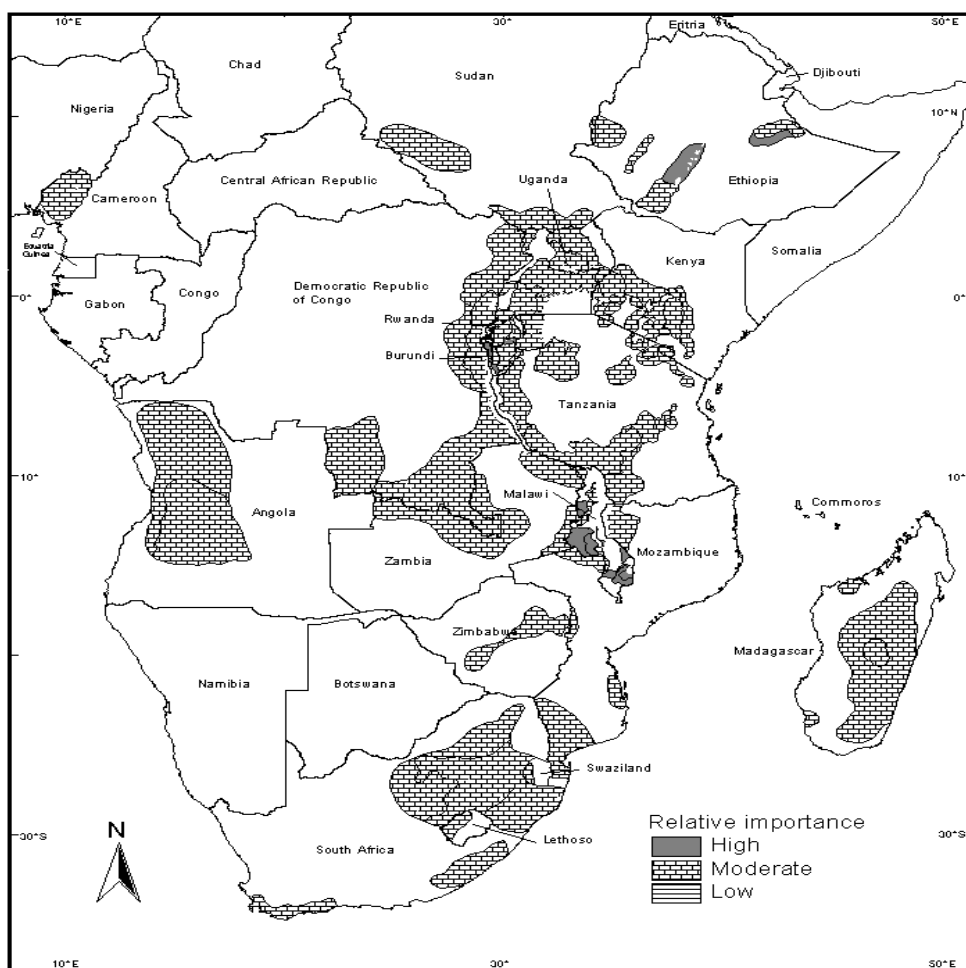


Fig. 2: Map of Africa showing the relative importance of bruchids in Malawi in relation to other sub-Saharan countries (Wortmann *et al.*, 1998).

The search for sources of resistance to the bean bruchid in Malawi is imperative. Martin and Adams (1987) and Mkandawire (1999) reported that there is wide diversity in beans in Malawi, which is recognized as a secondary centre of dry bean diversity. This diversity can be utilised to identify possible sources of resistance to the bean bruchid. Through this research, it was hoped that bean cultivars with improved levels of resistance to bruchids would be identified for use in breeding resistance, and the genetic inheritance of this resistance would be determined to enable the development of an effective breeding strategy. Subsequently, resistant varieties, incorporating traits preferred by farmers, could be developed and deployed to farmers. This will contribute significantly to the national goal of improving food security and nutrition in Malawi.

This study was guided by the following main research objectives:

- (i) Determine farmers' perceptions of the importance of damage by *Acanthoscelides obtectus* Say (bean weevil) and *Zabrotes subfasciatus* Boheman (Mexican bean weevil) to the dry bean both in the field and storage;
- (ii) Solicit farmers' views on what they consider important traits in a bean cultivar and their implication for breeding;
- (iii) Identify effective and adaptable sources of resistance to the two economically important bruchid species, using Malawian dry bean landraces, so that farmers can grow bean varieties that are resistant to bruchids, are well adapted to their agro-ecologies and have the preferred varietal traits;
- (iv) Determine the mode of gene action and inheritance of *A. obtectus* resistance in Malawian dry bean landraces.

8. Thesis structure

This thesis has been written in a composite form and thus there are overlaps either in context or literature citations. The above objectives are discussed in Chapters 2-4. Chapter 1 covers a literature review pertinent to the crop and the two bruchid species, including advances in breeding bruchid resistance. Chapter 2 presents a participatory rural appraisal (PRA) study on farmers' perceptions of the importance of bruchids in storage and in the field. Chapter 3 outlines laboratory screening to identify effective and adaptable sources of bruchid resistance. Chapter 4 presents a genetic analysis of Malawian bean landraces' resistance to *A. obtectus*. Chapter 5 provides a brief general overview of the main findings, challenges and implications for breeding, and recommendations for future research.

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Chapter One

Literature Review

1.1 Introduction

This chapter provides an overview of bean genetic diversity and the major gene pools and their implications from a breeding perspective. It briefly covers bean reproductive biology and its relevance in hybridisation. The importance of bruchid distribution and management aspects are covered. Strategies that have been used to breed for bruchid resistance in dry beans, the key role of germplasm collection and its use to provide sources of such resistance are also reviewed. The section ends with a review of the mechanisms, genetics and inheritance of bruchid resistance. In essence, this chapter creates a frame of reference for the research which has already been done and highlights research gaps.

1.2 Origin, gene pools and genetic variation in dry beans

Based on phaseolin seed storage protein variation (Gepts *et al.*, 1986; Gepts, 1990), marker diversity (Koenig and Gepts, 1989; Sheila-Dessert, 1991) and morphology (Gepts and Debouck, 1991), two major gene pools of wild dry beans (*Phaseolus vulgaris* L.) were identified. These commonly recognised gene pools are the large-seeded Andean and small seeded Meso-American beans, which correspond to the two centres of origin (Singh, 2001). The distribution for common bean extends from southern Mexico to northern Argentina, interrupted only by high- and low-altitude environments and constitutes the gene pool for the species. Independent domestications at the north and south ends of this distribution have created two genetically distinct germplasms centered on these geographic centres of domestication. Of the two major common bean gene pools, the Andean gene pool seems to have a narrower genetic base (Islam *et al.*, 2004). Although not widely accepted, Sauer (1993) suggested that six major races have resulted from six separate domestication events. Divisions between the two centres of origin are such that hybridisation between representatives of the two groups of plants often results in F1 hybrid weakness (Singh and Gutierrez, 1984; Gepts and Bliss, 1985). Progress in bean breeding programmes requires the exploitation of genetic variation present among races or through introgression across gene pools of *P. vulgaris* L.

The common bean presents an extraordinary range of diversity of phenotypic traits (Debouck, 1999; Singh, 2001) ranging from its morphology, cultivation, utilization to its ability to adapt in diverse environments (CIAT, 2001). Among major food crops, it has one of the highest levels of variation in growth habit, seed characteristics (size, shape and colour) and maturity. Consumer preferences for seed type, colour, shape and brilliance vary greatly even within the same environment (Hildago, 1991). From the breeding perspective, the development of common bean varieties is complicated by the many different environments and cropping systems under which the crop is grown. The grain type and colour preferences associated with its consumption imply that bean breeding programmes should emphasise developing bean varieties for specific regional and market requirements. Knowledge of the existing genetic diversity of beans and their origins should assist breeders to improve dry beans genetically against important biotic and abiotic production constraints (Allen *et al.*, 1989; Cardona, 1989; Karel and Autrique, 1989; Schoonhoven and Voysest, 1989; Zimmermann, 1989; Wortmann, 1998). The marked differences in genetic variation in the common bean may imply that there could also be large differences in combining ability and occasional problems affecting gene recombination and exchange between common bean races and gene pools (Schoonhoven and Voysest, 1991). This has made the task of maximising the use of the available germplasm a challenging one (Singh, 2001).

The bean is endowed with such genetic diversity that one could argue that this should have provided sufficient solutions to problems in bean production through research and development. However, it is evident from this review that although there is much genetic diversity in the *Phaseolus* species, the genetic base of commercial cultivars within specific market classes is still narrow (Singh, 2001). The average global yield of the common bean remains as low as 900 kg ha^{-1} and bean production continues to suffer from a wide range of biotic and abiotic constraints. This may possibly suggest that existing bean genetic diversity has been and still is being under-exploited to some extent.

1.3 Bean genetic diversity in Malawi

Dry beans probably reached Malawi from the eastern coast of Africa through the influence of traders and merchants (Muwowo *et al.*, 1972). Malawi is considered a secondary centre of bean genetic diversity in Africa (Mkandawire, 1999). The existence

of this diversity was confirmed by a principal component analysis (PCA) technique by Martin and Adams (1987a). A further analysis showed that this diversity was generated both biologically (Martin and Adams, 1987b) and socio-culturally (Barnes-McConnell, 1989). Significant regional variations in the number of bean varieties grown by smallholder farmers have been documented (Ferguson and Mkandawire, 1993). Greatest bean diversity was observed in the northern and central regions of Malawi, with less genetic diversity reported in the south (Martin and Adams, 1987a). A bean production survey carried out in Dedza district in central Malawi showed there was wide bean genetic diversity as most farmers planted up to 13 bean varieties, mostly of local types (Ferguson and Mkandawire, 1993). Bean germplasm collection in Malawi was first conducted in 1969 (Mloza-Banda and Ayeh, 1989). A follow-up collection was done in 1983 after the inception of the Bean-Cowpea Research Support Programme (CRSP) at Bunda College of Agriculture. The Bunda College of Agriculture bean programme had the largest number of collections compared to other important food crops in Malawi. (However, much of the collection, approximately 4,400 accessions, was lost due to poor storage facilities). This diversity provides an opportunity for plant breeders to develop new bean crop varieties with better traits or improve existing bean cultivars through crop research and development.

1.4 The genus *Phaseolus*

The genus *Phaseolus* consists of approximately 55 species with a wide range of growth habits, reproductive systems and adaptations throughout the world (Mercado-Ruaro and Delgado-Salinas, 2000). Gepts and Debouck (1991) and Debouck *et al.* (1993) reported that the genus contains five domesticated species: common bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), scarlet runner bean (*Phaseolus coccineus* L.), year-bean (*Phaseolus polyanthus* Greenman) and tepary bean (*Phaseolus acutifolius* A. Gray). Most prominent among these is the common bean, *P. vulgaris*, which today is cultivated worldwide in tropical, semi-tropical and temperate climates (De la Cruz *et al.*, 2005). The common bean occupies more than 90% of the production area devoted to all *Phaseolus* species globally (Popelka *et al.*, 2004). In this study, the focus is on the common bean, an important legume grown widely in Malawi. A comprehensive karyotype study of the genus *Phaseolus* showed that all species in the genus have a uniform chromosome number, $2n = 2x = 22$ possessing complete, papilionaceous flowers with coiled style and hairy stigma (Honma, 1956; Smart, 1976). This means that

hand pollination, with or without emasculation, is required to achieve the desired hybrid combinations. No naturally occurring polyploids have so far been reported (Hildalgo and Beebe, 1997).

1.5 Inter-specific and intra-specific hybridisation in dry beans

Inter-specific crossing is rare in nature, though hybridisation between *P. vulgaris* and *P. coccineus* (scarlet runner bean) does occur (Graham and Ranalli, 1997). The latter has been used to improve the common bean. Several species of *Phaseolus* can be hybridised with the common bean, though the hybrid seeds are likely to survive only when embryo-cultured on synthetic media. Smartt (1990) indicated that the first inter-specific hybridisation was a cross between *P. vulgaris* and *P. coccineus* by Mendel in 1866. However, the reciprocal crosses that produced hybrids have either been sterile or non-viable in most cases (Graham and Ranalli, 1997). At CIAT, a novel double congruity backcross technique has been developed and this has facilitated a generation of hybrids with excellent sources of resistance to bruchids, particularly the bean weevil (Cardona *et al.*, 2005).

1.6 Bean bruchids and their importance

Post-harvest losses are attributed to physical (during processing), technical (poor handling) and biological factors, with the latter being the principle cause of these losses. The bean weevil (*Acanthoscelides obtectus* Say) and the Mexican common weevil, also known as the spotted bean weevil (*Zabrotes subfasciatus* Boheman), are the two most important bruchid species for beans in storage. They are characterised by a high rate of reproduction and a short developmental period, enabling them to multiply rapidly and inflict damage (Bell *et al.*, 1999). Songa and Rono (1998) reported 40% weight loss and 80% quality loss, which made the beans not suitable for human consumption, after six months in storage on-farm. Previously losses ranging between 7% and 73% were reported in Colombia, Kenya and Tanzania (Karel and Autrique, 1989; Silim, 1990). In Uganda, damage levels have been estimated to be 3% and 8% respectively for storage durations of 3 and 6 months (Silim *et al.*, 1991). In Malawi, storage losses up to 38% have been reported (Chirwa, 2001). It has become more apparent therefore, that in most regions, realising the existing yield potential of commercial varieties, stabilising production and reducing crop losses have been of greater priority than increasing productivity potential per se (Bergvinson, 2000).

1.7 Composition and distribution of the two bruchid species

Zabrotes subfasciatus and *A. obtectus*, which cause damage to beans in storage are multivoltine in nature, i.e., they produce many generations in one year, developing in dry pods or seeds (Southgate, 1981). As many as six generations are produced in a single season, and breeding continues in storage as long as there is food available in the beans and the temperature is warm. *Acanthoscelides obtectus*, in particular, is known to be prolific and hence very destructive once they attack beans. The two bruchid species are known to be distributed worldwide in all bean growing areas. However, *A. obtectus* is widely distributed in Africa and Latin America (Schoonhoven and Voysest, 1991). In the tropics, it is mainly found in higher altitudes where cooler temperatures favour its life cycle (Howe and Currie, 1964). *Zabrotes subfasciatus*, on the other hand, has a limited distribution, perhaps because it was only recently introduced to the African continent (Abate and Ampofo, 1996). Surveys by Giga *et al.* (1992) in Uganda, Tanzania and Zimbabwe showed that *A. obtectus* was the predominant species on farms. In Uganda, although both species were present at the on-farm level, *A. obtectus* was the predominant species (Silim, 1990) and the only storage pest of beans in the highland areas of eastern Uganda (Silim *et al.*, 1991). In Malawi, preliminary survey reports showed that *A. obtectus* was more predominant than *Z. subfasciatus* (Chipokosa and Nyirenda, 1999).

1.8 Ecology and biology of the two bruchid species

Acanthoscelides obtectus and *Z. subfasciatus*, both cosmopolitan weevils, belong to the order Coleoptera and the family Bruchidae, commonly known as bruchids. In Latin America, it has been demonstrated that the two bruchid species differ in ecological adaptation and have defined distribution patterns influenced by the ambient temperature regimes (Schoonhoven *et al.*, 1986). *Acanthoscelides obtectus* exhibits a high tolerance of a range of temperatures, thus it is found in cool highland areas as well as warmer parts of the tropics. On the other hand, *Z. subfasciatus* prefers warmer climates in the lower altitudes and consequently is more important in the tropical and subtropical regions. Research work carried out in southern and eastern Africa showed that such climatic distinctions and preferences were less important (Giga *et al.*, 1992); because the African strain of *Z. subfasciatus* might be different from the Latin America strain. In Africa, factors other than altitude and temperature could influence their abundance.

In both species, the first-instar larvae penetrate and develop inside the seed. Larvae of both species moult four times before pupating (Schoonhoven *et al.*, 1986). There is some variability in the length of their life cycles and number of eggs laid, depending on the geographical region (Kornegay and Cardona, 1991). *Zabrotes subfasciatus* lays an average of 35 eggs while *A. obtectus* females lay up to 63 eggs. The life cycles of *A. obtectus* and *Z. subfasciatus* are completed in about 28 and 24 days respectively. Oviposition behaviour of the two species is different. The eggs of *Z. subfasciatus* are glued to the seed testa, while the eggs of *A. obtectus* are deposited loosely within the pod cavity or among stored seed. The larvae of *Z. subfasciatus* are white, whereas those of *A. obtectus* are a dirty white or pale yellow. The newly hatched larvae of *Z. subfasciatus* immediately bore directly into the seed while those of *A. obtectus* wander about before they penetrate the seed. Schoonhoven *et al.* (1986) further reported that *Z. subfasciatus* attacked stored seed, while *A. obtectus* began infestation in the field by ovipositing on the mature pods and continued to infest seed in storage. The optimum controlled conditions for the rapid development of *A. obtectus* eggs are 70% R.H and 30°C, when the insects spend 22-33 days inside the beans. Adult insects do not live long; their life span is only 12 days.

Understanding biological differences and the distribution of the two bruchid species is critical in order to develop management strategies through improved cultivar development, targeted distribution of resistant cultivars, cultural control methods or integrated pest management (IPM).

1.9 Bruchid management and control strategies

Because of the extent of the damage that bruchids may inflict on stored beans, and with a greater awareness of the hazards associated with the use of synthetic organic pesticides, small-scale farmers have tried a number of options to control or minimise bruchid damage in storage (Scott and Maideni, 1998; Giga, 2001). Botanical products such as plant ash, neem powder and tobacco leaves have been used (Ntoukam *et al.*, 2001; Escalada and Heong, 2002). These indigenous control methods have not been effective in most cases because of variations in application rates and differences in post harvest storage management practices (Giga *et al.*, 1992). Songa and Rono (1998) also reported that ash, for example, was not that effective. They attributed this to differences in quality of ash used with respect to plant origin and differences in bruchid susceptibility

among the bean varieties used. Farmers, in most cases, have been forced to sell their grain shortly after harvest due to inevitable losses experienced. Sustainable ways of managing these infestations are therefore required. Control strategies/methods must integrate plant resistance, cultural methods and biological control with limited use of pesticides, to minimise losses in storage.

1.9.1 Cultural and interference control methods

Cultural control methods are those practices that make the environment less attractive to pests and less favourable for their survival, dispersal, growth and reproduction (Casida and Quistad, 1998). For field insect pests such practices include modification of the plant density, crop rotation, intercropping, destruction of volunteer plants, sanitation and weed control. Varying planting time as reported by Teetes (1991) and Metcalf and Metcalf (1993), creates an asynchrony between the plant phenology and the population dynamics of the pest species, thereby retarding the rates of colonisation, reproduction and survival of the insect pest. Practically, this may reduce pest numbers below economic threshold level or sufficiently allow natural or biological controls to take effect (Hill, 1989). These field cultural practices might not offer effective control of the bruchids, which are mainly storage pests. For storage pests, the cultural methods like cleaning the seed and good sanitation in the store are the main requirements for reducing the initial insect population.

Dent (2000), quoting Evans (1987), indicated that there are approximately 20 economically important insect pests of stored products. Each insect species has a characteristic range of limiting and optimal temperatures and humidities (Evans, 1987). By modifying the temperature/humidity either above or below the tolerance limits of an insect species, its rate of development will be reduced to an acceptable level (Benz, 1987). For example, *Callosobruchus maculatus* requires temperatures above 20°C for normal development and reproduction (Dent, 2000), thus any reduction in temperature below this will have a significant effect on its population growth. In dry beans, temperatures of 26-32 °C and 75-80% relative humidity have been reported to favour rapid development of the two bruchid species (Schoonhoven and Voysest, 1991).

In summary, cultural control measures are considered cheap because they require only a modification of normal production practices, which sometimes does not require extra

labour. Such measures are dependable and usually specific. However, cultural control methods require long-term planning and need careful timing for them to be effective. The effectiveness of these methods does not always provide economic control of pests and, importantly, cultural control methods effective against one pest may be ineffective against a closely-related species (Watson *et al.*, 1976). For example, similar cultural control measures may not be applied effectively against *Z. subfasciatus* and *A. obtectus* as the two species have some differences biologically.

1.9.2 Chemical control methods

Pesticides have provided the principal means for, and an essential component of insect pest control (Casida and Quistad, 1998; Dent, 2000). The status which they have achieved provides ample evidence of their value (immediate response) and effectiveness (fast rate of kill) by the user (Webster and Bowles, 1996; Oakley *et al.*, 1998). However, insecticides have drawbacks associated with their use (Watson *et al.*, 1976). The use of insecticides is associated with insecticide resistance among insects, destruction of beneficial insects, environmental contamination and hazard to the user, particularly in developing countries due to high levels of illiteracy. In addition, insecticides are costly and hence unaffordable for subsistence farmers, while in rural settings, pesticides may not be easily accessible. Farm stores may have to be fumigated to control storage pests yet the nature of many farm structures (open warehouses) in villages renders fumigation impractical. In Malawi, a wide range of chemicals such as Super-actellic dust (1.6% Perimiphos methyl + 0.3% Permethrin), Super liquid actellic (1.6% Perimiphos methyl + 0.3% Permethrin), Superguard dust (1.6% Perimiphos methyl + 0.4% Permethrin) and Shumba Super (1.0% Fenitrothion + 0.13% Deltamethrin) are some of the recommended insecticides used to protect grain from storage pests.(Saka *et al.*, 2005)

1.9.3 Biological control methods: Use of parasitoids

Whereas bean varieties with resistance to *Z. subfasciatus* have been identified (Schoonhoven *et al.*, 1983) and developed for use (Cardona *et al.*, 1990), host plant resistance for the control of *A. obtectus* is still lacking. Biological control has been considered to be a viable tool where beneficial insects occur naturally or can be released by farmers when needed to parasitise bruchid larvae. Very little research has been done

to study the extent to which parasitoids can be used to control bruchids or to minimise damage to stored beans.

Dinarmus basalis (Rondani) has been found to be a promising control agent for bruchids, particularly *A. obtectus*. Schmale *et al.* (2002) reported a significant reduction in the bruchid population in the presence of the parasitoid. In a study to evaluate the effectiveness of *D. basalis* at different levels of introduction on red lentils, Islam and Kabir (1995) reported that the release of 50 pairs of parasitoids (highest density) was able to suppress the population of *Callosobruchus chinensis* (L.). However, the effectiveness or efficacy depended on the type of bag or container in which the product was stored. Schmale *et al.* (2001) studied the potential of three Hymenopteran parasitoid species against *A. obtectus* for longevity and progeny production and found that parasitoids have some limitations on their use. Schmale *et al.* (2006) found that *D. basalis* was effective for controlling natural infestation by *A. obtectus* on several farms depending on the developmental stage of the weevil. However, success with other type of parasitoids apart from *D. basalis* has not been impressive. *Horismenus ashmeadii* (Dalla Torre) was used to parasitise *A. obtectus* in the field and in storage. Schmale *et al.* (2002) reported that *H. ashmeadii* failed to develop and attack *A. obtectus* under storage conditions, while under field conditions it managed to attack the first generation of the bruchid with a parasitisation level of only 18%. This clearly shows that *H. ashmeadii* was not effective in controlling *A. obtectus* in storage.

Although parasitoids seem to have potential for controlling the bruchid, their use by and applicability for small-scale farmers could have some limitations. Firstly, farmers must have a steady supply of the parasitoids' nutrition such as honey, sugarcane or host larvae, in order to maintain their longevity and progeny production. Secondly, the effectiveness of these parasitoids depends on the timing of release and the stage of larvae development. Thirdly, the parasitoids are introduced at the larvae stage, at which time damage has already occurred within the seed. These constraints have made biological control using parasitoids, unpopular for bruchid control and management. Host plant resistance, where plant varieties resistant to bruchids are identified and used in an integrated manner with the other control methods, remains the best option.

1.9.4 Host plant or varietal resistance

Host plant resistance is defined as the inherent ability of crop plants to restrict, retard or overcome pest infestation, thereby improving the yield and/or quality of the harvested crop product (Kumar, 1984). It has been argued that varietal resistance to stored grain insects provides one basis on which to build an integrated pest management (IPM) programme (Semple, 1992). Integrated pest management can be defined as the acceptable use of practicable measures to minimise, cost-effectively the losses caused by pests in a particular management system (Taylor *et al.*, 1992; White, 1992). Dobie (1984) suggested that the use of improved grain cultivars with resistance to storage pests could provide a key element in IPM for stored grains. For IPM measures to be cost-effective they must be appropriate to and acceptable into that system (McFarlane, 1989). The IPM concept stresses the need to use multiple means to ensure that insect pest abundance and damage fall below levels of economic significance. Thus, a major advantage in the use of insect-resistant crop varieties as a component of IPM arises from ecological compatibility and compatibility with other direct control measures (Eigenbrode and Trumble, 1994). Insect-resistant cultivars synergise the effects of natural, biological and cultural insect pest-suppression measures. The built-in protection in resistant plants disrupts the normal association of the insect pest with its host plant (Van Emden, 1997).

Derera *et al.* (2000b) observed that the resistance of maize grain to the weevil (*Sitophilus zeamais* Motsch.) was not absolute but partial. The use of local bruchid control methods could presumably be more effective if used in combination with varieties that have good levels of resistance. The use of insect-resistant crop varieties is economically, ecologically and environmentally advantageous (Kogan, 1998). Economic benefits occur because crop yields are saved from loss to insect pests and money is saved by not applying insecticides that would have been used on susceptible varieties. In most cases, the seed of insect-resistant cultivars costs no more, or little more, than that of susceptible cultivars. Ecological and environmental benefits arise from increases in species diversity in the agro-ecosystem, in part because of the reduced use of insecticides.

Plant resistance to insect pests has advantages over other direct control methods. For example, plant resistance to insects is compatible with insecticide use, while biological control is not. Plant resistance to insects is not density dependent, whereas biological control is. Plant resistance is specific, only affecting the target pest. The effects of the use of insect-resistant cultivars are often cumulative over time. Usually the effectiveness of resistant cultivars is long lasting.

Though host plant resistance is a promising strategy for pest control, insect populations are able to develop biotypes that can attack formerly resistant varieties (CIMMYT, 1992). Host plant resistance to insect pests provides a potential and sustainable option to be utilised in insect pest management. In practice, however, it is a challenging task because to date there is no cultivar that is completely resistant to insect pests. Despite the emphasis on the identification of mechanisms of resistance to insect pests, relatively few cultivars have been produced that are resistant to insects. This is partly due to the nature of resistance to insects and due to the relatively late interest shown in developing insect resistant cultivars to storage pests in particular (Dent, 2000). Resistance to insects is often partial because it is often multigenic and difficult to combine with yield and hence presenting challenges for the breeder. The development of resistance to insects has therefore taken a second place to that of resistance to pathogens, where large discrete differences have been found and complete resistance is exhibited.

1.10 Breeding for bruchid resistance

Scientists at CIAT and elsewhere have attempted to understand bean resistance to the bean weevil and the Mexican bean weevil so that bean varieties with resistance to the two bruchid species could be developed. A study carried out by Schoonhoven and Cardona (1982) showed that there was a low level of resistance to the Mexican bean weevil in most of the cultivated dry beans. A follow-up study that focussed on wild bean accessions showed high levels of resistance to the two bruchid species (Schoonhoven *et al.*, 1983). This resistance in the wild bean accessions was due to the presence of arcelin-seed protein that has been reported to confer resistance against *Z. subfasciatus*.

The initial selection strategy consisted of infesting bulked F2 bean populations with large numbers of *Z. subfasciatus* and *A. obtectus* adult bruchids (Kornegay and Cardona, 1991). This resulted in the development of some lines with acceptable levels of

resistance (CIAT, 1985) to *Z.subfasciatus* but none showed acceptable resistance to *A. obtectus*. However, most of the developed lines were small-seeded and bruchids exhibited a marked preference for large-seeded bean lines. Misangu *et al.* (2001) also screened and identified some potential lines with resistance to *Z.subfasciatus*, which was conferred by arcelin. One of the outstanding lines, which outperformed the donor parents, had medium seeds which might be accepted by farmers in Africa.

It has been possible to transfer resistance from a wild accession to a cultivar by backcrossing (Cardona *et al.*, 1990; Myers *et al.*, 2000). This indicates that it is likely that bruchid resistance was present at a single genetic locus and may result from a single resistance factor. Where resistance is caused by a combination of seed factors encoding at different loci (Kornegay and Cardona, 1991) backcrossing may not be successful. However, resistance that results from a combination of seed factors, all present at suboptimal levels, may be more durable and may give the plant an evolutionary advantage.

1.11 Bruchid resistance testing methods

It is argued that if the amount of damage in a sample is to be used as a measure of resistance, there must be an assurance that the sample has adequately and equally been exposed to infestation (McCain *et al.*, 1964). Scientists have therefore exploited various resistance testing methods for this reason. Two methods commonly used to test germplasm for resistance to storage pests are free-choice (also known as cafeteria system) and no-choice tests. In a free-choice test, insects are at liberty to select the test sample of their choice. The free-choice test method measures antixenosis, which is the ability of a variety to repel insects, causing a reduction in oviposition or feeding. Antixenosis can be chemical or morphological. Antixenosis is generally measured by testing insect behaviour, for example comparing the number of insects landing on or laying eggs on different test varieties. Free-choice tests are probably more applicable when large numbers of varieties are to be tested, and a larger number of adults are released in the same container. In this case sex determination of males and females, to be used in a sample, may not be necessary. Horber and Mills (1976) reported that the free-choice test method can be used conveniently to eliminate obviously susceptible varieties. Another method, probably a modification of or an extension to the free-choice test, known as the weevil warehouse method, has been developed mainly for breeders

who can incorporate it into their yield trials screening process before variety release (CIMMYT, 2002). A screening facility, such as a shed, is constructed where there is a known high natural pressure of the primary storage pests. Samples are brought into the warehouse immediately after all field data have been taken. Samples are stored in the warehouse for 90 d, in the case of *Sitophilus zeamais*, or less depending on the type of storage pest, prior to damage assessment. Damage rating is done visually using a pre-determined scale. This method provides additional criteria for the identification of varieties that are likely to lead to greater adoption by farmers as grain storage quality is an important criterion when selecting a variety.

A no-choice test method is commonly used in laboratories to screen genotypes for storage pest resistance (Kanayo and Horber, 1975; Giga, 1995). In this method, insects are restricted in choice of grain from a sample. Samples are normally put in jars and insects of known age and sex are introduced and allowed to oviposit (lay eggs). Damage is then assessed after a pre-determined time period (Davey, 1965). The no-choice test method measures antibiosis and measurements of the development period, number of emerging insects and percent grain weight loss are taken. These parameters may then be used to calculate indices of susceptibility.

In this study, field infestation under the free-choice system was carried out and the no-choice test method was used under laboratory conditions to verify resistance to bruchids. These methods have previously been used to screen genotypes for weevil and/or bruchid resistance (Tipping *et al.*, 1989; Cardona *et al.*, 1990; Derera *et al.*, 2001b; Eddie and Amatobi, 2003; Cardona *et al.*, 2005).

1.12 Mechanisms of bruchid resistance

Understanding the mechanisms underlying bruchid resistance is essential for developing appropriate breeding strategies. The most widely accepted classification of mechanisms of resistance is that proposed by Painter (1951). He categorised insect resistance into non-preference, antibiosis and tolerance. Non-preference resistance is where features of a plant or grain prevent the insect pest from using it for oviposition (egg-laying), feeding and shelter or a combination of the three. Panda (1979) described two types of non-preference: one observed in the presence of the host and the other observed in the resistant plant or variety even in the absence of the preferred host. Factors that condition preference or non-preference of the host have been covered in detail (Painter,

1951). Genetic variation for weevil non-preference has been reported among maize cultivars (Kang *et al.*, 1995; Derera *et al.*, 2001b).

Antibiosis is the mechanism by which a colonised host is resistant, because it has an adverse effect on an insect's development, reproduction and survival (Dent, 2000). When antibiosis is operating, the host will injure, reduce the reproduction potential, slow the rate of development or kill the insect pest or indirectly affect the insect by increasing its exposure to its natural enemies (Painter, 1951). Allelochemicals and primary metabolites (phyto-toxins) are generally associated with antibiosis. Studies have shown that hydroxycinnamic acids (phenolics) are important in grain resistance to storage pests (Classen *et al.*, 1990; Arnasson *et al.*, 1992; Sen *et al.*, 1994). In maize, resistant hybrids have high levels of phenolic acids, which cause adverse effects in weevil feeding and survival (Sen *et al.*, 1994). In screening genotypes for bruchid resistance, biochemical analysis should be considered.

Tolerance is the ability of the host to rapidly recover, repair or withstand infestation. However, tolerance is viewed by others (Beck, 1965) to denote a mere biological relationship, while antibiosis and non-preference are chemical and physical resistance devices. Consequently, Horber (1989) concluded that host plant tolerance was inapplicable to storage pests, because damage inflicted on stored produce is irreversible. It is therefore antibiosis and non-preference mechanisms of resistance that are of relevance in this study. However, the three mechanisms of resistance, wherever applicable, will influence the population dynamics of insects either under laboratory or field conditions by their action on the life history parameters: initial colony size, fecundity of adults, developmental period and mortality of larvae or adults (Dent, 2000).

Several factors, including the physical and chemical, have been used to explain seed resistance to storage pests. It has been demonstrated that physical factors such as seed coat hardness and seed coat roughness confer resistance to bruchids (Giga, 2002). A hard seed coat may prevent larvae from successfully penetrating the seed, while a rough seed coat provides difficulties for *Z. subfasciatus* in particular, because it glues its eggs on the seed testa; hence rough seeds are less preferred for oviposition (Nwanze and Horber, 1976; Messina and Renwick, 1985). Lale and Kolo (1998)

suggested that the presence of biochemical factors in the seed coat, irrespective of coat texture, may cause reduced oviposition and the poor survival of bruchid eggs on some resistant cowpea varieties. Tannins in the seed coat (Deshpande, 1992) and trypsin inhibitors (Savelkoul *et al.*, 1992) have been implicated in the resistance of seed to bean weevils. Kemal and Smith (2000) found that the proportion of larvae entering faba bean varieties, and hence capable of completing their development and emerging as adults, was greater in decorticated seeds than in whole seeds. They concluded that the resistance of faba bean varieties could be due to the properties of seed coats or biochemical antibiosis as development was very successful on seeds without a seed coat. However, contrary to these findings Edde and Amatobi (2003) showed that cowpea seeds with intact seed coats were preferred to decorticated seeds for oviposition and therefore it was concluded that the seed coat may not be a useful aspect to consider when breeding for bruchid resistance in the cowpea.

Cardona *et al.* (1989) further observed that even though the testa may occasionally act as a physical barrier, factors responsible for resistance were chemical in nature and they were present in the cotyledon. It is therefore clear that resistance to post-harvest insect attack is a function of interrelated component factors of antibiosis and non-preference. Other chemical factors within the seed, such as phytohemagglutinin (PHA), have also been reported to confer resistance in dry beans (Ishimoto and Kitamura, 1989). In all these cases, the growth and development of larvae feeding is inhibited or retarded (Gatehouse *et al.*, 1979; Baker *et al.*, 1989), indicating the importance of antibiosis as a mode of resistance. Varietal differences in the degree of field infestations of some wild bean accessions have been demonstrated (Cardona and Kornegay, 1989; CIAT, 1996; Schoonhoven *et al.*, 1983). Resistance was expressed as reduced oviposition, prolonged larval development and reduced progeny weight. Semple (1987) indicated that crop varieties can display variable resistance depending on the crop's geographical origin and agronomic cultural practices. This suggests that different production environments may result in differing levels of resistance and affect crop varieties' susceptibility to storage pests.

There is a sufficient body of literature explaining the bean's resistance mechanism to bruchid infestation under controlled laboratory conditions (Hartweck *et al.*, 1991; Fory *et al.*, 1996; Guzmán *et al.*, 1996). The presence of arcelin, seed hardness, seed coat

thickness, tannins, lectins and trypsin inhibitors have been used to explain antibiosis or non-preference resistance mechanisms to bruchid infestation. Very little is known about or documented on the relationships of qualitative plant traits such as growth habit, pod colour and flower colour to understand field resistance mechanisms. The behaviour of the two bruchid species in the discovery and identification of their host plant for oviposition and factors that dictate host searching are not clear. It is argued that plant resistance is not dependent on a single mechanism, but that there are overlaps between the morphological and biochemical bases of resistance. In this study, the influence of qualitative traits such as flower colour, plant height, growth habit and pod colour on bruchid resistance or susceptibility in storage was investigated. Earlier studies suggested that pod colour had an influence on varietal preference for *A. obtectus* oviposition (Johnson, 1981; Labeyrie, 1981).

The relationship between bruchids and their host plant and, therefore, the mechanism of adaptation of these pests to their food base is complex (Taylor, 1981). Understanding the factors that influence a bean attack by bruchids in the field may assist breeders to develop appropriate intervention strategies (Teshome *et al.*, 2001). *Zabrotes subfasciatus* cannot perforate pods to lay its eggs nor can it lay eggs on pods as is the case with *A. obtectus* or *C. maculatus* (Ouedraogo and Huignard, 1981). This observation might have led to the understanding that *A. obtectus* is both a field and storage pest and that *Z. subfasciatus* can only attack beans once they are stored (Deborah *et al.*, 2003).

1.13 Resistance factors to storage pests

Present knowledge of the resistance of crops to storage pests has principally focused on establishing those factors that confer resistance. Plant breeders can successfully incorporate inherent varietal resistance to storage pests as integral and desirable selection criteria if they understand the factors that regulate insect resistance to insects. Though resistance in general is attributed to physical, chemical and nutritional factors, for storage pests, physical and chemical causes are more influential (Semple, 1992).

(a) Physical factors

In maize, the extreme value of a complete, well-fitting set of husk leaves reduce pre-shelling infestation by *Sitophilus zeamais*, while in sorghum the glumes mostly cover the

grain and reduce attack by bruchids (García-Lara *et al.*, 2004). Tight husks are an important resistance mechanism for maize, wherever climatic conditions encourage field infestations (Rogers and Mills, 1974). The seed coat of food grains may also be sufficiently thick and tough so as to inhibit penetration to a degree, even though primary feeders are well adapted to chew into whole undamaged kernels. Certain characteristics of the cellular structure of the seed coats of some cowpea varieties (*Vigna unguiculata* L.) partially prevented entry of first instar larvae of *Callosobruchus maculatus* (F.) (Shade *et al.*, 1996). Resistance in chickpeas to the adzuki bean weevil (*Callosobruchus chinensis* L.) was attributed to its rough, nearly spiny seed coat, which effectively inhibited oviposition (Brewer and Horber, 1983). The broadbean (*Vicia faba* L.) was not preferred for oviposition by *C. chinensis* (F.) even though it possessed a relatively smooth seed coat, and it was shown that most of the larvae died during the first instar due to their inability to penetrate the thick seed coat. Brewer and Horber (1983) concluded that larval antibiosis (the mechanism by which a colonised plant is resistant because it has an adverse effect on an insect's development, reproduction and survival) due to mechanical, physical or biochemical factors, and ovipositional antexinosis (the resistance mechanism employed by the plant to deter colonisation by an insect) were assumed to be present.

(b) Chemical factors

Most food crops do not contain substances toxic to insects, and if they are naturally present, they probably exist in concentrations that would not significantly affect insect or man (Dent, 2000). However, certain components that may be in levels toxic to insects and rendered harmless to man by preparation and cooking are known in legumes or pulses and in some root crops, notably cassava (Bollini *et al.*, 1999). Females of *C. maculatus* readily laid eggs on the seed surface of the cowpea seed, and larvae began feeding on the underlying cotyledons, but growth was extremely slow, and eventually most larvae died in cowpea seeds. In *P. vulgaris*, differing resistance responses to *C. chinensis* and *A. obtectus* have been explained by the differential digestion of soluble heteropolysaccharides, which contain arabinose, xylose, rhamnose, glucose, and galactose (Suzuki *et al.*, 1995). According to Horber (1983), breeding for higher concentrations of this heteropolysaccharide in beans would be logical to protect them from both bruchid species. Arcelin in the cotyledons, tannins in the seed coat, and phytohemagglutinin (PHA) within the seed including α -amylase inhibitors, have been

reported to confer resistance to storage pests (Schoonhoven *et al.*, 1983; Osborn *et al.*, 1986; Gatehouse, 1990).

1.14 Role of arcelin in bean bruchid resistance

It has been demonstrated that the high mortality of late instars of the bruchids in the wild bean accessions were primarily caused by high levels of antibiosis and non-preference for oviposition (Schoonhoven *et al.*, 1983). These findings demonstrated that, other than the seed coat which acts as a physical barrier to bruchids attack, chemical factors in the cotyledons were responsible for seed resistance to bruchids. Further research revealed that arcelin and the concurrent absence of phaseolin were indeed responsible for the resistance of the wild strains of beans to the Mexican bean weevil (Osborn *et al.*, 1986; CIAT 1996). Arcelins are abundant, lectin-like seed storage proteins that are present in wild *P. vulgaris* accessions. Researchers have characterised the wild common bean accessions containing these arcelin alleles which are designated as *Arl- 1* through to *Arl- 7* (Osborn *et al.*, 1986; Lilo and Bollini, 1989; Santino *et al.*, 1991; Kornegay *et al.*, 1993; Suzuki *et al.*, 1995; Goossens *et al.*, 2000). Misangu *et al.* (2001) evaluated bruchid resistance in bean hybrids in Tanzania and reported that presence of arcelin delayed bruchid progeny development and emergence, and also reduced the level of bruchid damage on seeds. Arcelin is inherited as a single gene and its presence is dominant (Osborn *et al.*, 1986) and six alleles with varying effects have been characterised (Acosta-Gallegos *et al.*, 1998). Electrophoresis tests can be used to detect the presence of arcelin in bean genotypes and it can be transferred by backcrossing or other breeding methods to improve bean resistance to the Mexican bean weevil (Hartweck *et al.*, 1991; Kelly, 2000). The discovery of arcelin has facilitated and expedited the breeding of bruchid resistance in common beans (Hartweck *et al.*, 1997). Hartweck and Osborn (1997) reported that the removal of phaseolin from common bean increased arcelin concentration in some of the lines which might improve resistance to bruchids.

Although high levels of resistance have been reported in four arcelin variants (*Arl- 1, 2, 4* and *5*), resistance levels were only maintained in lines generated from crosses with *Arl- 1* and *Arl- 5* parents (Cardona *et al.*, 1990). Studies on the analysis of the insecticidal activity of the *Arl- 5* variant, highly present in bean accession G02771, showed no correlation between the presence of *Arl- 5* and the insecticidal effects observed in that bean accession (Goossens *et al.*, 2000). This may imply that the resistance is not due

to arcelin, but to the presence of another factor, genetically closely linked to the *Arl- 5* allele. In any case, arcelin with its high biochemical stability still remains useful in impairing bruchid development. Furthermore, since *Arl- 1* and *Arl- 5* are linked to the presence of arcelin locus, they remain useful markers to introgress high levels of resistance to bruchids in common beans (Padgham *et al.*, 1992).

Until recently, arcelins were thought to be restricted to wild accessions of the common bean, but arcelin-like sequences were obtained from *Phaseolus acutifolius*, suggesting that they may also be present in other *Phaseolus* species (Mirkov *et al.*, 1994). In the current study, resistance based on arcelin is used in addition to exploring other sources of resistance.

The presence of arcelin, a seed protein of the phytohemagglutinin-arcelin- α -amylase inhibitor gene family, has been linked to bean resistance against *Z. subfasciatus* (Osborn *et al.*, 1986, 1988). Resistance was evidenced by a reduction and delay in adult bruchid emergence. Cultivated common bean cultivars lack these proteins, which were presumably excluded by the genetic selections that occurred during domestication (Myers *et al.*, 2000). An attempt has therefore been made to introgress resistant genes into dry bean cultivars by selecting for arc (Cardona *et al.*, 1990). However, the introgression of the arc into cultivars resulted in the replacement of α -amylase inhibitor 1 (AI-1) by α -amylase inhibitor 2 (AI-2), rendering arc non-effective against *Z. subfasciatus* (Minney *et al.*, 1990; Fory *et al.*, 1996). It has been reported that the larvae of *A. obtectus* are not affected by the presence of arcelin and the introgression of arcelin into cultivated dry beans did not yield resistant varieties (Cardona *et al.*, 1993). Further investigations to understand the effect of arcelin on the insect larvae of *Z. subfasciatus* revealed that *Arl- 1* (which is the most effective of the 7 arcelin variants) had deleterious effects, disrupting the epithelial structure in some regions on the midgut of *Z. subfasciatus* but not in the midgut of *A. obtectus* larvae (Paes *et al.*, 2000). This explains why arcelin is more effective against *Z. subfasciatus* and not *A. obtectus*.

Although SMARC lines (bean-containing arcelin) have been found to confer bruchid resistance to *Z. subfasciatus*, these lines are not well adapted to tropical conditions and as such do not perform well under local conditions. Importantly, they are small-seeded types and most farmers in Malawi would not prefer them. Cardona *et al.* (2005) reported that incorporation of arcelin in bruchid-resistant lines (coded RAZ) was effective against

the Mexican bean weevil but resulted in a depressing effect on yield. Most RAZ lines tested gave lower bean yields than their respective recurrent parents. It has also not been established whether arcelin-containing lines store well in farm storage. Furthermore, their long-term effects on human beings are not clear. A preliminary study of the nutritional properties of RAZ-2, a recently developed bean line which contains arc 1, fed to rats showed anti-nutritional effects on the rats' metabolism (Putzai *et al.*, 1993). It showed the stimulation of hyperplastic growth of the small intestine, enlargement of the pancreas and a decline in body lipid content (Putzai *et al.*, 1993). It has been argued, however, that most of the anti-nutritional effects of RAZ-2 beans could be eliminated by boiling fully-hydrated beans at 100°C for 10 minutes.

Clearly, this shows that there is still a need to search for adaptable and effective sources of bruchid resistance to develop varieties that are adaptable to local farming systems and preferences and are also safe for human consumption.

1.15 Factors mediating the expression of resistance and durability of resistance to stored products

Although resistance is governed primarily by genetics, it must be noted that physical and abiotic factors in the environment also influence its expression. Physical factors such as weather, plant architecture and cultural practices can influence the plant's physical environment (Panda, 1979). Varietal performance can be affected by temperature and changes in nutrient availability. Biotic factors such as the age of the plant, the intensity of insect infestation and insect pest biotypes affect the expression of resistance. This explains why breeding for insect resistance is a challenge to the breeder. Consequently, the resistance of varieties has to be validated under field conditions.

Horber (1983) stated that resistance proves effective where the resistant varieties are grown extensively in environments that favour insect infestation. Durability of resistance depends on the genetic control of the resistant factors, such as single gene or multiple gene resistance, where each has a small additive effect on the resistant genotype (Acquaah, 2007). Polygenic resistance causing antibiosis or antixenosis is preferable (and more stable) to monogenic resistance mechanisms (Welsh, 1981). Because resistance incorporating antibiosis or antixenosis is exerting a selective influence on insect populations, it is anticipated that new biotypes will develop in conditions of

prolonged use in isolated populations. This has become apparent in resistant cultivars developed for pre-harvest pest and disease prevention e.g., in cotton), but has not as yet been reported in the literature for stored-products insects.

1.16 Gene action and inheritance of bruchid resistance

Genes, comprised of DNA (deoxyribonucleic acid), are the basic units of inheritance. Gene action is the functioning of a gene in determining the phenotype of an individual. Gene action can be grouped into two categories, additive and non-additive. The non-additive gene expression may exhibit dominance, recessivity, no dominance, over-dominance and epistasis (Acquaah, 2007).

The effect of gene expression on phenotype is generally thought of as being either additive or non-additive (Falconer, 1981). Additive gene action occurs when the phenotypic effect of one gene adds to the phenotypic effect of another gene i.e., each of the two alleles contributes equally to the production of qualitative phenotypes; neither allele is dominant. The heterozygous genotype produces a phenotype that is intermediate between those produced by the homozygous genotypes. Traits affected by additive gene action are moderately to highly heritable and will be affected very little by outcrossing and inbreeding (Kearsey and Pooni, 1996). This type of gene action influences many of the traits a breeder is interested to select for in a breeding programme. Improvement through selection can be made effective when additive gene effects, which are fixable, are involved in conferring bean resistance to bruchids. When the gene expression is non-additive, the phenotypic expression of one gene does not necessarily add to the phenotypic expression of another gene. Non-additive gene action is observed when the additive model inadequately explains the variance (Falconer, 1981).

Epistasis is a form of non-additive gene action. It is the interaction between the genes at two or more loci, so that the phenotypic expression is masked. In epistatic interaction one gene may control the degree to which another gene is expressed. In quantitative traits, epistasis is described as non-allelic gene interaction. Epistasis is important in population genetics when the fitness effects of a genotype depend on what genotype it is associated with at the other locus. It is in this situation that natural selection can maintain linkage disequilibrium in a population (Hill *et al.*, 1998; Acquaah, 2007).

In plant breeding, studies of hybrid populations sometimes report the presence of traits or phenotypes that are extreme, relative to either of the parental lines (Rieseberg *et al.*, 1999). The generation of these extreme phenotypes in hybrids (i.e. phenotypes that exceed those of either parental line) is referred to as transgressive segregation (Grant, 1975; De Vicente & Tanksley, 1993). Transgressive segregation has been hypothesized as an important mechanism by which novel adaptations can arise in hybrids.

There are many mechanisms that could be responsible for transgressive segregation in hybrids such as: an elevated mutation rate reduced developmental stability, epistatic effects between alleles, overdominance caused by heterozygosity at specific loci or chromosome number variation (Xu *et al.*, 1998).

A recent review of phenotypic variation in hybrids indicates that transgressive segregation occurs frequently in segregating plants (Rieseberg *et al.*, 1999). Out of 171 studies reviewed, 155 (91%) reported at least one transgressive trait and 44% out of 1229 traits examined were transgressive. Transgressive segregation was found to be more frequent in plants than in animals, in intraspecific than in interspecific crosses, in inbred than in outbred populations and in domesticated than in wild populations (Darlington and Mather, 1949; Vega and Frey, 1980; Rieseberg *et al.*, 1999). Rick and Smith (1953) proposed three potential explanations for the occurrence of interspecific transgression including de novo mutation induced by hybridity, complementary action of genes from the two parental species and unmasking of recessive genes normally held heterozygous. However, genetic studies indicate that transgressive segregation mostly results from the appearance, in individual genotypes, of combinations of alleles from both parents that have effects in the same direction: complementary gene action (De Vicente & Tanksley, 1993; Rieseberg *et al.*, 1999).

There are many genes in plants without known effects besides the fact that they modify the expression of a major gene by either enhancing or diminishing it. These are known as modifying genes (Bhatnagar *et al.*, 2004). The effect of modifier genes may be understated, but they are very important because they influence phenotypic expression. These trait modifications are of concern to a plant breeder as they conduct breeding programmes to improve traits that involve many major traits of interest. To develop an efficient and successful resistance breeding programme, understanding the genes controlling resistance is fundamental. Information from the literature on bruchid

resistance inheritance studies is scanty. Arcelin was shown to be inherited as a single dominant gene and resistance to *Z. subfasciatus* was easily transferred to commercial bean types (Cardona, *et al.*, 1990). However, the monogenic inheritance of arcelin leaves open the possibility that biotypes of *Z. subfasciatus* may exist or evolve, which are able to overcome this resistance. In a study to understand the inheritance of resistance to *A. obtectus*, Kornegay and Cardona (1991) found that resistance was inherited in a recessive manner when crossed with two commercial cultivars.

In a study where 10 inbred lines of maize were evaluated to determine combining ability for weevil resistance, Kang *et al.* (1995) found that additive gene effects were more important than non-additive gene effects. In another study to investigate inheritance of resistance to oviposition by maize weevil, Tipping *et al.* (1989) reported that additive gene action was important. Derera *et al.* (2001a, b) investigated gene action for weevil resistance in both free-choice and no-choice tests and found significant additive, non-additive and maternal effects. Dhliwayo *et al.* (2005) reported that both general combining ability (GCA), which is defined as average performance of individual lines in crosses and specific combining ability (SCA), which is the deviation of some crosses from the expected value (sum of the GCA values of the two parents involved)¹, were also found to be important for resistance to maize weevil. Dhliwayo and Pixley (2003) reported the importance of reciprocal effects on maize weevil resistance. Cockerham (1963) suggested partitioning of reciprocal effects into maternal and non-maternal effects. This is useful in determining whether maternal or extranuclear factors are involved in the expression of a trait.

Variation in an individual's phenotype may be determined, not only by the genotype and environment of that individual, but also by maternal effects i.e., the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987). During angiosperm development, multiple fertilization occurs where one sperm nucleus fuses with the egg nucleus to form a zygote. The other sperm nucleus fuses with the two polar nuclei to form triploid (3n) endosperm nucleus. Although the endosperm is not always triploid, it always contains more doses of maternal than paternal genes (Huidong, 1987; Bogyo *et al.*, 1988). As a consequence of the differential dosage of male and female genes, the

¹ Kearsey and Pooni (1996)

female parent may have a more important role in determining the characteristics of the trait under study.

Genetic analysis of grain resistance to weevils is reportedly complicated (Widstrom, 1989; Serratos *et al.*, 1997) because the weevil feeds on diploid and triploid tissues, which are both maternal and biparental in origin due to the fact that grain tissues belong to two different generations and have different gene doses from the parents (Serratos *et al.*, 1997). While the endosperm persists in the mature seeds of maize grain, in dry beans the $3n$ endosperm disappears (absorbed by the embryo) during seed development and the cotyledon, and not endosperm, becomes food storage.

Maternal effects are considered sources of error because generally they are non-Mendelian and reduce the precision of genetic studies (Roach and Wulff, 1987). The consequences of maternal effects for the response to selection may be further complicated by the correlation between maternal and offspring environments (Singh and Murty, 1980; Rossiter, 1996). Actual influence of maternal effects on response to selection will depend on the type of maternal effect involved. Environmental maternal effects will increase the amount of environmental “noise” and thus slow the response to selection (Alexander and Wulff, 1985). On the other hand, cytoplasmic or nuclear genetic maternal effects inflate the response to selection if maternal effects are dominant (Naylor, 1964). Although Cockerham (1963) indicated that maternal effects in plants were minimal and did not generally require consideration, Roach and Wulff (1987) provided substantial evidence that maternal effects have significant effects on the phenotype of an individual.

1.17 Mating designs and the usefulness of the diallel

In plant breeding programmes artificial crossing or mating is done to generate information for the breeder to understand the genetic control or behaviour of the trait of interest and also to generate a base population to initiate a breeding programme (Acquaah, 2007). Several mating designs have been reviewed for estimating general variance (Comstock *et al.*, 1949; Falconer, 1981; Hallauer and Miranda, 1981). These mating designs include biparental or paired crosses, polycross, North Carolina designs I, II, III and Diallel. Sokol and Baker (1977) suggested that to use these designs and make

correct genetic interpretation of the data, certain assumptions should apply, summarised as follows:

- 1) The materials in the population have diploid behaviour. However, (Acquaah, 2007) suggested that polyploids that exhibit disomic inheritance (allopolyploids) can be studied;
- 2) The genes controlling the trait of interest are independently distributed among the parents (i.e., uncorrelated gene distribution);
- 3) The absence of: non-allelic interactions, reciprocal differences, multiple alleles at the loci controlling the trait and G x E interactions.

Diallel mating design provides information on GCA effects of individual lines. It also provides information regarding SCA effects of the crosses. As indicated by Ghosh and Das (2003), a cross between two lines has an expected value which is the sum of the GCA of its two parental lines. However, some crosses deviate from this expected value and this deviation is the specific combining ability of the two parents (Kearsey and Pooni, 1996). Statistically, GCA effects represent the main effects due to the parents and SCA is the interaction effects (Falconer, 1981).

Differences in reciprocal crosses detected in a diallel cross are the most direct quantitative evidence for unequal contribution by maternal and paternal to the phenotype of the offspring (Wall *et al.*, 2005). Kearsey and Pooni (1996) stated that diallel analysis provides information on average performance of individual lines in crosses known as general combining ability (GCA). It also gives information about the performance of crosses relative to the average performance of parents involved in the cross known as specific combining ability (SCA). Griffing (1956) proposed four methods of diallel analysis:

Method 1: Complete diallel, which includes parents, F1 and reciprocals (n^2 entries, where n is the number of parents).

Method 2: Parents and F1s without reciprocals [$n(n+1)/2$ entries].

Method 3: F1s and reciprocals used but not parents [$n(n-1)$ entries].

Method 4: Only F1s are used, no reciprocals or parents [$n(n-1)/2$ entries].

Griffing (1956) further postulated two models of analysis of variance of the diallel mating design. Model 1 (fixed effects) is used in assumption that the parents are the population i.e., parents are a fixed set of lines. With this model, estimates only apply to the genotypes included and cannot be extended to some hypothetical reference population (Hallauer and Miranda, 1988). Consequently, estimation of components of variance is not appropriate in this model, but GCA and SCA effects estimates are informative and necessary. Model II (random effects) is used where parents are a sample of randomly chosen lines from a reference population and the estimates of variance components are the main interest.

In the current study, a 6 x 6 complete diallel mating design (Griffing, 1956) was used where parents were crossed to generate 36 reciprocal crosses including parents. This design has been widely used in plant breeding research to obtain genetic information. It is used both in self-pollinating and cross-pollinating species as well as homozygous or inbred parents (Jinks and Hayman, 1953; Griffing 1956) and non-inbred parents (Gardner and Eberhart, 1966). Christie and Shattuck (1992) concluded that diallel analysis is a sophisticated form of progeny testing from which information can be obtained that is not available from any other analysis and can be used in plant breeding to assist in selection.

1.18 Assumptions of the diallel mating design

Christie and Shattuck (1992) outline the six assumptions that must be validated in a diallel mating design scheme. Failure to take into consideration such assumptions may result in incorrect interpretation of genetic data. The assumptions were listed as follows:

- a) Diploid segregation- The diallel analysis is only considered for diploid species. The dry bean is a diploid species consequently the assumption is valid.
- b) Homozygous parents- Failure to meet this assumption may under-estimate such genetic parameters as the proportion of genes with positive and negative effects while additive-dominance covariance could be over-estimated. Dry bean is a self-pollinating crop, therefore, it should be greatly homozygous.
- c) Absence of maternal effects or reciprocal differences- This is intended to estimate and evaluate the variances for maternal effects. Reciprocal differences were included in the model used in the current study.

- d) Absence of epistasis- Non-allelic gene interaction or no epistatic gene action is one of the important assumptions in a diallel mating design. In this study absence of epistatic effects was assumed and confirmed by testing the adequacy of additive-dominance model used in this study.
- e) Multiple alleles- Being a diploid species, dry bean has two alleles at a single locus hence this assumption was valid. A survey of literature did not yield any evidence for multiple allelism in dry beans. Christie and Shattuck (1992) also reported that the problem of multiple alleles in diallel analyses has been scarcely discussed in the literature.
- f) Independent distribution of genes among parents- Non-independent distribution of genes arise because of linkage between loci or because of selected parents. The parents used in the study were randomly selected based on degree of resistance or susceptibility, and it is acknowledged that a small sample of six parents was used, and therefore it may be difficult to validate this assumption. Baker (1978, as reported in Christie and Shattuck, 1992) suggested that independent distribution of genes in parents might not be a realistic assumption to make.

1.19 Summary

Although the genus *Phaseolus* is of American origin, it has spread widely and grown globally, in a broad range of environments and cropping systems. It has been demonstrated that *P. vulgaris* (the common bean) is the most predominant species, occupying more than 85% of the bean's production area worldwide. There is abundant diversity among the *Phaseolus* species and this diversity has been used for crop research and development. In spite of such an array of genetic diversity, the average global yield of dry beans still remains low and its cultivation continues to face a wide range of biotic and abiotic constraints. The question to ask is where have bean breeders/researchers gone wrong? Is this abundant genetic diversity being under-utilised? Whatever the case, what is clear is that there exists the potential to exploit the existing genetic diversity of beans to improve some of its major production constraints, such as low resistance to storage pests. Developing cultivars with resistance to bean bruchids is one area that still needs attention because no suitable resistant varieties have been developed so far. The two bruchid species, *A. obtectus* and *Z. subfasciatus*, are still a serious storage pest problem, wherever beans are grown. Although arcelin-

containing bean varieties resistant to *Z. subfasciatus* exist, they are likely not to be accepted by most African farmers because of their small-seed size, long cooking time and non-adaptability to tropical conditions. Importantly, recent research results have demonstrated that arcelin-derived bean lines are negatively correlated to seed yield.

Bruchid control strategies such as cultural, biological, chemical and mechanical methods, may not adequately address the problem of bruchid damage experienced by smallholder farmers. Host plant resistance, through the use of resistant varieties in combination with the other insect control strategies, seems to be the most effective and sustainable option. As breeders make use of the available bean germplasm to develop new improved varieties, it is necessary to take cognisance of the preferences of end users (farmers) through a participatory approach.

From this literature review, it is evident that scientists need to search for effective and adaptable sources of resistance, apart from the current arcelin-based strategy, which mainly confers resistance to *Z. subfasciatus* and does not prevent field infestation with *A. obtectus*. Furthermore, the development of an efficient and successful breeding programme requires knowledge of the genes controlling resistance. The nature of gene action and combining ability are pre-requisites for the development of new varieties. The combining ability estimates are useful for evaluating the genetic worth potential of lines to exploit the relevant type of gene action in a breeding programme.

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Chapter Two

Smallholder Farmers' Perceptions of Dry Bean Damage By Bean Weevils and Varietal Preferences in Malawi and Their Implications for Breeding

Abstract

Farmers' preferences and priorities in dry bean cultivar development have often been overlooked in the past. This has resulted in varieties that fail to perform under local farming conditions or varieties that are high yielding but with unacceptable culinary characteristics. The aim of this study was therefore to ascertain farmers' perceptions of the importance of *Acanthoscelides obtectus* Say (bean weevil) and *Zabrotes subfasciatus* Boh. (Mexican bean weevil) damage to the common bean both in the field and in storage. A participatory rural appraisal (PRA) was conducted in Bembeke, Mitundu and Mkwinda extension planning areas in August 2004. With a set of guide questions, a semi-structured interview technique was used for individual households and focus group discussions involving both male and female farmers. A direct matrix ranking technique was used to assist farmers to list bean varieties in order of preference. Results of this study showed that bruchids were important storage pests causing significant damage to stored beans in the absence of any control measures. Most households (93%) indicated that more than 50% of their stored beans were lost to bruchids, particularly where no control measures were applied, compared to a figure of 38% that has been reported in literature. This indicated that varieties grown by farmers were generally highly susceptible to bean weevils. An appraisal study on the effectiveness of indigenous control methods showed that some plant botanicals, such as bean plant ash, were effective. Results also showed that plant botanicals used in combination (Mphanjobvu + Tete) were more effective than that of single treatments (Mphanjobvu or Tete alone) and suggested that management and control methods were not effective. It was evident from this study that agronomic traits such as maturity period, adaptation and yield and culinary characteristics such as cooking time, flavour/taste and seed colour need to be considered by breeders in cultivar development. Farmers' participation is vital to enhance the adoption of improved common bean varieties.

2.1 Introduction

Bean research in Malawi is focused mostly on developing varieties that are high yielding and resistant to field insect pests and diseases. Not enough emphasis has been placed on improving those traits or characteristics that would make such new crop varieties acceptable to farmers. Unique qualities preferred in the traditional bean varieties, such as taste, flavour and resistance to storage pests, have often been overlooked. No detailed studies involving smallholder farmers have been carried out in Malawi to establish the extent to which farmers perceive the bruchid as a storage pest of economic importance. Surveys conducted in Malawi have mainly focused on field pests (Letourneau, 1994; Scott and Maiden, 1998), the primary objective being to establish the status and distribution of bean insect pests and methods used to control them. In all these cases, the farmers were not fully involved, although it has been demonstrated that valuable information on economic, social and agronomic aspects of different crops can be obtained if farmers and other community members play a part (Barahona, 2002; SARRNET, 2003). Participatory variety development would, in turn, enhance the adoption of newly developed crop varieties.

The need to understand farmers' conditions and to include farmers as partners in the development and evaluation of new agricultural technologies is increasingly being recognised (Manoharan *et al.*, 1993; DeVries and Toenniessen, 2001; Escalada and Heong, 2002). It is argued that farmers have extensive, well-developed and sound knowledge of their environment, crops and cropping systems (Bellon, 2001). Understanding this knowledge is a fundamental step to generating a dialogue between farmers and scientists so that appropriate technologies can be developed, which can eventually accelerate adoption of the improved varieties (Chambers, 1994a). Farmers can be engaged in dialogue with plant breeders through a participatory rural appraisal (PRA), among other methods. Participatory rural appraisal is a tool used to gain an overview of a rural community's main problems and opportunities. Conceptually, it is a methodology for learning about rural life and the rural environment (Pokharel, 1998) from the people themselves. The PRA concept is based on the principle that local people are creative and capable of doing their own investigations, information analysis and planning independent of scientists (David, 1995). Chambers (1992) argued that the PRA could

be used to interact with both rural and urban people to understand, learn and solicit their participation. Unlike most conventional research techniques, which are essentially extractive and conducted by outsiders, the PRA approach draws upon several participant-oriented traditions to bring another dimension to development-related research (Chambers, 1994b). It is assumed that local people are able to determine the important issues and the relevant information that is needed. The PRA emphasises open access to information and “avoids professional possessiveness” (Soleri *et al.*, 1999).

Participatory approaches that involve farmers in problem identification, priority setting and planning ensure the establishment of a long-term research agenda at the community level. Farmer participation improves the efficiency and effectiveness of problem-solving research (Witcombe *et al.*, 1996). Whereas some degree of farmer participation in technology development, testing and dissemination has increasingly been considered essential in agricultural research and development, David (1995) argued that systematic and meaningful farmer input into the research process and need identification has been weak. Sperling *et al.* (2001) suggested that dialogue between farmers and scientists should be strengthened to ensure the widespread adoption of developed technologies and to meet social needs both at the community and national level. Relevant information can be obtained from communities using a range of PRA techniques. These include semi-structured interviews, preference and wealth ranking, village resource maps, transect walks, trend analysis and Venn diagrams (Chambers, 1994b). In this study, matrix ranking and semi-structured interview techniques were used to obtain the information required.

Another way of ensuring that farmers become partners in technology development is participatory plant breeding (PPB) or participatory crop improvement (PCI). It is accepted that the adoption of new crop varieties by smallholder farmers, especially those in marginal areas, has been low (Cooper, 1999; Cleveland *et al.*, 2000; Ceccarelli *et al.*, 2001). Low adoption of new cultivars from research is attributed to a failure to involve farmers and the end-users in research and the development of new varieties (Almekinders and Ellings, 2003). Generally, researchers have often given little or no cognisance to the perception of local people in technology development. Nassif (1999)

argued that the adoption of technology depends mainly on how well it fits the purpose for which the end user needs it.

The poor adoption of developed agricultural technologies, particularly new crop varieties, continues to present a big challenge to breeding as a strategy for improving food security. Although a considerable amount of money has been spent on maize breeding in most African countries, only an estimated 37% of farmers regularly plant improved varieties (Morris *et al.*, 1999). DeVries and Toenniessen (2001), quoting Ahmed *et al.* (2000), also indicated that adoption of sorghum and millet varieties was very low in most countries in eastern and southern Africa. In a study to establish the uptake of modern crop varieties after the Asian green revolution, Evenson (2003) concluded that adoption of new bean varieties has been low in most African countries in spite of the large number of varieties that have been released.

Farmers' requirements for varieties have been investigated in many cereal and legume crops through both PRA and formal research methods. Bänziger (2004) indicated that farmers' selection criteria for maize varieties was based on the importance attached to grain poundability, earliness and high grain yield potential in southern Africa. Breeders tend to overlook the importance of poundability in their selection during the breeding process. A diagnostic survey conducted in Uganda by Adipala *et al.* (1999) revealed that farmers ranked bruchids as the most important constraint in cowpea production. A survey carried out in three states of Nigeria showed that farmers knew which pests were more important and had developed their own control strategies to combat these pests (Banjo *et al.*, 2003). Suitable post-harvest techniques to control the cowpea weevil were developed in Cameroon after taking farmers' concerns into consideration (Ntoukam *et al.*, 2001); thus confirming the effectiveness and importance of participatory approaches.

Although many bean varieties have been developed in Malawi, their uptake is generally very low. One reason for the low adoption rate is that farmers had very little or no input during the development process (Chirwa, personal communication). A study by Masangano (2000) concluded that most of the agricultural technologies developed were not demand driven and consequently failed to meet the needs and preferences of farmers in Malawi. A PRA was therefore considered to be necessary to identify farmers' bean varietal preferences and to solicit their perceptions on bean bruchid damage.

Study objectives

The objectives of the study were to:

- (i) solicit bean growers' perceptions on bruchid damage both in the field and in storage;
- (ii) identify traditional methods that farmers use to control bruchids in storage and their efficacy, and
- (iii) establish the criteria that farmers used to prioritise bean varieties in order of preference.

2.2 Materials and methods

Malawi is divided into eight agricultural development divisions (ADDs) that are considered areas of focus in programme delivery. The ADDs are further divided into rural development projects (RDPs) and extension planning areas (EPAs) principally on administrative grounds. Service delivery at the village level is through extension planning areas. The PRA was conducted in Bembeke, Mkwinda and Mitundu EPAs within Dedza and Lilongwe RDP, in the central region of Malawi (Fig 2.1). These two RDPs were selected because traditionally they are bean-growing areas. It was therefore envisaged that sufficient, relevant and useful information could be obtained from these districts. In addition, beans in these areas are considered the second most important crop after maize as a source of income for many farmers. Dedza, in particular, has unfavourable soils (highly weathered and leached ferralitic latasolils) for maize production as a result beans are an excellent complementary crop to mitigate against the effects of food shortage through consumption or sales.

2.2.1 Features of the study areas

(a) Bembeke EPA

Bembeke EPA falls within the Dedza RDP in the Dedza district. It stretches from the main Lilongwe-Blantyre road to the Rift Valley escarpment. The area is 1625 m above sea level (asl) receives an average annual rainfall of 936 mm and has average minimum-maximum temperatures of 13.2 - 24.9°C. It is at a latitude of 14.19 S and longitude of 33.39 E. Three villages, Juwa, Chimlambe and Kauye, were randomly selected from a list (sampling frame) provided by local extension workers. Bembeke has 12,567 farm families.

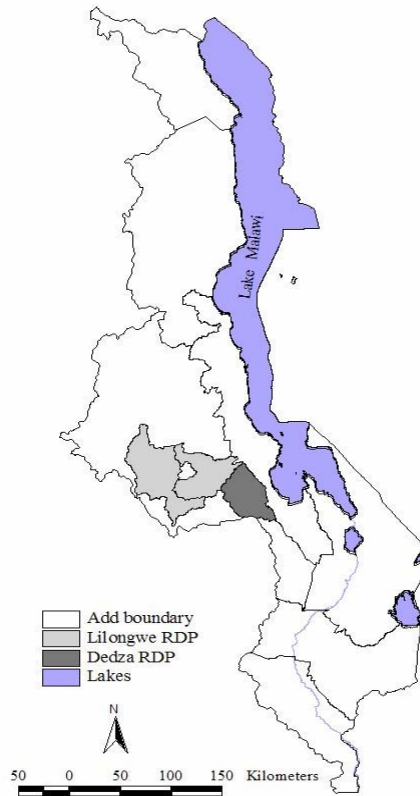


Figure 2.1: A map of Malawi showing Lilongwe and Dedza rural development projects under the Lilongwe agricultural development division, where the PRA was conducted.

(b) Mkwinda and Mitundu EPAs

These two EPAs are situated on the fertile Lilongwe-Kasungu plain within the Lilongwe rural development project in the Lilongwe district. The two areas are 1140 m above sea level, with temperatures of 13.9 - 26.4°C. They are at a latitude of 11.02 S and longitude of 33.86 E. The average annual rainfall is 931 mm and the soil can support most of the important crops grown by the majority of smallholder farmers in Malawi such as maize, tobacco, groundnuts, beans and root crops. In Mkwinda EPA, the study was carried out in three villages, namely Katuta, Chingala, and Zikadza, whereas in Mitundu EPA, the study was conducted in only two villages, Chalera and Khombe. In each EPA, these

villages were selected randomly with the guidance of an extension worker. Mitundu and Mkwinda EPAs have 14, 279 and 20, 270 farm families respectively.

2.2.2 Data collection and analysis

Secondary data were collected on crops grown, production, area planted while yield was estimated by calculating the ratio of production to area under production. The Crop Production Department of the Ministry of Agriculture and Food Security also provided data on annual crop estimate figures. Crop production figures were also obtained from famine and early warning systems (FEWS) from the Planning Department.

Primary data were collected by a field research team comprising the principal investigator, a social scientist, an enumerator and two extension officers, one from Bembeke EPA and the other from Mitundu and Mkwinda EPAs. The extension officers facilitated field visits and mobilised farmers for group discussions.

A pre-test visit (pilot study) was first made to the study areas. The pre-test visit was important for two reasons. Firstly, to brief the participating farmers on the study's purpose, importance and implementation plan. Secondly, the visit allowed the facilitators to test the prepared guide questions and amend them if necessary before the actual implementation of the study. In addition, general information on locally-grown legume crops other than beans, rainfall patterns, and crop production practices was also obtained during the pre-test visit. This information assisted the team to establish relevant discussion topics necessary for improving the guide questions. For the pre-test questionnaire, 15 individual bean growers from randomly selected households were interviewed. The pre-test questionnaire was used only in two villages in Bembeke EPA.

For the purpose of the main study, semi-structured interviews and matrix ranking and scoring were the two PRA techniques that were used to collect data from both individuals and focus groups. Farmers were put in groups (Fig 2.2) where issues outlined in Table 2.1 were dealt with.

Table 2.1: Summary of data obtained from bean growers from three extension planning areas (EPAs) in Lilongwe and Dedza Rural Development projects

Information gathered from bean growers	
	<ul style="list-style-type: none"> • List of commonly grown bean varieties, both landraces and improved varieties • Type of cropping systems normally practised • In relation to storage pests, whether intercropping minimises bruchid damage to stored beans or not • Proportion of stored beans that is destroyed by bruchids • Prevalence of bruchid damage - is it throughout the year or at certain times of the year? • Their experience in terms of whether some varieties are more susceptible than others to bruchids • Length of storage before bruchids are noticed • Views of farmers on whether infestation occurs both in the field as well as in storage • Experience of farmers on incidence of bruchids between beans produced in the winter and summer • Indigenous methods farmers use to minimise bruchid damage in storage • Their assessment of the effectiveness of those methods • Views of farmers on whether modern varieties are better than their old varieties in terms of bruchid resistance

Farmers of both sexes were included in the study to get the benefit of multiple insights on common issues and problems. A total of 30 households in each EPA, with varying group sizes (5 to 15 people) were engaged in focus group discussions.



Figure 2.2: Focus group discussions in one of the villages in Bembeke Extension Planning Area, Dedza RDP

Where it was felt necessary to obtain additional information or clarity on certain topics or issues, individual interviews were conducted. The interviews were conducted at random with key informants, such as local leaders or with some farmers considered to be ‘model or key farmers’ in their areas.

A direct matrix ranking technique was used to determine farmers’ priorities and preferences as to which bean varieties they considered most important and their reasons for this. The farmers identified the bean varieties they grew. The varieties were listed on a large sheet of white paper. After group discussions, farmers were asked to rank the varieties in order of preference using agronomic and culinary attributes. To probe for more details on each of the varieties, some questions were asked. Answers to all the questions were written on a flip chart. These were then listed on the left hand side of the matrix with scores on the top row rated from the highest to the lowest.

Fifty individuals selling dry beans, ten from each of the five markets in Ntcheu district, were interviewed. In Lilongwe and Dedza districts, three open markets were visited in each district where 30 individuals, ten from each market, were interviewed. A summary of the information obtained from bean traders in various market outlets is presented in Table 2.2.

Table 2.2: Summary of information solicited from bean traders from three market outlets in Lilongwe and Dedza districts

	Information gathered from bean traders
	<ul style="list-style-type: none"> • Names of varieties commonly bought from farmers • Varietal preferences or preferred variety characteristics • Timing of purchase whether they buy after harvesting or as farmers are harvesting • Time it takes to experience bruchid damage in storage • Whether or not they buy different bean varieties or one variety only • Their experience in terms of whether some varieties are more susceptible than others to bruchids

Data were analysed using the Statistical Package for Social Scientists (SPSS) version 11.5 to generate percentages and frequencies for the various variables. Cross tabulations were used to establish relationships between target variables.

2.2.3 Indigenous bruchid control methods validation study

Based on information obtained from farmers on indigenous bruchid control methods, an appraisal study of selected bruchid control methods was conducted in the laboratory. The aim of the study was to confirm what some respondents had indicated about the effectiveness of some bruchid control methods in controlling the two bruchid species in storage. Six local bruchid control methods were selected (Table 2.3). Two botanicals (neem leaves and dry bean plant ash), three botanicals (Mphanjobvu, Tete and Garlic were suggested by farmers) and super actellic dust was used as a control treatment. Two bean varieties, already known to be susceptible, were used in the study.

Table 2.3: Botanicals evaluated in the study

Scientific name	Local name	Collection source	Part used
<i>Azadirachta indica</i> L.	Neem	Salima along lake Malawi	Leaves
<i>Neorautanenia</i> sp.	Tete	Dedza	Tuber
<i>Neorautanenia mitis</i> L.	Mphanjobvu	Ntcheu	Tuber
<i>Allium sativum</i> L.	Garlic	Lilongwe Central Market	Bulbs
<i>Phaseolus vulgaris</i> L.	Dry beans	Field of dry beans (Chitedze)	Whole dry plant
Super actellic dust	-	ATC-Lilongwe	-

The study was conducted to validate the effectiveness of selected indigenous control methods that farmers use compared with commercial insecticides in controlling bruchid damage in susceptible varieties. Super actellic dust, a commercial insecticide which is recommended by the local extension services was therefore used as a control to compare its effectiveness against those indigenous methods used by farmers.

Using two susceptible bean varieties (variety 1- Kholophethe and variety 2- Nagaga) and six bruchid control methods (single treatments and their combinations), a total of 12 treatments were generated (Table 2.4). Five grammes of each of the botanicals was applied, in dry powder form, to each vial which contained 25 seeds, except for actellic dust, which was applied at the recommended application rate of 25 g to 50 kg of seed

and hence 0.05 g was used. Super actellic dust was used as a control treatment in this study. Ash was applied at a reduced rate of 2.5 g to 10 g of seed because of its volume. Insects were allowed to oviposit (lay eggs) for 10 d after which the bruchids were removed and then the processed botanicals were applied. The experiment was laid out as a completely randomised design with three replicates.

Super actellic dust was purchased from the Agricultural Trading Company (ATC) and garlic was bought already processed i.e. in powder form from Lilongwe Central Market. Bean ash was obtained by burning the dried stems and leaves of the bean plant without pods.

The tuberous part of tete and mphanjobvu were sun dried for 5 d. Neem leaves were dried inside the laboratory under shade for 9 d. The fully dried tubers and leaves of tete, neem and mphanjobvu were ground using a Thomas-Willey laboratory mill. A 600mm aperture sieve was used to sieve the ground plant products into usable fine powder (Fig.2.3).



Figure 2.3: Processed plant products (botanicals) including Super actellic dust in powder form ready for use

Table 2.4: Treatment description and quantities used in the study

Code	Treatment	Application rate (g) /25 seeds
01	Mphanjobvu	5
02	Neem powder	5
03	Actellic dust	0.05
04	Bean ash	5
05	Garlic	5
06	Tete	5
07	Mphanjobvu + Tete	5
08	Neem + Garlic	5
09	Bean ash + Neem powder	5
10	Neem + Tete	5
11	Garlic powder + Mphanjobvu	5
12	Bean ash + Neem powder	5

Bruchid control methods were tested on two bruchid species (*Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman) in separate experiments. The test treatments were applied after oviposition to determine whether the treatments were effective in controlling emerging insect progeny to simulate the farmers' situation. Infestation of seeds by storage pests occurs in the field. Twenty five seeds were placed in each vial, into which 10 unsexed F1 generation insects (1-3 d old) were introduced. Experiments were replicated three times. Data were recorded on bruchid median development period (calculated as the number of days from the middle of oviposition (d 5) to the first progeny emergence) and number of bruchids that emerged (dead and alive).

2.3 Results

2.3.1 Secondary data

Crops grown in the study areas included potatoes, cassava, soybeans, maize and beans. However, maize production is constrained by highly acidic soils in most areas of Bembeke EPA. Dry beans, cassava and potatoes remain crops of economic importance for most farmers in these areas. The importance of beans in terms of area planted is

shown in Table 2.5. Lilongwe ADD, where this study was conducted, has the largest area planted with beans.

Table 2.5: Bean production area and yield estimates for smallholder farmers in selected Agricultural Development Divisions (ADDs) in Malawi

ADD	Area planted (Ha)	Production (MT)	Yield estimate (t ha⁻¹)
Karonga	10, 036	5,017	0.50
Mzuzu	40, 191	13,967	0.35
Kasungu	49, 850	22,647	0.45
Lilongwe	78, 301	35,058	0.45
Blantyre	36, 819	16,444	0.45

Source: FEWS / Ministry of Agriculture Irrigation and Food Security: National crop production estimates (2003/4 season).

2.3.2 Damage caused by bruchids

Farmers confirmed the importance of bruchid damage as a significant constraint to bean production. Bruchids caused a substantial yield reduction in their areas. The results from Bembeke EPA showed that 28 households, representing 93.3%, perceived that more than 50% of their stored beans were lost due to bruchid-related damage, particularly where no control measures had been applied (Table 2.6). They also indicated that Maziraankhono and Sapatsika/Nanyati, as improved bean varieties, were very susceptible to bruchids in storage. Farmers also perceived that some dry bean varieties showed partial resistance to bean bruchids, one such variety being Phalombe, a landrace preferred by many farmers because of its large-seed size (37-40 g 100 seeds⁻¹) and seed colour (dark red).

The results for Mkwinda and Mitundu EPAs showed slight differences in farmers' perceptions regarding the proportion of beans that were damaged by bruchids in storage. More than 80% of the respondents indicated that less than 50% of their beans were damaged by bruchids in storage (Table 2.6). In Mitundu and Mkwinda areas, most farmers still preferred their landraces to improved bean varieties because the improved varieties were perceived as more vulnerable to bruchid damage in storage. Farmers around Bembeke EPA, on the other hand, grew both landraces and improved bean

varieties. The storage period varied between 3 and 6 months. Depending on the management level, the longer the storage period, the greater the damage.

Table 2.6: Farmers' responses regarding proportion of bean damage in storage by bruchids

EPA	% seed damage (50 kg bag)	Frequency	Percentage
Bembeke	25	2	7
	50	6	20
	75	22	73
Total		30	100
Mkwinda	25	18	60
	50	8	27
	75	4	13
Total		30	100
Mitundu	25	11	37
	50	16	53
	75	3	10
Total		30	100

2.3.3 Bruchid control methods used by farmers

Smallholder farmers used botanical products, such as vegetable oil, tobacco leaves and ash prepared from different plant parts and sources, as a means to reduce bruchid damage in storage (Table 2.7). The quantity applied and methods of application varied among farmers. This suggests that the use of local methods to control bruchids and their effectiveness need thorough investigation.

Table 2.7: Summary of indigenous bruchid control methods farmers used to minimise bruchid damage in storage in the three extension planning areas (EPAs)

EPA	Village	Control method used	Quantities applied
Bembeke	Chimlambe	Ash and actellic dust	Handful of ash to a bucket full of beans (approximately 20-30 kg) 20-30 g actellic dust applied to 50 kg of beans Two handfuls of tobacco powder to a bucket full of beans
	Kauye	Actellic dust & Sevin	
	Katsekaminga	Tobacco, Sevin & ash	
Mitundu	Katuta	Black pepper & ash from various crop plants	Depended on the quantity of beans Depended on the plant used to make the ash 500 g of ash to approximately 10 kg of beans A tea mug full of neem powder (200-300 g) to 20 kg of beans
	Chingala	Ash	
	Juwa	Ash Neem leaves	
Mkwinda	Chalera	Paraffin & ash	Quantity not specified
	Khombe	Ash, tobacco & sand	Quantity dependent on the level of observed damage

Less than 5% of the farmers used insecticides, such as actellic dust (1.6% pirimiphos methyl and 0.3% permethrin). Most households interviewed used botanical products as well as inert dusts such as ash to control bruchids in storage. Traders mostly used super actellic dust to treat their beans to protect them from bruchids in storage as most of them buy beans from farmers during harvesting when prices are still low. They reported that if beans are stored untreated, weevil infestation becomes high within a period of 3-6 months, causing substantial damage.

2.3.4 Appraisal of farmers' bruchid control methods

The results for testing the effectiveness of the various bruchid control methods that farmers use to minimise damage of stored beans by bruchids are presented in tables 2.8, 2.9 and 2.10 and are summarised as follows:

- Adult bruchid emergence was significantly affected by the application of the botanicals (Tables 2.8 and 2.9). On the other hand, the development period for

both bruchid species on the two bean varieties was not significantly different (Table 2.10) when applied after the insects had laid eggs.

- Bean plant ash was more effective on *A. obtectus* (Table 2.8) than *Z. subfasciatus* (Table 2.9), applied after the insects had laid eggs. This could be explained by the fact that larvae of *Z. subfasciatus* are not exposed to ash or actellic dust because they penetrate the below the eggs into the seed.
- Super actellic dust was effective on both bruchid species (Table 2.8 and 2.9) as it was lethal to almost all the bruchids that emerged.
- *A. obtectus* had a higher adult bruchid emergence (Table 2.8) than *Z. subfasciatus* (Table 2.9), suggesting that in the absence of any control measures, this storage pest can damage stored beans extensively.
- Mphanjobvu + Tete and Neem + Tete, in combination, were lethal for *Z. subfasciatus* (Table 2.9) but were completely ineffective against *A. obtectus* (Table 2.8), suggesting that the effectiveness of plant botanicals on bruchids could be species-specific considering that the two species have different ovipositional behaviour.

A method was considered effective if it killed emerged bruchids as this would reduce the re-infestation rate if the beans were kept a little longer in storage. Few adult bruchids would emerge in the next generation and this would result in reduced damage.

Table 2.8: Effect of botanicals applied after oviposition on *Acanthoscelides obtectus* adult bruchid emergence in two susceptible bean varieties§

Control Method	Variety 1		Variety 2	
	Adult bruchid emergence No. Dead	Adult bruchid emergence No. Live	Adult bruchid emergence No. Dead	Adult bruchid emergence No. Live
Mphanjobvu	1	73	1	118
Neem	1	71	1	89
Super actellic dust (control)	43	1	46	2
Bean plant ash	40	22	48	44
Garlic	1	96	1	136
Tete	1	54	2	134
Mphanjobvu + Tete	1	83	1	78
Neem + Garlic	2	45	2	38
Bean ash + Mphanjobvu	3	40	4	44
Neem + Tete	1	47	1	64
Garlic + Mphanjobvu	1	76	1	99
Bean ash + Neem	4	48	3	54
Mean	8	55	9	75
Significance	**	**	**	**

** Significant at $P \leq 0.01$

§Variety 1- Kholophethe and variety 2- Nagaga

Table 2.9: Effect of botanicals applied after oviposition on *Zabrotes subfasciatus* adult bruchid emergence in two susceptible bean varieties§

Control Method	Variety 1		Variety 2	
	Adult bruchid emergence No. Dead	Adult bruchid emergence No. Live	Adult bruchid emergence No. Dead	Adult bruchid emergence No. Live
Mphanjobvu	2	32	1	29
Neem	0	41	0	37
Super actellic dust (control)	35	1	30	0
Bean plant ash	7	21	19	18
Garlic	1	30	1	34
Tete	6	11	10	13
Mphanjobvu + Tete	48	2	31	1
Neem + Garlic	1	41	4	20
Bean ash + Mphanjobvu	27	20	30	18
Neem + Tete	26	1	34	2
Garlic + Mphanjobvu	2	92	4	87
Bean ash + Neem	15	8	39	26
Mean	14	25	17	24
Significance	***	***	***	***

*** Significant at $P \leq 0.001$

§ Variety 1- Kholophethe and variety 2- Nagaga

Table 2.10: Effect of botanicals applied after oviposition on the median development period[†] (days) of the two bruchid species in two susceptible bean varieties[§]

Control method	<i>Zabrotes subfasciatus</i>		<i>Acanthoscelides obtectus</i>	
	Variety 1	Variety 2	Variety 1	Variety 2
Mphanjobvu	35	32	32	33
Neem	32	31	33	32
Super actellic dust (control)	32	31	34	32
Bean plant ash	23	31	35	33
Garlic	34	31	34	32
Tete	34	32	34	32
Mphanjobvu + Tete	31	31	32	31
Neem + Garlic	32	31	36	35
Bean ash + Mphanjobvu	32	31	35	35
Neem + Tete	34	31	34	33
Garlic + Mphanjobvu	31	31	31	33
Bean ash + Neem	34	31	33	31
Mean	32	31	34	33
Sign_(0.05)	ns	ns	ns	ns

[§] Variety 1- Kholopethe and variety 2- Nagaga

2.3.5 Varieties grown and farmers' preferences

In the three EPAs farmers grew both local and improved bean varieties. However, Table 2.11 shows that farmers mostly grew their local landraces. Landraces are well adapted to local environment; they are tolerant to drought and have good flavour/taste preferred by consumers. The Malawi bean improvement programme has released up to twenty varieties of improved bean varieties and non-governmental organizations have actively been involved to create awareness and disseminate these new varieties. In Dedza, although non-governmental organisations introduced improved bean varieties, farmers grew only seven out of the 20 varieties released by the national bean improvement programme (representing 35%). In Mitundu EPA only five of the 20 improved varieties (representing 25%) were grown by farmers, while in Mkwinda EPA farmers grew only six of the 20 improved bean varieties, representing a 30%. Some improved varieties such as Maluwa (CAL 143), Namajengo (336) and Nagaga (A197) and landraces locally named Phalombe, Kayera/Zoyera and Bata were popular varieties commonly grown in all the extension planning areas.

From this study, it was evident that farmers grew a wide range of both landraces and improved bean varieties. The improved varieties were those developed by the National Bean Programme in collaboration with other institutions such as Centro Internacional de Agricultura Tropical (CIAT) and University of Malawi (Bunda College of Agriculture).

Table 2.11: Dry bean varieties commonly grown in Bembeke, Mitundu and Mkwinda

Extension Planning Area	Bean varieties commonly grown [†]	
	Landraces	Improved
Bembeke	(i) Nanyati (ii) Phalombe (iii) Mkhalatsonga (iv) Domwewalirira (v) Sadyakwenda (vi) Kayera (vii) Khakhi/Bata (viii) Kaulesi	(i) Napilira (<i>CAL 143</i>) (ii) Kamtedza (<i>97/1</i>) (iii) Kambidzi (<i>A 286</i>) (iv) Namajengo (<i>336</i>) (v) Sapatsika (<i>DRK 57</i>) (vi) Chimbamba (<i>25-2x-8-7</i>) (vii) Maluwa (<i>CAL 143</i>)
Mitundu	(i) Kachitosi (ii) Phalombe (iii) Mkhalatsonga (iv) Kayera (v) Maziraankhono (vi) Khakhi (vii) Msandionekuda (viii) Nanyati	(i) Usiwawatha (ii) Namajengo (<i>336</i>) (iii) Nagaga (<i>A 197</i>) (iv) Maluwa (<i>CAL 143</i>) (v) Kalima (<i>PVA 692</i>)
Mkwinda	(i) Msandionekuda (ii) Phalombe (iii) Zoyera (iv) Khakhi (v) Kankhono (vi) Galang'ande (vii) Nanyati (viii) Kamuyanga	(i) Namajengo (<i>336</i>) (ii) Sapatsika (<i>DRK 57</i>) (iii) Kalima (<i>PVA 692</i>) (iv) Maluwa (<i>CAL 143</i>) (v) Nagaga (<i>A197</i>) (vi) Usiwawatha

[†]Names in brackets (italicised) are breeding codes

Farmers' ratings of bean variety trait preferences were slightly different from that of the traders (Table 2.12). Farmers rated yield as the most important trait in a bean variety. On the other hand seed colour, bruchid resistance and flavour were considered the most important by the traders. Phalombe, Maluwa and Napilira are the most widely grown and popular dry bean varieties that middlepersons (bean traders) prefer to buy from farmers. Most of these varieties are red seeded or red speckled, have an excellent flavour and cook quickly.

Table 2.12: Summary of varietal trait preference among bean growers and traders

Variety Trait	Rating Scores [†]					
	Bean growers			Traders		
	Extension planning areas			Name of district market		
	Mkwinda (n=10)	Mitundu (n=10)	Bembeke (n=10)	Lilongwe (n=10)	Ntcheu (n=10)	Dedza (n=10)
Yield	1	1	1	5	3	3
Seed size	1	1	1	2	1	1
Seed colour	2	1	2	1	1	1
Bruchid resistance	1	1	2	1	1	1
Flavour	1	1	1	1	1	1
Cooking time	1	1	1	1	1	2
Earliness	3	3	3	5	5	5

[†]scores used were: 1= most important and 5 = least important

For bean traders, genetic traits and culinary characteristics were important criteria when deciding which varieties to market (Table 2.13). Medium to large-seeded sizes were preferred. Dark red kidney, red speckled and khaki (cream mulatinho) were the preferred seed colours for both traders and bean growers. Green and black seed colours were the least preferred.

Table 2:13: Summary of preferred bean varieties/landraces and their desirable attributes as rated by traders from various open markets

Name of Market	Preferred Varieties	Attributes
Ntcheu District		
Masonkhano	<i>Phalombe</i>	Large-seeded size, reduced cooking time, good taste/flavour
Tsangano	Kayera, Thyolo, <i>Khakhi</i>	Reduced cooking time and large-seeded size
Mphate	Phalombe, Kayera, <i>Khakhi</i>	Reduced cooking time, large-seeded size, good seed colour [†]
Lizulu	<i>Napilira</i> , <i>Khakhi</i> , <i>Phalombe</i>	Good flavour, reduced cooking time, large-seeded size
Kanyimbo	<i>Napilira</i> , Kayera, Phalombe	Reduced cooking time, good flavour, good seed colour [†]
Lilongwe District		
Msundwe local market	Kayera, <i>Phalombe</i> , <i>Khakhi</i> , <i>Napilira</i> , Namatchola	Reduced cooking time, good flavour, large-seeded size
Mitundu local market	Thyolo/Phalombe, <i>Khakhi</i> , <i>Napilira</i> , <i>Maluwa</i>	Large-seeded size, good flavour, cooks fast, seed colour [†]
Lilongwe central market	<i>Khakhi</i> , Thyolo, <i>Kankhono</i> , <i>Napilira</i> , Kayera, <i>Kalima</i>	Reduced cooking time, good flavour, good seed colour [†]

Note: Preferred improved varieties are bolded and italicised

† The preferred seed colours were dark red kidney, red speckled and khakhi

2.3.6 Perceptions of bruchid incidence and severity

Farmers' views were captured as to whether bruchids attacked beans in the field as well as in storage. Results showed that the majority of farmers (63.3%) believed that infestation occurred only in storage. Some farmers were ignorant of the fact that bean weevil infestation started in the field and expressed a strongly held belief that bruchid infestation occurred only in storage. An improved bean cultivar (CAL 143), known

locally as Napilira (or Chiudzu in some places), was mentioned by some farmers as susceptible to bruchid infestation in the field but that infestation was observed only if harvesting was delayed.

It is not well established by particularly farmers, whether beans obtained from a pure stand would be more prone to attack by bruchids than those from an intercrop. This question was put to farmers in Mitundu and Mkwinda EPAs, where beans are mostly grown as an intercrop. The results showed that most households (67%) were of the opinion that cropping systems reduced the intensity of bruchid infestation in storage. On the other hand, 33% of the households perceived that it did not matter whether beans were intercropped or grown in a pure stand, the level of bruchid infestation was similar.

Questions aimed at eliciting farmers' views on whether seasonal changes influenced the incidence of bruchid infestation and level of damage revealed that 83.3% of respondents believed strongly that seasonal changes, i.e., erratic rainfall, delayed on-set of rain and high temperatures, have no influence on the level of damage by bruchids (Table 2.14).

Table 2.14: Farmers' perceptions of seasonal changes and level of damage by bruchids over time

Seasonal changes affect intensity of damage by bruchids	Frequency	Percent
Yes	5	16.7
No	25	83.3
Total	30	100
Damage by bruchids worse now than before		
Yes	21	70
No	9	30
Total	30	100

Furthermore, 70% of respondents believed that bruchid infestation has become more severe than in the past. The farmers attributed this trend to three main reasons:

i) Influx of new varieties in the farming community

Some farmers believed that the introduction of new improved bean varieties in their areas by research institutions and food relief programmes by non-governmental

organisations such Action Aid, Concern Universal and Inter Aide, has increased the number of varieties that are more susceptible to storage pests than some of their landraces.

ii) Increased area under bean production

Some farmers argued that there has been such an increase in bean production over the last 5 to 10 years that the old traditional way of storing beans in mud pots was no longer feasible. Currently, beans are harvested in bulk, processed and stored in sacks. These modern storage practices, may have contributed to bruchid adaptability and severity of attack in storage.

iii) Change in weather patterns

Some farmers believed that rainfall patterns have changed completely and that the hot periods had become abnormally long. These changes are believed to have created a more conducive environment for bruchids to develop and multiply quickly.

2.4 Discussion and conclusion

The results have shown clearly that bruchids as storage pests seriously affect smallholder farmers in Malawi. However, farmers from the three extension planning areas gave varied responses regarding the proportion of harvested beans affected by bruchids in storage. Their overall perception was that more than 50% of stored beans may be lost to bruchids if no control measures are put in place. The stated difficulties associated with storage problems, as mentioned in this study, confirm those previously reported by Scott and Maiden (1998), who conducted a similar study which focused on general constraints to bean production.

The average gross yield based on secondary data presented is $\pm 0.45 \text{ t ha}^{-1}$. Farmers' perceive that 50% of this, as opposed to the 38% reported in literature (Chirwa, 2001), may be lost in storage to bruchids in the absence of proper control measures. The net yield, after these storage losses, is therefore very low. This has serious implications for food security and potential income for Malawian farmers. This analysis of the severity of crop damage by bruchids for smallholder farmers is more serious than initially thought and calls for immediate intervention strategies. Clearly, there is a need to come up with effective control measures to reduce yield losses.

According to farmers, almost all of the improved bean varieties succumb to bruchid infestation, which may explain the huge losses caused by bean weevils. This impacts negatively on the adoption of improved bean varieties. This study has shown that farmers grow 65% of bean landraces and only 35% of the improved varieties. These findings support the hypothesis that there is low adoption of the improved bean varieties in Malawi. These results further explain why bean production figures are generally low. Farmers predominantly grow landraces, which sometimes are more resistant to insect but they have a lower yield potential than the improved varieties. Improved bean varieties, under good crop management on -station, have a yield potential of up to 2.0t ha⁻¹ compared to the 0.45t ha⁻¹ that smallholder farmers obtain from landraces (Malawi Government, 2003). In spite of the low yield, farmers grow landraces because they combine all the other preferred traits such as medium to large seed size, red to red speckled seed colour, good flavour, high storability and adaptability to the local environments.

It is apparent that screening for bruchid resistance has not been a major focus of the breeding and bean research programme in Malawi. The challenge for breeders is that potential bean lines should be screened for resistance to bruchids before release. It is possible that failure to engage farmers in breeding programmes could have resulted in breeders overlooking the importance of bruchid resistance in varieties already deployed in the smallholder sector. Acceptance and adoption of any new agricultural technology can be increased if participation becomes an ingredient of technology development (Monyo *et al.*, 2001). The PRA can be used to improve communication between farmers and breeders so that farmers' concerns and preferences are incorporated into the research objectives (Sperling *et al.*, 1993; Eyzaguirre and Iwanaga, 1996; Snapp *et al.*, 2003). In future, farmers in Malawi should be involved in testing for resistance to storage pests, especially at the resistance validation stage.

Realising the extent of the damage that bruchids inflict on stored beans, farmers have taken deliberate steps to develop and use various indigenous control methods, such as vegetable oil, ash from various plant parts, neem leaves and kernels, tobacco powder and paraffin, to minimize this damage. Farmers, however, noted that most of these indigenous control methods were not totally effective. This could be attributed to variability in quantities applied and the time of application. In addition, quantities applied were dependent on the level of observed damage and the quantity of beans stored.

Clearly, there are no recommended application rates for most of these indigenous bruchid control methods. In this study, ash from the bean plant was very effective in controlling bruchids, particularly against *A. obtectus*, even when applied after the bruchids had laid their eggs. Tete, on the other hand, was effective against *Z. subfasciatus*. As a single treatment, it reduced adult bruchid emergence. When used in combination with Neem or Mphanjobvu, it effectively killed the emerged adults of *Z. subfasciatus* (Table 2.9). This presumably could be attributed to the dosage rate used. It may be that the dosages used were slightly low as a combination of botanicals proved more effective, and compared well with super actellic dust (an inorganic pesticide), than when applied as a single treatment. However, this occurred under laboratory conditions whereas it would be more appropriate to prove this under farm conditions.

The appraisal study has shown that some of the control methods that farmers use are more effective against *Z. subfasciatus* than *A. obtectus*. It is not clear why this should be the case. However, one reason could be the oviposition behaviour of the two insects. The eggs of *Z. subfasciatus* are glued onto the seed and hence are in continuous contact with the botanicals, whereas the eggs of *A. obtectus* are scattered around the seeds (Schoonhoven and Cardona, 1982) and this could reduce contact with the botanicals. However, one would argue that *A. obtectus* larvae were even in more contact with the botanicals since the larvae move around. The results of the current study on the effectiveness of some of the indigenous bruchid control methods, such as using plant ash, agree with what was reported by Rahman and Talukder (2006) on black gram seeds. In their study, Bablah ash was very effective and significantly reduced the adult emergence of *Callosobruchus maculatus* (F.). Neem, though reportedly known to be effective against storage pests, was not effective in the appraisal study to control bruchids, when used as a single treatment. Sunita (2006) also reported that neem was only effective when used in combination with other botanicals to control the two bean bruchid species.

Some of the botanicals were effective in killing adult larvae of *Z. subfasciatus* (Table 2.9) when used in combination. For example, Mphanjobvu + Tete; Neem + Tete were more effective in combination than when used as single treatments. The standard practice is that farmers use only one control method. These results suggest that indigenous bruchid control methods would be more useful if applied timely (before egg laying) and used in combination.

Farmers' variety and trait preferences were evident in the study. Farmers preferred large-seeded bean varieties with good flavour. In Malawi, before the inception of the Bean Improvement Programme, most of the varieties released were the small-seeded (18-25g/100 seeds) (Mesoamerican) type. Most of them were not popular among smallholder farmers in spite of their high yield potential. Three new bean varieties released in 1993 were medium (26-35g/100 seeds) to large-seeded (>35g/100 seeds) (Andean) types and were therefore preferred by many farmers, and are still popular, e.g., Chimbamba (25-2x 8-7) and Kalima (PVA 692) (Masangano, 2000). Farmers still prefer local landraces over new improved bean varieties. Seed colour preferences were also observed among some farmers. Dark red and red speckled (calima type) beans were the most preferred. Consumers in Malawi's central region prefer khaki or "sugar beans" (tan with brown, black or red speckles). Those in southern Malawi tend to prefer red beans. There is evidence of a premium price for red beans in some markets because of the demand. The preference for seed colour implies that breeders cannot overlook its importance in developing new cultivars. Again, this underlines the importance of conducting a participatory rural appraisal to assist the breeder to set relevant breeding objectives to develop cultivars that are area - or region-specific.

In general, discussions with farmers and traders revealed their concerns about risk avoidance and yields of good quality beans. Bean cultivars should be well adapted over a wide range of environmental conditions. Results of this study have shown that both traders and producers were concerned about culinary quality, taste and selected traits such as seed size, colour and plant growth habits. It is important for a breeder to be aware that apart from agronomic traits such as growth habit and yield, culinary characteristics such as cooking time and flavour are also important and are used by farmers in selecting bean varieties. This further underscores the need to involve farmers in variety development.

The level of bruchid infestation, according to farmers, has changed over time. Bruchid damage is recently rated more severe than has been the case in the past, suggesting that breeder-farmer interaction should be encouraged. It may be necessary to explore the factors that may have contributed to this change in order to develop practical intervention strategies. Although the adoption rate for improved varieties is low, a few varieties have been adopted and are widely grown by farmers in the two study areas,

and elsewhere. As most improved varieties succumb to bruchid infestation in storage, their widespread production may exacerbate storage losses. Some respondents indicated that the weather pattern has changed. Rainfall is now erratic and mean temperatures are higher. High temperatures and humid conditions favour rapid multiplication of the insects.

In conclusion, the results of this study show that the two bruchid species are very important storage pests for smallholder farmers in Malawi, inflicting severe damage on stored beans in the absence of any effective control measures. The perceived 50% damage in storage and the ineffectiveness of some of the indigenous bruchid control methods call for immediate action. The high levels of damage caused by bruchids compromise the food security and nutritive quality of the beans. Apart from agronomic traits such as high yield and disease resistance, breeders must also take into consideration varietal preferences such seed colour, seed size and the flavour of bean varieties. Screening and selecting for bruchid resistance should be an essential part of the breeding strategy; farmers must be involved at all stages of the breeding programme and crop development to ensure uptake and adoption of new varieties.

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Chapter Three

Resistance of Malawian Dry Bean (*Phaseolus Vulgaris* L.) Landraces to *Acanthoscelides Obtectus* Say and *Zabrotes Subfasciatus* Boheman

Abstract

Seed damage by storage pests cause substantial losses in Malawi and most of Africa. The existing genetic diversity of dry beans has not been exploited fully in terms of the sources of resistance to bruchid strains found in Malawi. In a search for adaptable and effective sources of resistance to the two economically important bruchid species in Malawi, *Acanthoscelides obtectus* and *Zabrotes subfasciatus*, Malawian bean landraces were screened for both laboratory and field infestations. Due to limited seed numbers, the 135 genotypes examined were randomly divided into two sets. The first set comprised 42 and 56 genotypes which were tested separately for resistance to *A. obtectus*. The second set comprising of 37 genotypes, mostly improved varieties, were tested for resistance to *Z. subfasciatus*. Two SMARC bean lines, SMARC 2 and SMARC 4, containing arcelin, which confer resistance to *Z. subfasciatus*, were included as resistant checks. First generation insects from a laboratory culture of both species were used to infest beans in the laboratory at 27-30°C and 66-70% relative humidity (RH) using a no-choice test. In other tests, insects collected from the farmers' fields were used to infest genotypes under field conditions at 100% flowering, full podding and 95% physiological maturity. Field infestation was via a free-choice test whereby the insects had some room to choose the bean genotypes they preferred. At harvest, the pods were brought to the laboratory and were incubated for 120 d at 27-30°C and 66-70% RH. The emerging adult insects were counted daily and grain weight loss measured. Bean resistance was measured using the Dobie susceptibility indices, which combine the number of bruchid emergence and the development period. Results of the study showed that there was wide variation among the bean genotypes for resistance/susceptibility to both insects, thus providing an opportunity for selection. A few genotypes displayed high levels of resistance but 83% of the tested lines were highly susceptible to *Z. subfasciatus*, while 50% of the tested lines were highly susceptible to *A. obtectus*. None of the improved cultivars were resistant. However, a landrace, KK35 consistently showed high resistance to the two bruchid species. Two

other landraces, KK73 and KK90, displayed stable resistance to *A. obtectus* under both laboratory and field infestations. Performance of most genotypes was not consistent between laboratory and field tests, suggesting that resistance mechanisms could be different and that laboratory screening should always be validated by field screening. The seed coat played a significant role in conferring resistance to both bruchid species in this study.

3.1 Introduction

Dry beans are an important source of food and income to smallholder farmers. In many areas, dry beans are an integral part of small-scale cropping systems and the bean production directly benefits both the urban and rural poor. In Malawi, annual bean production figures vary, depending on the season, but during favourable seasons up to 100,000 MT can be produced (Malawi Government, 2003). Nutritionally, dry beans constitute the second most important source of dietary protein in Malawi. Globally, the dry bean is the world's most important food legume, far outweighing chickpeas, faba beans, lentils and cowpeas (CIAT, 2003). Research has shown that a single serving of dry beans contributes significantly to the minimum daily requirements of folic acid, dietary fibre and other essential inorganic dietary micronutrients such as Zn, Fe, Ca and Cu (Bressani, 1985; CIAT, 2003).

In spite of the economic and nutritional benefits that dry beans provide for many households, their yield is drastically reduced by pre- and post-harvest attacks by two coleopteran insects of the family bruchidae: the common bean weevil (*Acanthoscelides obtectus* Say) and the Mexican bean weevil (*Zabrotes subfasciatus* Boheman), which is also known as the spotted bean weevil (Cardona, 1989). Given that yields of dry beans are typically low in Africa (0.45 t ha^{-1}), subsequent losses to bruchids can be devastating for a resource-poor farmer. *Acanthoscelides obtectus* attacks beans both in the field and in storage, while *Z. subfasciatus* attacks beans only in storage (Schoonhoven and Voysest, 1991). This study, however, has demonstrated that *Z. subfasciatus* can also attack beans in the field. Damage due to bruchid infestation, in the absence of control or preventative measures, can be severe. This damage can also reduce seed viability, quality and nutritional value. The commercial value of beans is reduced when seeds are covered with eggs and frass materials such as insect fragments and excreta, and also due to seed perforations and the presence of adult insects.

Due to high variability in infestation levels from season to season, a quantitative assessment of losses due to damage by these storage pests has been difficult. However, depending on the storage period, losses up to 100% have been reported for beans and cowpeas in the smallholder sector (Lienard and Seck, 1994; Wongo, 1996). Farmers have a tendency to store some of the beans on-farm for household security. Inevitably, they want to store a part of the harvest for a long time in anticipation of favourable market prices occurring in the future, especially when a substantial crop has been produced. In the absence of proper control measures, annual storage losses of up to 38% due to bruchids have been reported in Malawi (Chirwa, 2001), which translates to 38,000 MT, a monetary loss of about US\$11.4 million. Managing post harvest losses both at subsistence and commercial levels is therefore crucial.

To date, bean entomological studies conducted in Malawi have concentrated more on field than storage pests, and previous breeding programmes have not focused on developing bruchid resistant lines. Smallholder farmers use indigenous control methods such as plant ash, sand and other botanical pesticides such as neem (both leaves and kernels) to minimise bruchid damage in storage, but it appears that these methods have been less effective. In desperation, some farmers are using dangerous insecticides such as Dichlorodiphenyltrichloethane (DDT) or Carbarly (sevin) to control bruchids in storage, despite their known undesirable ecological and health consequences (Ogbuinya, 1997; Dorn, 1998).

Breeding resistance to bruchids in bean varieties would be valuable for providing a sustainable method to reduce bean losses. In the Americas, an attempt has been made to develop bean varieties that contain arcelin, a protein that confers resistance to *Z. subfasciatus* (Cardona *et al.*, 1990; Myers *et al.*, 2000). Unfortunately, these varieties are not well adapted to tropical conditions. As they are small-seeded, they are not acceptable to most Malawian farmers, who prefer the large-seeded bean (see Chapter two) types. Wide use of improved bean varieties with a relatively high degree of resistance to bruchids would assist farmers in storing their beans for longer in order to sell them later in the season when market prices are more attractive. Long-term storage is particularly important in areas where a crop of beans is produced only once a year,

forcing farmers and consumers to store the crop until the next harvest. The utilisation of genetic resistance against bruchids is a more environmentally friendly and sustainable approach.

In order to establish a viable breeding programme for resistance to bruchids in dry beans, an adaptable and effective source of resistance needs to be identified. Martin and Adams (1987) reported rich genetic diversity within the landraces grown by farmers in Malawi. Evidence of substantial genetic variation in the large-seeded types has also been reported by Ferguson and Mkandawire (1993) and Mkandawire (1999). In these studies, genotypic variation was evident because most farmers planted up to 13 different local bean types. This genetic diversity could provide valuable germplasm for bean cultivar improvement. It is believed that farmers have selected preferred cultivars (landraces) that have not originated from any formal breeding programmes. These selected landraces have survived for many years against the vagaries of nature, including pests and diseases and have successfully grown in different agro-ecological zones. Such characteristics should make landraces appropriate and adaptable sources of resistance for exploitation in breeding. This study was conducted to search for an effective and adaptable source of resistance to *Z. subfasciatus* and *A. obtectus*, in a collection of dry bean landraces in Malawi and a range of cultivars, including those landraces or improved cultivars obtained from other institutions.

Specific objectives

The objectives of this study were to:

- (i) identify bean landraces with resistance to the two bruchid species.
- (ii) screen a range of cultivars to determine the susceptibility levels and identify those that have some levels of resistance/lower susceptibility in the field or under laboratory infestations
- (iii) establish the role of physical or chemical properties of the bean seed in bruchid resistance.

3.2 Materials and methods

3.2.1 Bean germplasm collection

The germplasm used in this study comprised landraces that were collected from local farmers in Malawi and improved cultivars provided by collaborating local and international institutions. Germplasm collection was carried out in the three administrative regions of Malawi (northern, central and southern), targeting selected bean growing areas (Fig 3.1). Main collection sites were smallholder farmers' households in villages and local roadside markets. Improved genotypes were obtained from Pannar Seed Company (South Africa), Centro Internacional de Agricultura Tropical (CIAT) in Malawi, Bunda College of Agriculture (Bean/Cowpea Research Support Programme) in Malawi, Pro-Seed in South Africa, Oregon State University in the United States of America (USA) and the Malawi National Bean Improvement Programme (Table 3.1). SMARC lines (SMARC 2 and 4) were used as resistant checks against *Z. subfasciatus* and genotype resistance was compared to the resistant checks and the trial mean. A detailed list of the collected germplasm, source of collection and their seed characteristics are presented in Appendices 1 to 3. The bean genotypes were planted at Chitedze Agricultural Research Station during the 2003/2004 season, to generate sufficient seed for laboratory testing. Some genotypes, particularly those obtained from other institutions, were not well adapted to Malawi's climatic conditions and therefore only 135 genotypes out of the total collected (142) were used in the study.

Table 3.1: Sources of germplasm used to screen for resistance to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*

Source of Germplasm	Number of genotypes	Type	Comments
Farmers	77	landraces	A mixture of seed colours and types
Pannar Seed Company	17	Improved	High yield potential
CIAT	15	Improved	Mostly small seeded
Pro-Seed Seed Company	10	Improved	High yield potential
Oregon State University	2	Improved	Contain arcelin, which confers resistance to <i>Z. subfasciatus</i>
Malawi National Programme	8	Improved	Released varieties
Bunda College	13	Improved	Advanced bean lines
Total	142		

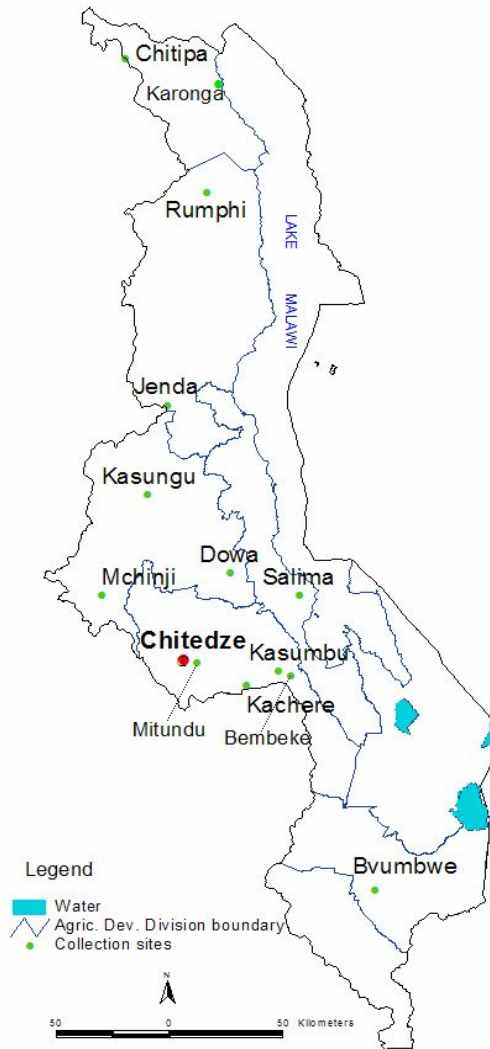


Figure 3.1: A map of Malawi, showing in green dots the specific collection sites of bean germplasm

3.2.2 Bruchid laboratory culture

Adult insects of *A. obtectus* and *Z. subfasciatus* were collected in the central region of Malawi from farmers with naturally infested beans. A laboratory culture was then established at Chitedze Research Station by allowing the collected samples of insects to lay eggs (oviposit) on two commercially grown susceptible varieties, Nagaga and Maluwa. The two bruchid species were maintained separately in the laboratory after collection. Approximately 800 insects were reared in 1kg of seed of each of the two varieties using 1L Kilner glass bottle jars. The jars were covered with muslin cloth,

which allowed adequate ventilation but prevented insects from escaping. These insects were allowed to oviposit for 10 d, after which they were discarded, and their progeny were used to form the F1 generation of the laboratory population. This population was maintained by regularly replacing the infested grain with fresh seeds. The introduction of new bruchids from the field into the laboratory culture was done every 120 d to restore variability and increase the vigour of the laboratory population.

The laboratory's controlled temperature and humidity (CTH) was maintained at 66-70% RH by an automatic Defensor 3001 humidifier. A Singer oil-filled radiator (model HUM-009, 1500w) with a thermostat was used to keep the room at 27-30°C. Depending on the environment inside the room, an automatic fan heater was used in addition to maintain the required temperature and relative humidity. A wet and dry bulb thermometer (hygrometer) and maximum and minimum thermometers were used to measure relative humidity and temperature respectively. Laboratory conditions were monitored every day to ascertain that the CTH conditions were constant throughout the experiment.

3.2.3 Bruchid resistance testing

A no-choice test was conducted in the laboratory by infesting dry bean samples in closed jars with bruchids. A free-choice test was done by infesting bean genotypes in the open field. In the no-choice test, the insects were allowed to oviposit and develop on the bean samples provided. In the field experiment, insects could feed and/or oviposit on the bean genotypes of their choice. These experiments were conducted as follows:

(a) Laboratory infestation (no-choice test)

Most genotypes did not yield sufficient seed for screening against both bruchid species, hence genotypes were tested for resistance against either *Z. subfasciatus* or *A. obtectus*, in independent experiments. However, a few genotypes, such as Maluwa and KK35, produced enough seed for testing against both bruchid species.

The first set of 42 genotypes (Appendix 1) was screened for resistance to *Z. subfasciatus* in the first experiment, and the second set with a total of 93 genotypes (56 landraces and 33 improved varieties) (Appendix 2 and 3, respectively) were tested for resistance to *A. obtectus*, in two separate experiments. The SMARC lines, SMARC 2 and SMARC 4, were tested as resistant checks against *Z. subfasciatus* only because

there was not enough seed to test them against both bruchid species. SMARC lines contain arcelin, which is known to confer resistance against *Z. subfasciatus*.

Prior to commencement of the test, seed samples were disinfested by freezing at -20°C (Horber, 1989) for at least 10 d. This was done to ensure that any eggs or adult insects from the field were killed. Seed samples were then removed from the deep freezer and were placed in the CTH room (CTH conditions as already described in 3.2.2) for conditioning for 7 d. Seed samples weighing $20 \pm \text{g}$ (approximately 20-25 seeds depending on seed size) were then placed in transparent plastic bottles (Fig 3.2). To prevent the bruchids from escaping, a muslin cloth was used to cover the mouth of each bottle and held in place by a rubber band. Transparent bottles allowed light to enter and allowed ease of observation on a daily basis. The bottles were labelled clearly indicating genotype name, replication number, the date the experiment began and dates of the daily sieving and adult emergence counts.



Figure 3.2. Bean genotypes in transparent bottles placed in the CTH room

In all three experiments, the bean samples in each bottle were infested with 10 randomly selected adult insects of 1-3 d old, but without sex determination. Bottles were laid out in a randomised complete block design with four replications on the shelves in the CTH room. Each shelf was large enough to contain a complete replicate. Bruchids were allowed to oviposit on the bean samples for 10 d, after which they were removed and discarded. The samples were further incubated in the CTH room. The adults *A. obtectus* and *Z. subfasciatus* started to emerge 28 d and 21 d after the oviposition period,

respectively. The bruchids that emerged were removed by sieving. The bruchids were counted daily until no more emerged (indicating the end of the first generation) at roughly 40 d after infestation.

(b) Field infestation (free-choice test)

A total of 88 bean genotypes were grown in the field at Bembeke experimental site in Dedza district in Malawi during the 2004/05 summer. The two resistant check lines, SMARC 2 and SMARC 4, failed to establish in the field because of poor adaptability to the climate. The experiment was established by planting two seeds per station spaced at 200 mm apart, with 2 ridges spaced at 750 mm apart and plot size was 4 rows x 4 m long with 2 border rows. The net plot comprised 2 middle rows of 3.5 m i.e., 0,25 m on each side were omitted during data collection. Ten genotypes (KK71, KK37, KK01, Nagaga, Maluwa, KK39, KK72, KK87, B91 and KK18) with varying maturity, flower colour, plant height and growth habits were mixed to make a composite variety. This composite variety was used as an infestor row and was planted in a staggered pattern around the experimental field and between test plots, at 3 d intervals, to ensure a steady supply of bruchids to attack the test genotypes (Fig 3.3). Due to limited availability of seed in this first cycle of field screening, the plots were not replicated.

Field layout

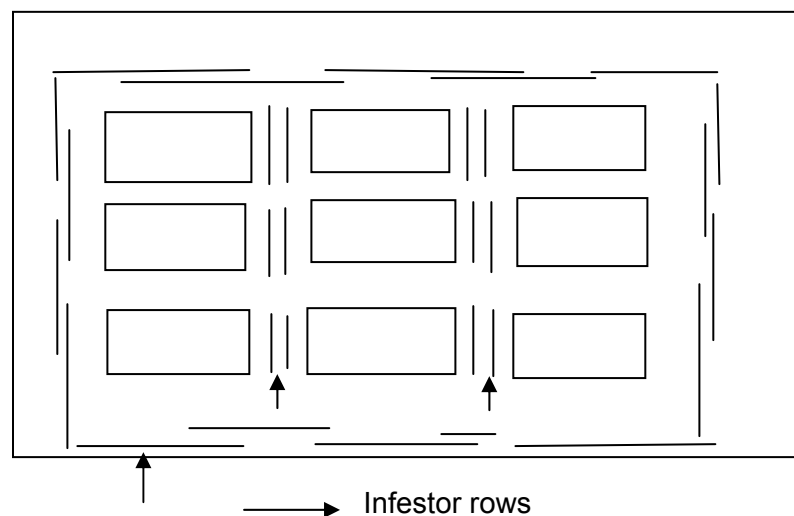


Figure 3.3: Field layout showing infestor rows between plots and around the experimental field

The two bruchid species were collected from farmers' stores together with infested beans in villages around Bembeke experimental station. Bruchids were collected a day before infestation was done. The bruchids comprised of mixed age adults. Approximately ±500 g of beans, were collected on which the insects were held temporarily. The bruchids were then carefully counted and placed into vials, ready for artificial infestation in the field.

Bruchids were physically introduced in the field on three occasions; at 100% flowering, full podding and 95% physiological maturity of the bean crop. Forty insects, consisting of 20 *Acanthoscelides obtectus* and 20 *Zabrotes subfasciatus*, were introduced in the infestor rows and at the centre of each plot each time.

For uniformity, each accession was harvested when fairly dry, 1 wk after attaining 95% physiological maturity. Harvesting all genotypes simultaneously was important to ensure that the beans were given the same length of exposure to bruchid infestation in the field. The harvested beans, in pods (unshelled), were kept in 10 kg jute twine bags and placed in the CTH room for 60 d at 27-30°C and 70-80% RH, during which time the progeny insects developed. The samples were checked daily to observe any insect emerging. The counting of emerged adult bruchids was done on a daily basis, after first emergence of bruchids, and until no more insects emerged at 120 d after incubation.

3.2.4 Bruchid resistance rating

In the field and laboratory experiments, resistance was measured by the grain weight loss incurred due to damage caused by bruchids, the number of adult bruchids that emerged, and the period required for the insects to develop within the bean seeds. Grain weight loss, which is an economic loss indicator, was calculated as follows:

$$\text{Grain weight loss (\%)} = 100 * (\text{IGW-FGW}) / \text{IGW of sample}$$

where; FGW = final grain weight

IGW = initial grain weight for the sample.

The data on the number of adult bruchid that emerged and the median development period were combined to calculate the susceptibility index (Dobie, 1974) for each genotype using the formula:

$$\text{DSI} = \text{Log}_e Y * 100 / t$$

where; Y = total number of adult bruchid emerged

t = median development period.

The median development period was calculated as the number of days from the middle of oviposition (d 5) to the first progeny emergence. If no insect emerged over the test period, the Dobie susceptibility index value was equal to zero (DSI=0). There was a slight modification of what others (Derera *et al.*, 2001b) have used previously. They defined mean developmental period as the number of days from d 5 of oviposition to 50% emergence of progeny. Genotypes with a high susceptibility index were considered susceptible and those with a low susceptibility index as resistant. This is based on the assumption that a few insect progenies would emerge out of a resistant genotype and insect progeny development would take a longer time in a resistant than in a susceptible genotype.

3.2.5 Classification of genotypes for resistance to bruchids

To facilitate comparison of materials tested in different experiments, the Dobie relative index of susceptibility (Dobie, 1977) was calculated based on the trial mean of the laboratory experiments. The trial mean was used because there was no established susceptible check. Furthermore, we assumed that breeders would seek to improve on the available resistance and would use materials that perform below the mean in each experiment.

Dobie's index of susceptibility was not calculated for the field infestation study as the median development period could not be established. Consequently, the actual number of adult bruchids that emerged was used to assess the performance of the genotypes and the relative adult bruchid emergence (RABE) was calculated for each genotype by taking the actual value of each genotype as a percentage of the overall mean using the formula:

$$\text{RABE (\%)} = \text{Number of bruchids emerged in a sample} \times 10 / \text{Trial mean.}$$

A similar method was used by Dhliwayo *et al.* (2005). In terms of the Dobie susceptibility Index, bean genotypes were grouped into four resistant/susceptible classes as follows:

Dobie susceptibility index	Resistance class
1- 5	Resistant
6-10	Intermediate/moderate resistant
11-15	Susceptible
16-21	Highly susceptible

The above classification for genotype resistance/susceptibility was done for convenience of comparison. Other researchers may classify differently depending on the range of Dobie susceptibility indices obtained.

Performance of genotype resistance under laboratory and field infestation was compared using the mean rank of the genotypes under the two test conditions. A small value for the mean indicates superior performance, whereas large values indicate inferior performance. A similar method is used to measure performances of maize genotypes in CIMMYT's regional maize trials (Vivek *et al.*, 2005).

3.2.6 Testing the resistance mechanism

(a) Confirmation of resistance

After some accessions expressed consistently high levels of bruchid resistance under laboratory infestation in 3 separate tests, it was considered necessary to carry out a separate study to validate the observed resistance. The six resistant landraces and five improved varieties used as susceptibility checks were re-subjected to *A. obtectus* in a no-choice test method, as described above (see Section 3.2.3a).

(b) Role of the seed coat in conferring resistance

The resistance of bean seeds to a bruchid attack could be due to the presence of some chemical compounds in the seed structure. The physical nature of the seed coat and its constitutive elements can deter bruchids from attacking beans in storage. An experiment was therefore set up to test the hypothesis that the seed coat played a crucial role in conferring resistance to a bruchid attack. Twenty five (25) seeds of each of the selected bean genotypes were evaluated against the two bruchid species, with and without their seed coats. The seed coat was removed manually soaking the seeds in water for 6 h. Using sharp tweezers, the shrunken seed coat was removed carefully. The seeds were then placed outside in the shade for 2 h, in the sun for 48 h and finally

in the CTH room for 4 h, to condition them. The samples were then infested with the bruchids, as described in Section 3.2.3. The experiment was laid out as a completely randomised block design with three replications.

(c) Role of arcelin

Though arcelin was initially thought to be present only in wild bean types, arcelin-like sequences have recently been reported to be present in the other *Phaseolus* species (Mirkov *et al.*, 1994). In view of this, the 11 genotypes (8 resistant and 3 susceptibles) selected for further screening (Appendix 4) were biochemically tested for the presence of arcelin. Arcelin tests were conducted at Oregon State University. A sample of 10 seeds of each genotype, coded A-K (Appendix 4), was prepared as described by Osborn *et al.* (1986). Cotyledon "flour" was collected by scraping the end of the seeds on coarse (80 grit) sandpaper, which was cut into disposable 25 mm squares. The flour was then transferred to microfuge tubes and 20 mg were weighed out and suspended in 200 μ l of 0.5 M NaCl at pH 2.4 and allowed to sit at room temperature for 30 min. The samples were centrifuged (2 min. at approximately 10,000 \times g) and 5 μ l was added to 25 μ l of sample loading buffer (Laemmli, 1970) and denatured at 94°C for 5 min. The samples were loaded immediately and electrophoresed on a 15% polyacrylamide gel (BioRad ReadyGel cat. # 161-1103), using a Laemmli (Tris/glycine/SDS) buffer system for approximately 2 h at 100 V constant. The gels were stained overnight in 10% methanol, 10% acetic acid, 0.1% coomassie blue, followed by 2-3 h of destaining in 40% methanol and 10% acetic acid. The gels were finally rinsed with distilled water and dried between cellulose sheets on a vacuum dryer.

3.3 Results

3.3.1 Resistance of bean germplasm to *Zabrotes subfasciatus* in the laboratory test

Forty-two genotypes evaluated under laboratory conditions, displayed significant variation in expression of resistance to *Z. subfasciatus*. Significant differences were observed in grain weight loss (%), number of adult bruchids emerged and Dobie susceptibility index among the genotypes (Table 3.2). The landrace KK35 had a zero DSI, and no bruchids emerged over the test period. The other landrace, KK25 (DSI=5.9), performed better than SMARC 4 (DSI=10.3). Though not significantly

different ($P >$ landrace KK35 (DSI=0) performed better than SMARC 2 (DSI=2.1) and SMARC 4 (DSI=10.3) and was highly resistant to *Z. subfasciatus*. Bruchids on SMARC 4 had a significantly longer development period (40 d) than all the other genotypes. Thirty six genotypes, representing 86% of the total tested, had DSI ranging from 15.4 to 20.9. This shows that most of the genotypes were highly susceptible to *Z. subfasciatus*. Improved varieties (B117, PAN 19, B49, B88, B122, B22, B19, B83, PAN 7, B126 and B25) and landraces (KK47, KK38, KK21, KK16, KK31, KK29, KK43, KK63, and KK68) had the highest adult bruchid emergence with high DSI values, and were classified as highly susceptible (Table 3.2).

Table 3.2: Evaluation of bean landraces for resistance to *Zabrotes subfasciatus* (data sorted by DSI)

Genotype	% weight loss	No. of bruchid adults emerged	Median development period (d)	Dobie's susceptibility index (DSI)
KK35	0.0	0	na†	0.0
SMARC 2	2.3	2	33	2.1
KK25	0.8	3	28	3.9
KK36	3.3	11	24	10.0
SMARC 4	5.8	61	40	10.3
KK50	2.2	58	25	11.9
PS 11	5.0	51	25	15.4
KK24	6.0	46	24	15.9
KK87	6.7	75	27	16.0
KK15	6.5	74	26	16.5
KK44	3.0	55	29	16.6
KK08	7.3	95	27	16.9
KK48	5.3	84	25	17.3
KK01	7.2	92	26	17.4
KK46	5.2	74	30	17.9
KK63	8.7	108	26	17.0
B117	4.0	77	24	18.1
KK30	6.3	93	25	18.1
PAN 19	7.0	99	30	18.1
KK05	8.3	94	25	18.2
ACC1611	9.5	98	25	18.3
B49	5.8	99	25	18.3
B88	7.6	81	24	18.3
KK68	7.8	100	25	18.4
B122	5.8	100	25	18.4
KK43	8.2	105	25	18.6
B22	7.8	109	25	18.8
KK19	8.3	91	26	18.8

Genotype	% weight loss	No. of bruchid adults emerged	Median development period (d)	Dobie's susceptibility index (DSI)
KK29	8.0	113	25	18.8
KK31	9.3	117	25	18.8
B19	5.8	82	28	18.9
KK16	8.0	112	25	18.9
KK21	7.7	113	25	18.9
KK32	5.5	93	29	18.9
KK79	6.8	116	25	19.0
KK34	5.5	107	24	19.4
B83	6.0	102	25	19.7
PAN 7	9.2	137	25	19.8
KK38	9.0	122	24	20.0
B126	6.3	108	24	19.9
B25	10.3	122	24	20.0
KK47	7.8	127	24	20.2
Mean	6.4	86	26	16.5
F(Sig.)	***	***	**	***
LSD (0.05)	3.6	27.57	6.61	3.4

** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

†na= Zero F1 bruchid had emerged after 40 d from infestation date

A frequency distribution of the 42 genotypes, based on the DSI, showed that 83% of the genotypes tested were highly susceptible, 5% susceptible, 5% moderately resistant and 7% were resistant to *Z. subfasciatus* (Fig. 3. 4). The resistant check SMARC 4 was in the moderate resistance class, whereas SMARC 2 was in the resistant category. Landraces KK35 and KK25 were in the resistant category, whereas KK36 displayed moderate resistance.

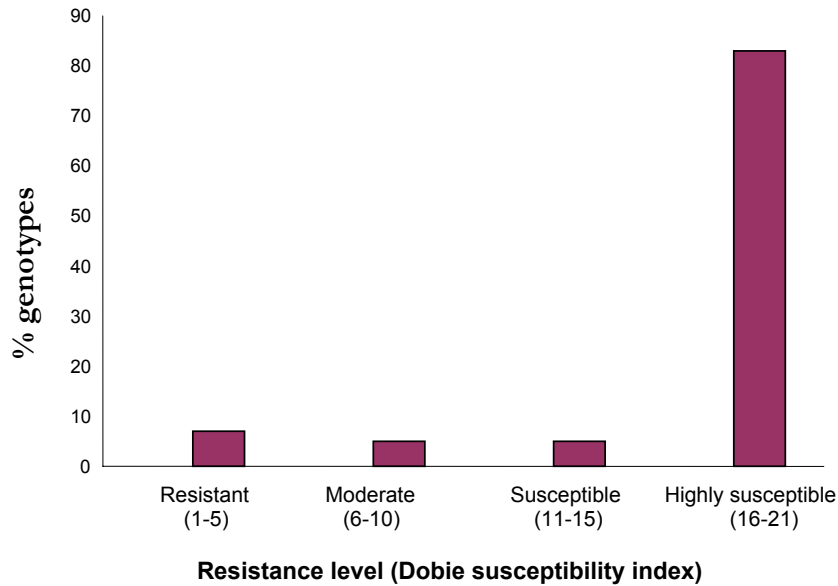


Figure 3.4: A frequency distribution of 42 bean genotypes for resistance to *Zabrotes subfasciatus* under laboratory infestation

3.3.2 Resistance of bean germplasm to *Acanthoscelides obtectus* in the laboratory test

The second set of 56 genotypes (Table 3.3) showed highly significant variation for resistance to *A. obtectus*. There were highly significant differences among the genotypes for grain weight loss, Dobie's susceptibility index (DSI), adult bruchid emergence and median development period (MDP). Genotypes KK35 (DSI = 0.0), KK93 (DSI = 0.0), KK90 (DSI = 2.5), KK03 (DSI = 1.7), KK73 (DSI = 2.4), and KK64 (DSI = 3.4) were highly resistant to *A. obtectus* (Table 3.3). Larvae of *A. obtectus* feeding on three genotypes, KK64, KK91 and KK35, exhibited a long median development period.

Table 3.3: Evaluation of bean landraces for resistance to *Acanthoscelides obtectus* (data sorted by DSI)

Genotype	% weight loss	No. of bruchid adults emerged	Median development period (d)	Dobie's susceptibility index (DSI)
KK 35	0.0	0	na†	0.0
KK 93	0.0	0	na†	0.0
KK 03	0.3	2	39	1.7
KK 73	1.5	2	28	2.4
KK 90	0.7	2	27	2.5
KK 64	0.5	6	53	3.4
KK 02	0.2	7	40	5.4
KK 36	2.5	11	37	6.5
KK 84	0.3	11	37	6.5
KK 59	1.7	15	40	6.8
KK 78	2.0	18	41	7.0
KK 58	1.5	19	39	7.5
KK 37	2.5	20	39	7.7
KK 48	3.7	24	39	8.1
KK 04	1.3	25	39	8.3
KK 80	4.5	22	37	8.4
KK 91	3.3	43	45	8.4
KK 40	3.3	30	39	8.7
KK 88	1.8	27	37	8.9
KK 95	13.3	32	40	8.9
B-94	3.8	33	39	9.0
KK 57	5.0	40	40	9.2
KK 61	5.8	34	38	9.3
KK 69	0.8	16	27	10.2
KK 13	3.2	31	33	10.0
KK 42	4.0	33	33	10.0
KK 20	5.3	33	35	10.1
KK 49	3.7	28	34	10.1
KK 71B	1.2	61	40	10.3
KK 85	5.0	37	35	10.3
KK 23	3.3	32	33	10.5
KK 89	6.3	43	35	10.7
KK 96	8.0	59	38	10.7
KK 76	7.8	45	35	10.9
KK 24	4.0	41	34	11.0
B-136	6.5	51	35	11.2
KK 75	4.8	45	34	11.2
KK 75	4.8	45	34	11.2
KK87	1.3	29	30	11.2
KK 81	5.2	49	34	11.4
KK 77	4.5	65	36	11.5
KK 06	3.3	46	33	11.6
KK 97	2.8	32	30	11.6

Genotype	% weight loss	No. of bruchid adults emerged	Median development period (d)	Dobie's susceptibility index (DSI)
KK 86	3.5	64	35	11.8
KK 74	2.3	28	28	11.9
KK 39	6.3	55	34	12.0
KK 45	5.0	64	34	12.2
KK 82	2.8	28	27	12.3
KK15	3.7	28	26	12.8
Maluwa	5.8	87	35	13.0
KK 83	11.2	98	34	13.5
KK 94	9.7	101	33	13.9
KK 72	10.3	112	39	14.0
KK 65	7.7	105	33	14.8
B-91	8.8	137	34	14.9
KK 71A	13.3	119	31	15.4
Mean	4.2	41	34.0	9.3
F(Sig.)	***	***	**	***
LSD 0.05)	4.65	29.99	12.82	4.82

** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

†na= Zero bruchids had emerged after 50 d from infestation date

A frequency distribution of the genotypes for resistance to *A. obtectus*, based on DSI, showed that 13% were resistant, 41% moderately resistant, 46% susceptible and none in the highly susceptible category (Fig 4.5). The most susceptible genotypes were KK15, Maluwa, KK83, KK94, KK72, KK65, B-91 and KK71A, which had DSI of 12.8, 13.0, 13.5, 13.9, 14.0, 14.8, 14.9 and 15.4, respectively (Table 3.3).

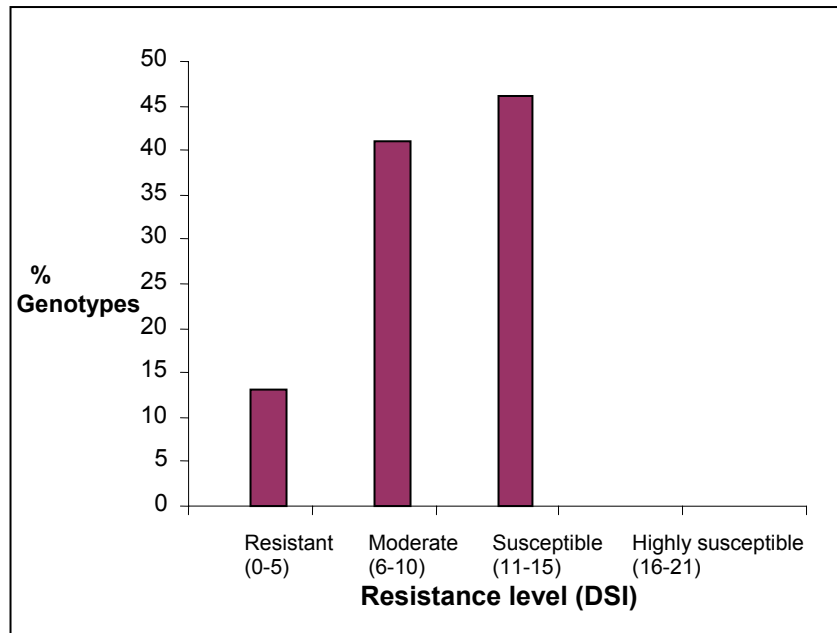


Figure 3. 5: A frequency distribution of 56 bean genotypes for resistance to *Acanthoscelides obtectus*

The relationship between grain weight loss (%) and adult bruchid emergence was highly significant ($P < 0.001$) and positively correlated ($r = 0.803$). There was also a highly significant ($P < 0.001$) and positive correlation ($r = 0.734$) between weight loss (%) and Dobie's susceptibility index (DSI). However, the correlation between median developmental period (d) and adult bruchid emergence was not significant ($P = 0.1181$).

3.3.3 Resistance of improved genotypes to *Acanthoscelides obtectus* in the laboratory test

Based on the DSI values and median developmental periods, there was no significant variation among the improved genotypes for resistance to *A. obtectus* (Table 3.4). However, there were significant differences among the genotypes for grain weight loss and number of adult bruchid emergence ($P < 0.05$). The adult bruchid emergence was positively correlated with % weight loss ($r = 0.942$). Grain weight loss, which is a measure of the extent of damage inflicted on stored grain by bruchids, was high ($\geq 15\%$) in some improved genotypes, for example, PS 25 and PN 1 (Table 3.4).

Table 3.4: Evaluation of improved bean genotypes for resistance to *Acanthoscelides obtectus* (data sorted by DSI)

Genotype	% weight loss from the bean sample	No. of adult bruchids emerged	Median development period (d)	Dobie susceptibility index (DSI)
KK35 (resistant check)	0.0	0	na†	0.0
PN 13	4.2	36	30	12.0
NBYT 11	4.2	44	31	12.2
NBYT 15	5.0	47	31	12.4
NBYT 8	4.7	48	31	12.5
PS 23	6.2	60	32	12.8
NBYT 2	5.2	54	31	12.9
NBYT 16	6.5	63	32	13.0
NBYT 5	8.2	58	31	13.1
PS 21	7.8	79	32	13.2
NBYT 10	5.2	66	31	13.5
NBYT 14	8.2	76	32	13.5
B 3	5.5	75	31	13.9
NBYT 12	8.5	87	32	14.0
PN 19	8.7	88	32	14.0
B 2	5.8	78	31	14.1
NBYT 13	6.3	69	30	14.1
NBYT 7	8.7	84	31	14.3
NBYT 4	10.5	89	31	14.5
B 179	8.0	91	31	14.6
PS 3	9.3	93	31	14.6
PS 4	9.7	94	31	14.7
PS 5	13.8	94	31	14.7
NBYT 3	10.8	110	32	14.7
PS 16	11.8	100	31	14.7
KK 22	11.3	106	31	15.0
PN 3	9.3	92	30	15.1
PS 7	13.5	130	32	15.2
B 34	10.0	115	31	15.3
PS 10	13.2	116	31	15.3
BELDAK	14.0	142	32	15.5
KK 41	12.2	123	31	15.5
Maluwa	7.6	81	30	15.6
PN 18	10.8	116	30	15.9
PS 25	15.0	144	31	16.0
KK 27	14.3	154	30	16.2
PN 1	15.2	147	30	16.6
Mean	9.1	93	32	14.7
F(sig.)	*	*	ns	ns
LSD(0.05)	6.85	29.03		

* Significant at P = 0.05

Note: na†= No bruchid had emerged after 50d from infestation

A local landrace, KK35, used as a resistant check in this experiment, was highly resistant to *A. obtectus*, and incurred no grain weight loss. All the improved genotypes were classified as susceptible to highly susceptible. In particular, PN1, PS25, PN18, NBYT3, B34, PS10, PS7 and PS16 were very susceptible. A frequency distribution of 33 improved genotypes, based on the DSI showed that 88% of the genotypes were in the susceptible category whereas 12% of the genotypes were in the highly susceptible category (Fig 3.6).

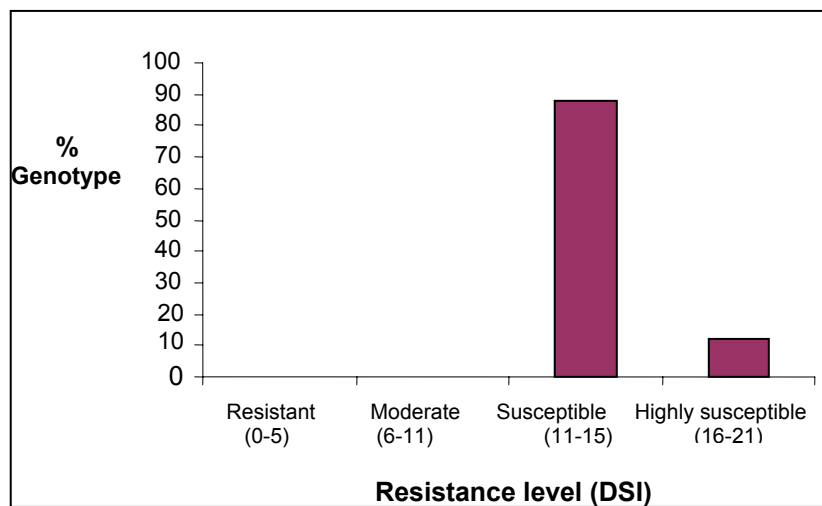


Figure 3.6: A frequency distribution of 32 improved commercial genotypes for resistance to *Acanthoscelides obtectus*

3.3.4 Confirmation of genotypes' resistance to *Acanthoscelides obtectus* under laboratory conditions

Resistance verification results for those genotypes subjected to a no-choice test (laboratory infestation) confirmed the resistance of the local landrace KK35 to *A. obtectus* attack. It was also confirmed on the basis of high DSI values that the improved varieties were susceptible (Table 3.5). Amongst the landraces, KK73 had the highest adult bruchid emergence (53) and a high percent grain weight loss (18.4%) when re-subjected to *A. obtectus*. However, it had the longest median development period (55 d) amongst all the genotypes.

Results from the biochemical analysis (banding pattern for arcelin) showed that arcelin was not found in any of the Malawian bean landraces (Appendix 5). There could be some other chemical compounds, in the seeds, which could be responsible for the observed resistance.

Table 3.5: Verification data for genotype resistance to *Acanthoscelides obtectus*

Genotype	Type	% weight loss of bean sample	No. of adult bruchids emerged	Median development period (d)	Dobie's susceptibility index (DSI)
Maluwa(CAL113)	Improved	6.7	25	34	9.5
Mkhalira (A344)	Improved	13.5	55	36	11.1
Nagaga (A197)	Improved	38.2	83	33	13.4
Napilira (CAL143)	improved	16.9	67	31	13.6
Sapatsika(DRK57)	improved	19.1	26	32	10.2
KK03	Landrace	3.6	8	33	6.3
KK25	Landrace	6.8	7	37	5.3
KK35	Landrace	0.0	0	na†	0.0
KK36	Landrace	5.8	11	34	7.0
KK73	Landrace	18.4	53	55	7.2
KK93	Landrace	2.5	2	31	2.2
Mean		11.9	30.6	32	7.8
SD		10.97	29.03	12.67	4.32
t-sign.		**	**	***	***

** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$
na† = Zero bruchid had emerged after 70d from infestation date

3.3.5. Resistance of genotypes under field infestation

Although the two bruchid species were introduced artificially at the same time in the field *A. obtectus* was more aggressive in attacking beans than *Z. subfasciatus*. The different bean genotypes displayed highly significant variations for resistance to *A. obtectus*, based on adult bruchid progeny that emerged (Table 3.6). For example, genotypes B2, KK25, KK81, KK54, PS7, PS10, KK89, KK65, KK84, KK08, KK63, PS23 and KK18 had the highest number of *A. obtectus* emergence whereas KK36, KK97, PS25, PS3, KK28, KK57, KK90, B22, KK20, KK88, KK18 and KK86 had either very few or no adult emergence of both bruchid species. However, *Z. subfasciatus* managed to attack KK73,

PS10, KK24, KK48 and KK48 during field infestation. Genotypes were significantly different in terms of phenotypic traits.

Table 3.6: Number of adult bruchid emergence in bean genotypes after field infestation with *Acanthoscelides obtectus* and *Zabrotes subfasciatus* and the morpho-physiological traits

Genotype	Plant Height (mm)	Days to first Flower	Days to 50% flower	Days to pod set	Days to physiological maturity	Adult bruchid Emergence (<i>A. obtectus</i>)		Adult Bruchid emergence (<i>Zabrotes</i>)	
						Live	Dead	Live	Dead
KK 04	140	34	37	41	73	0	8	0	0
KK 36	160	35	37	41	78	0	0	0	0
KK 83	110	35	37	41	76	5	0	0	0
KK 71	390	33	35	38	73	38	7	0	0
KK 69	370	33	35	38	72	0	0	0	0
KK 76	420	34	37	39	76	32	36	0	0
KK 96	142	35	37	42	72	22	3	0	0
KK 97	270	37	40	42	83	0	1	0	0
KK 73	250	36	39	42	84	3	2	10	6
KK 29	720	36	40	42	87	0	0	0	0
PN 13	180	36	39	42	83	8	0	0	0
KK 88	240	36	39	41	78	2	0	0	0
KK 75	250	36	40	42	75	5	2	0	0
KK 65	260	36	39	40	79	147	46	0	0
KK 74	240	36	40	41	83	83	12	0	0
KK 34	240	36	40	41	81	63	4	0	0
KK 23	210	36	40	50	88	23	0	0	0
KK 89	240	37	40	45	84	115	17	0	0
PS 10	310	37	41	43	83	133	26	3	2
KK 59	230	38	41	45	85	36	4	0	0
PS 25	230	38	41	46	85	0	0	0	0
PS 7	120	39	42	45	82	147	13	0	0
KK 37	350	38	44	45	81	2	7	0	0
KK 57	220	38	49	59	90	0	0	0	0
KK 54	270	38	42	42	81	137	14	0	0
KK 61	440	39	43	45	84	0	0	0	0
KK 03	220	39	46	47	85	6	8	0	0
KK 94	590	42	45	47	90	29	8	0	0
PS 5	450	43	48	50	91	5	3	0	0
KK 01	730	44	49	50	91	65	7	0	0
KK 39	850	42	47	49	90	10	0	0	0
KK 90	230	42	48	50	89	0	0	0	0
KK 35	240	42	46	50	90	9	20	0	0
PN 18	450	45	49	52	92	28	12	0	0
KK 81	610	36	41	43	84	165	155	0	0

Genotype	Plant Height (mm)	Days to first Flower	Days to 50% flower	Days to pod set	Days to physiological maturity	Adult bruchid Emergence (<i>A. obtectus</i>)		Adult Bruchid emergence (<i>Zabrotes</i>)	
						Live	Dead	Live	Dead
KK 72	500	36	39	40	82	9	11	0	0
KK 25	320	36	39	41	87	112	20	0	0
KK 87	680	37	41	42	83	17	2	0	0
KK 31	780	37	40	43	85	0	11	3	0
KK 24	750	37	40	43	84	9	19	20	0
KK 05	370	37	41	42	84	8	18	2	0
B 2	370	37	42	43	81	901	54	0	0
B126	610	37	41	43	90	20	0	0	0
B22	640	37	41	42	89	0	1	0	0
KK 47	550	38	43	44	82	10	25	0	0
KK22	690	39	44	50	87	158	49	0	0
B3	630	39	45	46	85	34	4	0	0
KK 46	430	39	45	46	85	23	35	11	3
KK 85	530	39	46	47	85	90	52	0	0
B19	750	39	47	50	90	17	9	0	0
B83	400	39	47	47	89	12	3	0	0
KK 40	700	40	44	46	90	27	2	0	0
KK 27	670	40	46	47	91	39	15	0	0
KK 48	640	40	46	54	89	48	17	93	3
B117	690	40	46	46	88	0	12	0	0
KK 15	560	44	49	49	83	0	18	0	0
KK 20	570	42	49	50	96	0	0	0	0
KK 13	510	43	49	50	96	17	20	0	0
PS 3	660	43	48	50	92	1	0	0	0
KK84	570	44	48	50	104	20	2	0	0
BELDAK	620	43	49	50	90	8	19	0	0
KK 42	520	42	46	50	89	12	3	0	0
PS 7	750	44	48	50	92	27	5	0	0
KK 93	490	43	47	50	90	18	11	0	0
KK 82	690	42	46	50	89	3	0	0	0
KK 08	770	44	51	53	104	0	20	0	0
KK 77	390	42	46	47	89	127	1	0	0
KK 38	580	44	49	52	92	0	38	0	0
KK 30	530	44	48	50	91	95	34	0	0
PN 3	600	44	49	49	91	13	23	0	0
KK 63	730	49	55	54	111	0	5	0	0
KK 80	470	42	46	47	85	7	0	0	0
KK 91	470	42	49	49	89	53	36	0	0
PS 23	600	45	49	51	102	43	0	0	0
B88	510	45	46	56	90	355	14	0	0
KK18	620	53	59	55	115	0	0	0	0
KK 86	880	36	38	42	83	2	3	0	0
KK 78	530	33	36	39	79	15	4	0	0
KK 68	650	35	41	42	84	314	44	0	0

Genotype	Plant Height (mm)	Days to first Flower	Days to 50% flower	Days to pod set	Days to physiological maturity	Adult bruchid Emergence (<i>A. obtectus</i>)		Adult Bruchid emergence (<i>Zabrotes</i>)	
						Live	Dead	Live	Dead
PS 16	200	36	39	44	79	6	3	0	0
B 136	220	36	40	42	81	0	10	0	0
KK 02	790	42	47	48	90	4	5	0	0
PN 1	570	43	49	50	92	0	5	0	0
KK 58	420	34	39	41	87	10	10	0	0
B91	470	36	39	44	87	2	36	0	0
PS 4	200	36	39	42	77	35	20	0	0
Mean	480	39	44	46	87	47	14	2	2
SE(±)	2.68	0.51	0.63	0.61	1.0	15.0	2.8		
SD	20.06	3.83	4.70	4.54	7.51	112.5	20.67		
Significance	***	**	**	**	***	***	***	ns	ns

** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

3.3.1 Comparison of genotypes' resistance to *Acanthoscelides obtectus* under laboratory and field infestation

Under both laboratory and field conditions, bruchid infestations were investigated to check whether bruchid infestation levels in laboratory were representative of or comparable to the field situation. Generally, results indicated that there was no significant rank correlation ($r = 0.210$) between bean genotypes for the number of bruchid progeny emergence after the field infestation (in a free-choice test for field resistance) and laboratory infestation (under a no-choice test condition), indicating that different resistance mechanisms exist to confer resistance under field and laboratory conditions. Similarly, the no-choice and free-choice test data for relative adult progeny emergence were not significantly correlated ($P = 0.336$; $r = 0.107$). However, a few genotypes (KK90, KK73, KK69, KK02 and KK03), with lower mean rankings were resistant to both in laboratory and field infestations, and showed a stable performance. These genotypes were consistently ranked in the top 10 for laboratory and field infestation. Similarly, some of the worst genotypes such as KK68, KK65, KK22, PS10 and PS7, were highly susceptible both under field and laboratory infestation. Genotypes B2 and B88 were more susceptible to field than laboratory infestation. Generally, genotype ranking for resistance showed that most genotypes were ranked differently under a free-choice test in the field (with the exception of those previously rated superior under no-choice laboratory trials). This clearly shows that the expression of bruchid

resistance mechanism in the field is different to that displayed in the laboratory. For example, KK35 and KK25 were ranked 1 and 4 under no-choice laboratory infestation, whereas they were ranked 23 and 43 for resistance, respectively to free-choice field infestation. There was effectively a zero progeny emergence from KK35 seeds under laboratory infestation, but there were some 29 emergent progenies when subjected to field infestation. Similarly, some genotypes that developed zero or very few progeny under field conditions, had a high progeny emergence under laboratory infestation, e.g., PS25 (0 in field vs. 144 in laboratory), KK29 (0 in field vs. 113 in laboratory), KK57 (0 in field vs. 40 in laboratory), KK61 (0 in field vs. 34 in laboratory), KK97 (1 in field vs. 32 in laboratory), PS3 (1 in field vs. 93 in laboratory) and B22 (1 in field vs. 109 in laboratory).

Table 3.7: Comparison of genotype resistance to *Acanthoscelides obtectus* under laboratory and field infestation (data sorted by average rank)

Genotype	No. of adult bruchid emergence (actual no.)		Relative adult bruchid emergence (RABE)		Genotype rank for resistance	
	(%)					
	Field	Lab	Field	Lab	Field	Lab
KK 90	0	2	0	3	1	3
KK 73	5	2	8	3	5	3
KK 69	0	16	0	25	1	8
KK 02	9	7	15	11	8	5
KK 03	14	2	23	3	12	3
KK 88	2	27	3	42	3	14
KK 80	7	22	11	34	6	12
KK 37	9	20	15	31	8	11
KK 82	3	28	5	43	4	15
KK 04	8	25	13	38	7	13
KK 97	1	32	2	49	2	18
KK 20	0	33	0	51	1	19
KK 61	0	34	0	52	1	20
KK84	22	11	36	17	17	6
KK 57	0	40	0	62	1	22
KK 35	29	0	48	0	23	1
KK 78	19	18	31	28	15	9
KK 93	29	1	48	2	23	2
KK 58	20	19	33	29	16	10
KK 75	7	45	11	69	6	25
KK 59	40	15	66	23	29	7
KK 23	23	32	38	49	18	18

Genotype	No. of adult bruchid emergence (actual no.)		Relative adult bruchid emergence (RABE)		Genotype rank for resistance	
	(%)					
	Field	Lab	Field	Lab	Field	Lab
B 136	10	51	16	78	9	27
KK 39	10	55	16	85	9	28
KK 36	10	11	9	17	21	16
KK 86	5	64	8	98	5	32
KK 42	25	33	41	51	19	19
KK 40	29	30	48	46	23	16
KK 13	37	31	61	48	27	17
PS 3	1	93	2	143	2	42
KK 24	28	41	46	63	22	23
KK 25	132	3	216	2	43	4
B117	12	77	20	118	11	36
KK 96	25	59	41	91	19	29
KK 15	18	74	30	114	14	34
KK 87	19	75	31	115	15	35
PS 5	8	94	13	145	7	43
KK 83	5	98	8	158	5	45
B83	15	92	25	142	13	41
PS 16	9	100	15	78	8	46
B22	1	109	2	168	2	52
KK 74	95	28	156	43	40	15
KK 29	0	113	0	174	1	54
KK 63	5	108	8	166	5	51
B19	26	82	43	126	20	39
PS 23	43	60	70	92	30	30
KK 08	20	95	33	146	16	44
KK 76	68	45	111	69	37	25
KK 71	45	61	73	94	31	31
KK 91	89	43	146	66	39	24
B3	38	75	62	115	28	35
KK 05	26	94	43	145	20	43
PS 25	0	144	0	221	1	62
KK 85	142	37	233	57	44	21
KK 31	11	117	18	180	10	56
KK 89	132	43	216	66	43	24
PN 3	36	92	59	142	26	41
B126	20	108	33	166	16	51
KK 46	58	74	95	114	34	34

Genotype	No. of adult bruchid emergence (actual no.)		Relative adult bruchid emergence (RABE)		Genotype rank for resistance	
	(%)					
	Field	Lab	Field	Lab	Field	Lab
PN 1	5	147	8	226	5	63
KK 72	20	112	33	172	16	53
KK 77	128	65	210	100	41	33
KK 94	37	101	61	155	27	47
KK 48	65	84	106	129	35	40
KK 81	320	49	525	76	50	26
PS 4	55	94	90	145	33	43
KK 01	72	92	118	142	38	41
BELDAK	27	142	44	218	21	61
KK 47	35	127	57	195	25	58
PS 7	32	130	52	200	24	59
KK 30	129	93	211	143	42	42
PN18	40	116	66	178	29	55
KK 38	38	122	62	187	28	57
KK 34	67	107	110	165	36	50
B91	38	137	62	210	28	60
B 2	955	78	1566	120	53	37
B88	369	81	605	125	52	38
KK 65	193	105	316	162	48	48
KK 27	54	154	89	237	32	64
KK 68	358	100	587	153	51	46
KK22	207	106	339	163	49	49
PS 10	159	116	260	178	46	55
PS 7	160	130	262	200	47	59

3.3.2 Relationship between phenotypic traits in the field and adult bruchid emergence in the laboratory

Phenotypic traits such as days to flower initiation, duration of flowering, plant height and physiological maturity did not significantly influence a bruchid attack on host plants in the field (Table 3.8). Although the correlation between days to physiological maturity (DPM) and adult bruchid emergence (ABE) was not statistically significant ($r = -0.110$; $P = 0.3119$), early maturing varieties might be preferred by bruchids than late maturing varieties due to having reached the moisture content levels suitable for storage pests

and also exposed longer in the field. Timely harvesting of beans, therefore, becomes an important cultural practice to prevent or reduce early bruchid population build up.

Table 3.8: Relationship between phenotypic traits and adult bruchid emergence of *Acanthoscelides obtectus* in 88 bean genotypes under field infestation

Trait	Correlation co-efficient (r)	P-value
Days to first flower	-0.080	0.3119
Days to 50% flower	-0.070	0.5202
Plant height (cm)	0.474	0.7412
Days to physiological maturity	-0.110	0.3119

Although seed size and number of adult bruchid emergence for both *A. obtectus* and *Z. subfasciatus* were significant ($P = 0.007$), the correlation ($r = 0.0163$) between these traits was too weak to suggest any significant role of seed size in influencing resistance of genotypes. The number of *Z. subfasciatus* adult bruchid emergence and hence susceptibility of selected bean genotypes was not influenced to any extent by seed size (Fig 3.7). Small, medium and large-seeded genotypes had 78, 90 and 89 F1 progeny emergence, respectively and had almost similar DSI values of 16.4, 16.6 and 18.0 for small, medium and large-seeded genotypes, respectively.

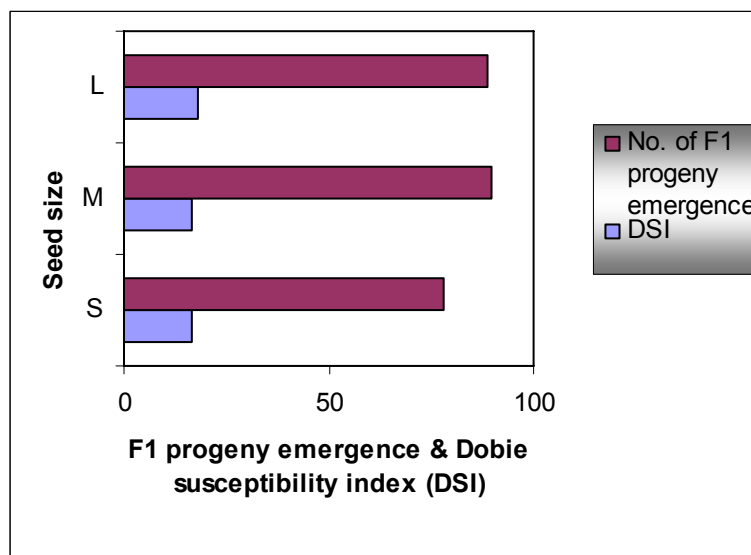


Figure 3.7: The influence of seed size (S = small, M = medium and L = large) on the expression of resistance of selected bean genotypes to *Zabrotes subfasciatus* (Large (>37g/100 seeds); Medium (26-36g/100 seeds); Small (<25g/100seeds))

3.3.3 Role of seed coat in influencing resistance of bean genotypes to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*

The seed coat was shown to play a significant role in the expression of resistance by bean genotypes to attack by the two bruchid species (Table 3.9). There was a drastic reduction in the number of *Z. subfasciatus* adult bruchid emergence when the seed coat was removed from the seeds. Conversely, there was an increased number of *A. obtectus* adult bruchid emergence when the seed coat was removed.

Evidence of the resistance of the arcelin-containing SMARC lines to *Z. subfasciatus* was clearly confirmed by these results. While *A. obtectus* managed to attack the SMARC lines, *Z. subfasciatus* did not. Clearly, dead adult *A. obtectus* were recorded on Smarc 2 lines after the test period whilst no adult *Z. subfasciatus* emerged from the Smarc lines (Table 3.9). Possibly, *Z. subfasciatus* laid the eggs on Smarc lines which might have failed to develop due to antibiosis effect conferred by arcelin. Future studies should consider the number of eggs laid on the seeds in addition to adult progeny emergence count.

Evidence of the seed coat as a possible physical and/or chemical barrier to seed attack by bruchids was observed. For example, the genotype KK35, which was consistently resistant to laboratory infestation, was found to be susceptible to *A. obtectus* after removal of its seed coat. Similarly, the resistance levels of the resistant checks, SMARC 2 and SMARC 4, were significantly reduced after removing the seed coat: progeny emergence increased by 500% and 800% respectively. On average there was more than 300% increase in *A. obtectus* progeny emergence when the seed coat was removed (Table 3.9). The testa provides first physical line of defence from attack by the bruchids. It is also possible that some chemicals within the testa might also confer the resistance. Biologically, *Z. subfasciatus* glues its eggs onto the seed coat and consequently, leaving the seed coat intact made oviposition possible. On the other hand, removal of the seed coat negatively affected the insect's oviposition behaviour presumably because there could have been reduced oviposition or the eggs laid were not securely attached which made hatching larvae have difficulties to penetrate the seed. The soaking of seed during the removal of the testa might possibly affect the

resistance through leaching of some chemicals which might play a role in conditioning resistance. Probably, when the seeds are soaked for a long time the germination process might be triggered resulting in the mobilization and metabolism of proteins such as arcelin which are important in conditioning seed resistance to bruchids. Therefore, it is recommended that the method should be appraised.

Observed resistance is probably not only associated with the seed coat but also with the cotyledon as evidenced in the parent lines. It is clear from the data in Table 3.9 that even after removal of seed coat KK35 and KK73 were more resistant to *Z. subfasciatus* whereas the removal of the seed coat, did not have any effect on the susceptible parents Maluwa and Nagaga, indicating that cotyledons also contained some resistance factors. Even SMARC 2 and SMARC 4 were more resistant than the susceptible checks when the seed coat was removed, supporting that the cotyledons also contained some resistance factors which affected the bruchids. In fact there was a slight change in number of *A. obtectus* adult larvae that emerged in KK73 after removal of the seed coat 11 versus 13 (Table 3.9).

Table 3.9: Effect of the seed coat on adult bruchid emergence in 12 dry bean genotypes

Genotype	<i>A. obtectus</i>			<i>Z. subfasciatus</i>		
	Change [§] (%)	Without seed coat (No.)	With seed coat (No.)	Change [§] (%)	Without seed coat (No.)	With seed coat (No.)
Nagaga	270	37	10	-94	1	18
Maluwa	671	54	7	-60	4	10
Napilira	59	32	13	-50	3	6
SMARC 2	800	9	1	0	0	0
SMARC 4	500	12	2	0	1	0
KK64A	570	67	10	-100	0	9
KK64B	500	48	8	-83	2	12
KK35	Infinite high [†]	18	0	-100	0	4
KK73	18	13	11	-96	1	28
KK25	233	20	6	-100	0	2
KK03	500	24	4	300	4	1
KK93	288	35	9	-100	0	8
Mean	342	31	7	-40	1	9
SD		18.28	4.20		1.56	8.27
t-sign		***	***		**	**

** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

[§] Change (%) = $[100(A - B)/A]$, where A = number of insects emerged from seeds with seed coat and B in seeds without seed coat.

[†] Infinite high means that number not divisible by zero

3.4 Discussion

3.4.1 Expression of resistance in landraces to the two bruchid species

Of 42 dry bean genotypes screened under laboratory conditions for resistance to the Mexican bean weevil (*Z. subfasciatus*), only two landraces (KK35 and KK25) were found to be resistant based on Dobie's susceptibility index (DSI) as a measure of resistance. According to Dobie (1974), the susceptibility index is linearly correlated with the intrinsic rate of increase and the logarithm of the number of insects that emerge over a given time period hence it provides a reliable estimate of resistance levels. KK35 had a zero DSI value indicating that no bruchids had emerged over the test period. It was highly resistant and performed better than the two SMARC lines (SMARC 2 and SMARC 4), which contain arcelin. Arcelin is a lectin-like seed storage protein present in the wild bean accessions known to confer high levels of resistance to the Mexican bean weevil (Osborn *et al.*, 1988). Although arcelin-like seed proteins were known only to be present in wild bean types, further studies (Mirkov *et al.*, 1994) demonstrated that arcelin could also be present in the other *Phaseolus* species. The relatively high levels of resistance to the Mexican bean weevil displayed by some of the Malawian bean landraces cannot be due to arcelin because a biochemical analysis of the bean genotypes showed no evidence of its presence. However, the seed coat played a significant role in the expression of resistance although it was not established in this study whether the laid eggs were normally attached and that hatching was successful. Evidence of the seed coat as a possible physical or chemical barrier to seed attack as a mechanism of resistance against the *C. maculatus* has been demonstrated (Kemal and Smith, 2001). However, similar studies in Mexican bean weevil are scarcely reported in literature. The consistently resistant landrace (KK35) in the screening tests (zero DSI) succumbed attack when the seed coat was removed.

Although the seed coat was important in conferring resistance, factors within the cotyledon may also play an important role. It is clear from the data in Table 3.9 that even after removal of the seed coat KK35 and KK73 were still resistant to *Z. subfasciatus* than the susceptible parents Maluwa and Nagaga, indicating that cotyledons also contained some resistance factors. The results indicated that even after

the removal of the seed coat, there was a negligible number of *Z. subfasciatus* that emerged from the seeds of KK73 confirming that the cotyledons also contained some important resistance factors. Even SMARC 2 and SMARC 4 were more resistant than the susceptible checks when the seed coat was removed, supporting the view that cotyledons also contained some resistance factors which affected *Z. subfasciatus*. Thus models used to evaluate the resistance should include both the seed coat and the cotyledons. There is no evidence in the literature that has supported the importance of embryo in conferring weevil resistance even in cereals (Widstrom and McMillan, 1992).

Except for three genotypes (SMARC 2 and the two resistant landraces), all the other genotypes had DSI values that ranged from 10.0 to 20.2 indicating that most of the genotypes were very susceptible to Mexican bean weevil. A frequency distribution of the 42 bean genotypes for resistance under no-choice laboratory conditions showed a skewed pattern towards susceptibility. Very few genotypes (approximately 12%) were rated resistant to moderately resistant whereas 88% were rated susceptible to highly susceptible. In a study to establish the comparative value of four arcelin variants in breeding for resistance to the Mexican bean weevil, Cardona *et al.* (1990) found that 94% of the genotypes screened were susceptible and only 6% were resistant using a DSI range of 9 to 11 as susceptible.

The 56 genotypes (Table 3.3) that were tested for resistance to the bean weevil (*A. obtectus*) showed a slightly different pattern. The level of damage, based on numbers of adult bruchid emergence and Dobie susceptibility index, was lower on genotypes that were subjected to *Z. subfasciatus*. However, adult bruchid emergence was significantly higher in storage after field infestation than that recorded for *Z. subfasciatus*. Clearly, showing that *A. obtectus* multiplies fast in storage and can be very destructive in the absence of any protective measure. *Acanthoscelides obtectus* infests beans in pods in the field as such the starting population in storage is much higher than with *Z. subfasciatus*. Schmale *et al.* (2002) reported that *A. obtectus* caused an average storage loss of 14% on dry beans over a storage period of 16 weeks and that two generations of bruchids had emerged. Porca (2003) indicated that in the absence of any control strategy, *A. obtectus* is capable of causing up to 100% damage on stored seeds. *Zabrotes subfasciatus* also multiplies very rapidly in storage, however in this case the populations taken into storage were low due to its lower efficiency of attacking the pods

in the field. Except for a few cases *Z. subfasciatus* were low for all the genotypes and does not appear to be due to inherent factors in the bean seeds.

The landrace, KK35, which was resistant to the Mexican bean weevil (*Z. subfasciatus*), was also resistant to a bean weevil (*A. obtectus*) infestation. This is a significant finding as a genotype resistant to both species has not previously been recorded. This would be used in breeding for resistance in high yielding cultivars, locally adapted that are susceptible to both bruchid species. Although all the test genotypes in the current study were not screened against both bruchid species, a study by Hartweck *et al*, (1997) suggested a positive relationship between susceptibility indices of bean germplasm to *A. obtectus* and *Z. subfasciatus*.

Thirty three improved dry bean cultivars (Table 3.4), that is, cultivars developed in a formal breeding programme or officially released for commercial production, were found to be susceptible or highly susceptible to *A. obtectus*. All of the genotypes had DSI values ranging from 12.0 to 16.6, indicating that they all fell in the susceptible to highly susceptible categories and that none were rated in the resistant to moderately resistant classes. This pattern of distribution seems to suggest that breeders have indirectly or unwittingly been selecting for susceptibility to storage pests by targeting certain traits such as high yield, reduced maturity period or seed size. These results confirm farmers' perceptions (see Chapter two) that most of the newly-introduced bean varieties succumb severely to bruchid attack. Results also confirmed the high levels of losses (38%) reported (Chirwa, 2001) and 50% loss perceived by farmers. Perhaps breeders preferred to improve disease resistance, which is relatively easy compared to insect resistance, which is difficult. A concurrent, integrated approach to screening for desirable agronomic traits as well as for bruchid resistance in early stages of the bean breeding programme is vitally important.

3.4.2 Bruchid resistance mechanisms: role of arcelin and the seed coat

The SMARC lines that were used as the resistant checks showed different resistance levels to *Z. subfasciatus* attack as SMARC 2 had a lower susceptibility index and was classified as more resistant than SMARC 4, which falls into the moderately resistant category. This suggests some differences in the expression of insect resistance by

arcelin. These findings agree with an earlier report by Cardona *et al.* (1990), who showed variation in the effectiveness of four arcelin variants in dry beans to the Mexican bean weevil. Hartweck *et al.* (1997) reported that SMARC 2 lines were more resistant to both bruchid species than SMARC 4 lines which were the least resistant of all the SMARC lines tested. Goossens *et al.* (2000) also reported no insecticidal activity by arcelin 5. The resistance of the SMARC lines, which in this study were used as checks for resistance to *Z. subfasciatus*, compared well with the local Malawi landrace, KK35, which was found not to contain arcelin. The genotypes that were tested for presence of arcelin showed that arcelin was not present in the resistant materials, suggesting that factors other than arcelin might contribute to the observed resistance. Further studies are required to investigate factors that confer resistance in KK35.

A decortication study confirmed that the seed coat played a significant role in conferring bean resistance to bruchids, particularly *A. obtectus*, as removal of the seed coat made resistant genotype such as KK35 susceptible. However, these results were not in agreement with what has been reported for cowpeas (*Vigna unguiculata* L.). Eddie and Amatobi (2003) reported that the seed coat did not affect resistance to *Callosobruchus maculatus* F. in the cowpea varieties they tested. Cowpea seeds with intact seed coats were preferred to decorticated seeds for oviposition by *C. maculatus* and therefore they concluded that the seed coat may not be a useful aspect to consider when breeding for bruchid resistance in the cowpea. It can be argued, however, that biological differences that exist between *C. maculatus* and the two bruchid species could have brought about these discrepancies. However, using a different bruchid species, Kemal and Smith (2001) found that seeds without seed coats reduced the development and adult bruchid emergence of *Callosobruchus chinensis* (F.). Sinha *et al.* (1988) assessed the vulnerability of common wheat cultivars to nine major stored-product beetles. They found that, in general, whole seeds were less susceptible to insects than crushed seeds, with kernel hardness accounting for the variability observed between cultivars for intact seed susceptibility.

Z. subfasciatus depends on the good surface area provided by the seed coat for successful oviposition, where eggs are glued onto the seed (Schoonhoven and Voyses, 1991), hence it may not easily lay eggs when the seed coat is removed. This explains the decline in the number of progeny insects that emerged when the seed coat was

removed. The adult female determines the quality and quantity of food for its immatures (Dendy and Credland, 1991), because larvae are restricted to the place where the female had laid the eggs. On the other hand, *A. obtectus*, which naturally scatters its eggs around the seed and the larvae are not restricted, had no problem in penetrating into the decorticated seeds.

Since the observed bruchid resistance was not associated with arcelin content in the cotyledons, the physical properties of the seed coat or other chemical factors could be implicated for the resistance reported in this study. Silva *et al.* (2004) reported the presence of vicillins (7S globulins) isolated from both the embryo and seed coat of *P. vulgaris* to be detrimental to *C. maculatus*. They concluded that the seed coat is an important barrier to infestation of *P. vulgaris* by storage pests. Sales *et al.* (2000) observed that legumins and vicillins are present in the seed coat of the broad bean (*Vicia faba*) and further demonstrated that the dry seed coat of the common bean contains sufficiently high levels of vicillins to deter development of the first instar larvae of *C. maculatus*. It is not clear whether similar mechanism is effective against the Mexican bean weevil in common beans because nothing has been reported in literature. Therefore, it is recommended that future study should validate the significance of the testa in conferring resistance to *A. obtectus*.

3.4.3 The influence of morpho-physiological traits on bruchid resistance

There was no evidence of the role of phenotypic traits in influencing resistance to bruchids in the bean germplasm, as has previously been suggested in other studies. Porca *et al.* (2003) reported that red-seeded bean cultivars were more susceptible to *A. obtectus* than white seeded bean cultivars. Although there was a range of seed colours: red kidney, cranberry, khakhi, white, calima and black, seed colour was not directly linked to the observed resistance to or susceptibility of the accessions in this study. Ofuya and Credland (1996) stated that although the colours are important for host selection to many insects such as pollinators, they found that colours were not that important for bruchids, in this case *Z. subfasciatus*. The size of the seed has been considered an important factor for the choice of hosts by the Bruchidae (Janzen, 1969; Simmonds *et al.*, 1989) and seed size has been reported to correlate with the susceptibility of bean genotypes: varieties that are large-seed being more susceptible than small-seeded bean varieties (Schoonhoven and Voysest, 1991). The argument is

that small seed size presents a barrier as mortality, size and fecundity of bruchid progeny are strongly affected by overcrowding within seeds (Schoonhoven *et al.*, 1983; Cipollini and Stiles, 1990). However, in this study seed size did not influence susceptibility of bean genotypes. Teixeira and Zucoloto (2003) in a study to determine whether there were differences in host suitability for *Z. subfasciatus*, reported that high number of eggs were laid on all *P. vulgaris* varieties despite their variations in seed size and colour. In the current study, resistance was not influenced by any of the morpho-physiological traits that were measured. Furthermore, there was no relationship between flower or pod colour, plant height and growth habit to bruchid resistance of the genotypes. This was not consistent with earlier observations by Biemont and Bonet (1981), who reported that *A. obtectus* females had a preference for yellow pods. However, none of the genotypes evaluated in this study had yellow pods; the majority had green and red speckled pods.

Days to physiological maturity is an important parameter in determining field resistance to bruchids. It is expected that infestations could be higher in early maturing varieties which attract the bruchids when they attain lower moisture content earlier than the late maturing varieties. Bruchids are attracted to seeds of lower water content (less than 35%). As a result the seeds would carry a higher population of bruchids to the store. However, the correlation between adult insect emergence and the number of days to physiological maturity was not significant in the current study.

3.4.4 Field resistance of bean genotypes

Although the two bruchid species were introduced artificially in the field during different plant growth stages, *A. obtectus* was more aggressive in attacking beans in storage. This confirms earlier findings that *A. obtectus* is a field as well as in-store pest and that the level of fecundity is very high with *A. obtectus* (CIAT, 1986). This may explain why *A. obtectus* is rated the most damaging storage pest compared to *Z. subfasciatus*. In the field bruchids attack pods and in storage they attack shelled beans. In the laboratory bean seeds were tested while in the field pods were attacked. The pod wall and the seed coat may have two different lines of defence, possibly with different resistance mechanisms. Two mechanisms of bruchid resistance are evident when observing the expression of genotype resistance after subjection to bruchid infestation under no-choice (laboratory) and free-choice (field) conditions. The importance of non-

preference/preference and antibiosis mechanisms of resistance explains why *A. obtectus* feeding levels on most genotypes were not consistent between laboratory and field infestation. Some genotypes were resistant to laboratory infestation but became susceptible under field conditions, or vice versa. Although KK35, KK25 and KK03 were attacked by *A. obtectus* when infested in the field, the physical characteristics/properties of the seeds restricted further multiplication of this bruchid in storage. Unlike PS3, KK57, B22, KK29, PS25 and PN1, which had no or low adult bruchid emergence after field infestation (restricted attack in the field), but bruchids eventually managed to multiply in storage causing severe damage.

Different environmental conditions during plant growth and/or seed development can influence levels of resistance significantly. Intermediately resistant lines could become fully susceptible, depending on the origin of the seed (Goossens *et al.*, 2000). This means that when screening bean genotypes for bruchid resistance, both laboratory and field infestation methods should be used to validate results before deriving any meaningful conclusions and committing to breeding the selected lines. Attack of pods in the field depends on many factors (pod wall characteristics, thickness, hairiness, pod splitting) and results of this study have shown/confirm that *A. obtectus* is better adapted for this than *Z. subfasciatus*. Whilst the field study was interesting and provided breeding opportunities, it is important that validation of resistance should be performed in farmers' stores where the insect pressure and conditions are different compared to laboratory glass jar experiments. Obviously the amount of seed would determine the scale of the experiment. Small samples placed in stores amongst the farmers' seeds would suffice providing the intensive insect pressure experienced under practical conditions. Clearly, the genotypes KK90, KK73, KK69 and KK02, which showed stable resistance both in field and in the laboratory including KK35, KK03 and KK25 with restrictive characteristics for bruchid multiplication in storage, would be recommended for use in breeding for bruchid resistance.

3.5 Conclusion

In general, all improved bean varieties and most of the landraces that farmers grow in Malawi are susceptible to *A. obtectus*. An effective source of bruchid resistance to the two species has been identified in a Malawian landrace (KK35). Another local landrace, KK90, has shown to have good bruchid resistance to *A. obtectus* to both laboratory and

field infestations. Reduced bruchid emergence and extended larval development periods suggest that antibiosis or antifeedant activity may be the actual resistance mechanisms, and that the seed coat also plays a significant role for the resistance reported in this study. In screening bean genotypes for bruchid resistance, both laboratory and field infestation methods should be utilised in order to identify effective resistant sources for use in breeding for bruchid resistance. Using the identified resistant genotypes, breeders can use recurrent selection or backcross breeding to improve bruchid resistance in the commercial genotypes.

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APPENDICES

Appendix 1: 42 dry bean genotypes tested in experiment 1 for resistance to *Z. subfasciatus* and some of their characteristics

Genotype	Characteristics			
	Seed size	Cultivar status	Seed colour	Collection source
KK30	Medium	Landrace	Red kidney	Dowa- Malawi
KK29	Medium	Landrace	Red kidney	Dowa- Malawi
KK16	Medium	Landrace	Sugar	Dedza-Malawi
KK34	Medium	Landrace	Red kidney	Dowa- Malawi
B88	Medium	Improved	Red kidney	CIAT [†]
B122	Small	Improved	Red kidney	CIAT
B83	Small	Improved	Red kidney	CIAT
KK19	Large	Landrace	Red kidney	Dedza-Malawi
KK63	Medium	Landrace	White kidney	Kasungu-Malawi
KK31	Medium	Landrace	Khakhi	Dowa- Malawi
B19	Small	Improved	Red	CIAT
KK15	Large	Landrace	Khakhi	Dedza-Malawi
KK87	Medium	Landrace	Mwezimoja	Rumphu-Malawi
KK46	Large	Landrace	White kidney	Mchinji-Malawi
KK50	Medium	Landrace	Khakhi	Mchinji-Malawi
KK36	Medium	Landrace	Cream yellow	Ntchisi-Malawi
KK48	Small	Landrace	Black	Mchinji-Malawi
KK21	Medium	Landrace	Calima (speckled)	Dedza-Malawi
KK35	Medium	Landrace	Calima (speckled)	Ntchisi-Malawi
KK32	Medium	Landrace	Calima	Dowa- Malawi
KK43	Medium	Landrace	White kidney	Ntchisi-Malawi
ACC 1611	Medium	Landrace	Black	MW [§] gene bank
B117	Small	Improved	Red	CIAT
PS 11	Small	Improved	White	RSA [†] (Pro-Seed)
SMARC 4	Small	Improved	White	USA*
PN 19	Small	Improved	Sugar	RSA (PANNAR)
B49	Medium	Improved	Red	CIAT
B126	Small	Improved	Red kidney	CIAT
PN 7	Medium	Improved	Sugar	RSA (PANNAR)
B22	Small	Improved	White	CIAT
KK79	Large	Landrace	Green	Mzimba-Malawi
KK24	Small	Landrace	Red	Dedza-Malawi
KK68	Medium	Landrace	Sugar	Lilongwe-Malawi
KK25	Medium	Landrace	Red kidney	Dedza-Malawi
KK08	Medium	Landrace	Khakhi	Dedza-Malawi
KK05	Medium	Landrace	Mixture	Dedza-Malawi
KK38	Medium	Landrace	Pale red	Ntchisi-Malawi
KK01	Medium	Landrace	Sugar	Dedza-Malawi
KK47	Medium	Landrace	Calima	Mchinji-Malawi
SMARC 2	Small	Improved	White	USA
KK44	Small	Landrace	White	Ntchisi-Malawi
B25	Small	Improved	Dark red	CIAT

Note: Large (>37g/100 seeds); Medium (26-36g/100 seeds); Small (<25g/100seeds)

[†] CIAT=Centro internacional de agricultura tropical

[§] MW= Malawi gene bank

[†]RSA= Republic of south Africa

* USA= United States of America

Appendix 2: 56 dry bean genotypes tested in experiment 2 for resistance to *A. obtectus* and some of their characteristics

Genotype	Seed size	Seed colour	Cultivar status	Collection source
KK02	Large	Khakhi	Landrace	Dedza-Malawi
KK03	Large	Calima	Landrace	Dedza-Malawi
KK04	Large	White kidney	Landrace	Dedza-Malawi
KK06	Large	Red kidney	Landrace	Dedza-Malawi
KK13	Large	Red kidney	Landrace	Dedza-Malawi
KK20	Large	Sugar	Landrace	Dedza-Malawi
KK23	Large	Calima	Landrace	Dedza-Malawi
KK24	Small	Red kidney	Landrace	Dedza-Malawi
KK35	Medium	Calima	Landrace	Ntchisi-Malawi
KK37	Large	Coffee orange	Landrace	Ntchisi-Malawi
KK39	Large	Khakhi	Landrace	Ntchisi-Malawi
KK40	Large	Red	Landrace	Ntchisi-Malawi
KK42	Large	White	Landrace	Ntchisi-Malawi
KK45	Medium	White kidney	Landrace	Mchinji-Malawi
KK48	Small	Black	Landrace	Mchinji-Malawi
KK49	Medium	Red kidney	Landrace	Mchinji-Malawi
KK57	Small	Calima	Landrace	Mchinji-Malawi
KK58	Large	Calima	Landrace	Mchinji-Malawi
KK59	Large	Coffee orange	Landrace	Mchinji-Malawi
KK61	Large	Coffee orange	Landrace	Mchinji-Malawi
KK64	Medium	Red kidney	Landrace	Lilongwe-Malawi
KK65	Large	Black speckled	Landrace	Lilongwe-Malawi
KK69	Large	Black speckled	Landrace	Lilongwe-Malawi
KK71A	Large	Black speckled	Landrace	Mzimba-Malawi
KK71B	Large	White	Landrace	Mzimba-Malawi
KK72	Large	Calima	Landrace	Lilongwe-Malawi
KK73	Large	Sugar	Landrace	Mzimba-Malawi
KK74	Small	Sugar	Landrace	Mzimba-Malawi
KK75	Large	Dark red kidney	Landrace	Mzimba-Malawi
KK76	Small	Green	Landrace	Mzimba-Malawi
KK77	Medium	Green	Landrace	Mzimba-Malawi
KK78	Large	Pale pink	Landrace	Mzimba-Malawi
KK80	Large	Pinto	Landrace	Mzimba-Malawi
KK81	Medium	Sugar	Landrace	Mzimba-Malawi
KK82	Large	Cream yellow	Landrace	Mzimba-Malawi
KK83	Large	White speckled	Landrace	Mzimba-Malawi
KK84	Large	White speckled	Landrace	Rumphi-Malawi
KK85	Large	Purple	Landrace	Rumphi-Malawi
KK86	Large	Calima	Landrace	Rumphi-Malawi
KK88	Medium	White	Landrace	Rumphi-Malawi
KK89	Large	Calima	Landrace	Rumphi-Malawi
KK90	Large	Calima	Landrace	Rumphi-Malawi
KK91	Large	Yellow	Landrace	Chitipa-Malawi
KK93	Medium	Khakhi	Landrace	Chitipa-Malawi
KK94	Large	Sugar	Landrace	Salima-Malawi

Genotype	Seed size	Seed colour	Cultivar status	Collection source
KK95	Medium	White kidney	Landrace	Salima-Malawi
KK96	Medium	Red kidney	Landrace	Salima-Malawi
KK97	Medium	White kidney	Landrace	Thyolo-Malawi
B136	Medium	Small reds	Improved	CIAT [†]
B91	Medium	Small reds	Improved	CIAT
B61	Medium	Small reds	Improved	CIAT
B94	Medium	Small reds	Improved	CIAT
Maluwa	Medium	Calima	Improved	Malawi National bean programme
Sapatsika	Medium	Red	Improved	Malawi National bean programme
Mkhalira	Small	Khakhi	Improved	Malawi National bean programme

Note: Large (>37g/100 seeds); Medium (26-36g/100 seeds); Small (<25g/100seeds)

[†] CIAT=Centro internacional de agricultura tropical

Appendix 3: 33 improved bean genotypes tested in experiment 3 for resistance to *A. obtectus* and some of their characteristics

Genotype	Characteristics			
	Seed size	Cultivar status	Seed colour	Source
PS 3	large	Improved cultivar	sugar	RSA [†] -PROSEED
PS 4	large	Improved cultivar	sugar	RSA-PROSEED
PS 5	large	Improved cultivar	sugar	RSA-PROSEED
PS 7	large	Improved cultivar	sugar	RSA-PROSEED
PS 10	large	Improved cultivar	sugar	RSA-PROSEED
PS 16	large	Improved cultivar	white kidney	RSA-PROSEED
PS 21	large	Improved cultivar	sugar	RSA-PROSEED
PS 23	small	Improved cultivar	sugar	RSA-PROSEED
PS 25	medium	Improved cultivar	calima	RSA-PROSEED
PN 1	large	Improved cultivar	sugar	RSA-PANNAR
PN 3	large	Improved cultivar	sugar	RSA-PANNAR
PN 6	large	Improved cultivar	sugar	RSA-PANNAR
PN 13	large	Improved cultivar	sugar	RSA-PANNAR
PN 18	large	Improved cultivar	carioca	RSA-PANNAR
BELDAK	medium	Improved cultivar	pinto	RSA-PANNAR
NBYT 2	medium	Breeders' material	red	CRSP [§] -Bunda
NBYT 3	small	Breeders' material	cream yellow	CRSP-Bunda
NBYT 4	medium	Breeders' material	khakhi	CRSP-Bunda
NBYT 5	small	Breeders' material	carioca	CRSP-Bunda
NBYT 7	large	Breeders' material	sugar	CRSP-Bunda
NBYT 8	large	Breeders' material	calima	CRSP-Bunda
NBYT 10	large	Breeders' material	sugar	CRSP-Bunda
NBYT 11	medium	Breeders' material	pink	CRSP-Bunda
NBYT 12	large	Breeders' material	calima	CRSP-Bunda
NBYT 13	large	Breeders' material	red	CRSP-Bunda
NBYT 14	large	Breeders' material	carioca	CRSP-Bunda
NBYT 15	large	Breeders' material	calima	CRSP-Bunda
NBYT 16	large	Breeders' material	red	CRSP-Bunda
NBYT 17	large	Breeders' material	red	CRSP-Bunda
B 2	large	Improved cultivar	dark red	CIAT [¶] -Malawi
B 3	small	Improved cultivar	purple	CIAT-Malawi
B 34	large	Improved cultivar	red kidney	CIAT-Malawi
B 179	small	Improved cultivar	red small	CIAT-Malawi
KK 22	medium	Landrace	white spot	Dedza-Malawi
KK 27	large	Landrace	khakhi	Dedza-Malawi
KK 41	large	Landrace	white	Ntchisi-Malawi
UNKNOWN	large	Landrace	red kidney	Mzimba-Malawi

Note: Large (>37g/100 seeds); Medium (26-36g/100 seeds); Small (<25g/100seeds)

[†]RSA= Republic of South Africa

[§]-CRSP= Collaborative Research Support Programme

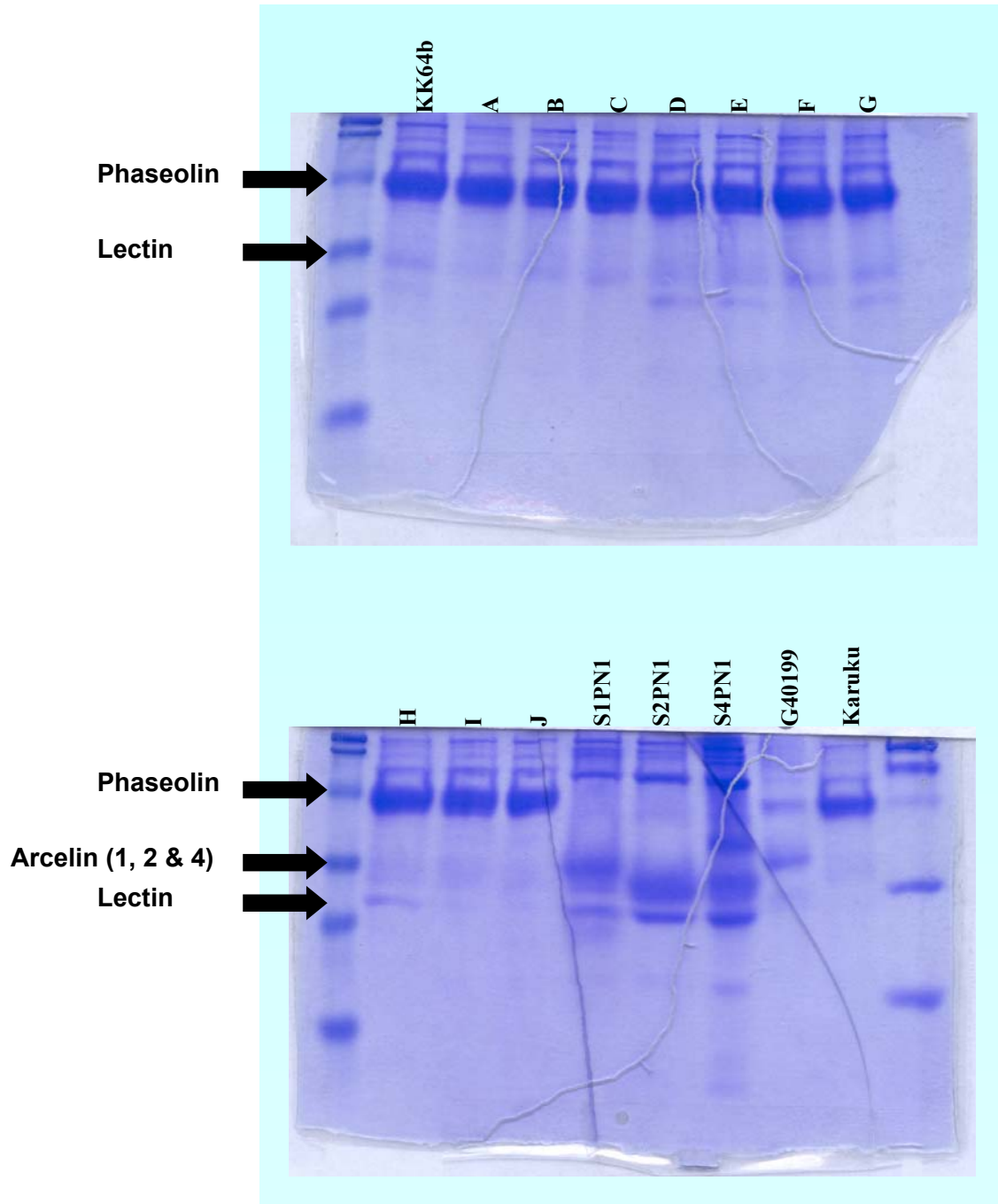
[¶] CIAT=Centro internacional de agricultura tropical

Appendix 4: Dry bean genotypes selected for further biochemical analysis

Code	Accession #/ name	Seed size	Level of observed resistance	Cultivar status
A	KK93	Medium	Intermediate	landrace
F	KK35	Medium	High	landrace
E	Maluwa	Large	Susceptible	improved
B	KK25	Medium	High	landrace
C	KK64	Medium	Intermediate	landrace
D	KK73	Large	Intermediate	landrace
G	KK03	Medium	Intermediate	landrace
H	Nagaga	Large	Susceptible	improved
I	PS 10	Medium	Susceptible	improved
J	KK36	Medium	Intermediate	landrace
K	KK64b	Medium	Intermediate	landrace

Note: Large (>37g/100 seeds); Medium (26-36g/100 seeds)

Appendix 5: Electrophoretic banding pattern of selected bean genotypes[§] showing absence of arcelin in the tested genotypes: starting with marker similar in size to phaseolin downwards in kDa: 45, 31, 21 and 14



[§] Test genotypes: A to J are as defined in Appendix 4

Control genotypes (Phaseolin null): S1PN1=SMARC 1-PN1, S2PN1=SMARC2-PN1, S4PN1=SMARC4-PN1; these lines possess Arl-1, Arl-2, Arl-4, respectively; G40199=wild tepary bean (*P. acutifolius*) accession with resistance to bruchid

Chapter Four

Genetic Analysis of Dry Bean Resistance to the Common Bean Weevil (*Acanthoscelides Obtectus* Say)

Abstract

The common bean weevil (*Acanthoscelides obtectus* Say) causes substantial post-harvest losses in susceptible varieties of dry beans (*Phaseolus vulgaris* L.) grown without insecticides by small-scale farmers. The development of a strategy to breed for resistance to bean weevils requires knowledge of the inheritance of resistance in local sources. The inheritance of resistance to the common bean weevil (bruchid) was therefore studied in a 6 x 6 F₂ diallel analysis of Malawian dry bean landraces. F₂ and F₃ generation and parental seeds were evaluated for bruchid resistance using no-choice tests in the laboratory. Data were recorded on insect progeny development periods, the Dobie susceptibility index, the number of adult bruchid emergence, seed damage and grain weight loss (%), which were significantly correlated. There was a significant variation among genotypes for number of adult bruchid emergence and Dobie susceptibility index in F₂ and F₃ generations, respectively, and for grain weight loss and seed damage due to bruchids in both F₂ and F₃ generations. The additive-dominance model was adequate to explain the variation among genotypes, indicating that epistatic effects were not important in controlling bruchid resistance. Dominance effects were significantly larger (>80%) than the additive effects, consequently heritability of resistance was very low (35%). Generally, results indicated over-dominance in F₂ generation; but partial dominance was also exhibited in five of the 13 populations in F₃ generation. Dominant genes in the parental lines were equally positive and negative for bruchid resistance implying that some genes enhanced resistance; while others increased susceptibility. The non-significance of χ^2 for single and two gene models and the observed transgressive segregation in F₃ generation suggests that many genes were involved in controlling bruchid resistance. Transgressive segregation observed in F₃ populations also suggests a quantitative mode of inheritance and confirms importance of additive effects, further suggesting that selection can be used to improve resistance. Reciprocal differences were not significant in the F₂ population but were significant in four crosses involving KK35, KK73, KK25 and Maluwa in the F₃ generation seed, suggesting that maternal effects or cytoplasmic genes might play a role in controlling

bruchid resistance. Resistant parental lines KK35 and KK73, displayed significant negative general combining ability (GCA) effects and would thus be valuable sources in breeding for resistance.

4.1 Introduction

Two bruchid species, *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman, destroy the nutritional, economic and planting value of stored beans. Dry beans in storage suffer irreversible and irretrievable quantitative and qualitative losses from bruchid infestation. Infestation may begin in the field, during which eggs are laid, and continues in storage. In storage, both bruchid species can cause severe damage resulting in substantial yield losses. Post-harvest losses exceeding 38% have been reported in Malawi (Chirwa, 2001). Improved varieties that are currently grown are very susceptible to the weevils (see Chapter 3), hence there is need to breed for bruchid resistance. Although chemical, cultural and biological control methods exist, genetic resistance still remains the most satisfactory and sustainable method of minimising losses due to insect pests, particularly as a basic element in an integrated pest management approach (Fory *et al.*, 1996).

The search for sources of resistance to bruchids in common beans must precede the study of the inheritance of resistance. Once the sources of resistance have been found, the next step is to determine the genetics of resistance. Therefore, prior to the current study good sources of bruchid resistance were identified in some local bean landraces with adaptable traits and characteristics preferred by farmers in Malawi (see Chapter 3). The development of an appropriate breeding strategy to improve resistance to bean weevils in dry bean varieties requires knowledge of the inheritance of resistance in local sources. For example, the gene action controlling resistance in the local sources has not been studied.

Knowledge of the way in which genes act and interact will determine which breeding system optimises gene action more efficiently and will elucidate the role of the breeding systems in the evolution of crop plants (Fasoula and Fasoula, 1997). Understanding the inheritance of resistance to *A. obtectus* and genes controlling it would potentially lead to more efficient deployment of host plant resistance. Using the identified resistant bean accessions, a breeding programme will be initiated to introduce resistance from an

adaptable source into the susceptible, but high yielding commercial cultivars currently grown in Malawi.

Inheritance studies of resistance to storage pests have previously been conducted in legume and cereal crops. Kornegay and Cardona (1991) investigated inheritance of resistance to *A. obtectus* in the reciprocal F1 and F2 generation dry beans seeds and concluded that resistance was controlled by two recessive complementary genes. R'omero Andreas *et al.* (1986) found that the inheritance of arcelin, a seed protein which confers resistance to *Z. subfasciatus* in wild beans, was controlled by a single dominant gene. The inheritance of resistance to the bean pod weevil (*Apion godmani* W.) in dry beans was conditioned by two genes that were segregating independently (Garza *et al.*, 1996). They further reported that one gene pair in each of the accessions was non-allelic. In an F2 segregation analysis of black gram (*Vigna mungo* L.) for resistance to *Callosobruchus maculatus* F, Dongre *et al.* (1996) reported a 15:1 ratio of resistant to susceptible, indicating epistatic gene action for resistance controlled by duplicate genes.

Genetic studies are applicable to the specific germplasm, specific crop and the set of testing environments; hence results from such studies cannot be generalised (Falconer, 1989). Therefore, the findings from other areas may not have a direct application for bruchid resistance in dry beans in Malawi. No inheritance studies of resistance to bruchids have been conducted in Malawi using the Malawian dry bean landraces. The current study focused on understanding the inheritance of resistance to *A. obtectus* for two reasons. Firstly, it was reported that *A. obtectus* was a more predominant species in Malawi (Chipokosa and Nyirenda, 1999) and secondly, *A. obtectus* is known to attack beans both in the field and quickly multiplies in storage once conditions are favourable, causing severe damage to stored beans.

Study objectives

The specific objectives of this study were to:

- (i) determine the mode of gene action and inheritance of *A. obtectus* resistance in Malawian dry bean landraces;
- (ii) determine the segregation ratio and frequency distribution of bruchid resistance in dry beans; and

- (iii) determine the role of maternal influence or effects on bruchid resistance in Malawian dry bean landraces.

4.2 Materials And Methods

4.2.1 Germplasm

Six bean genotypes comprising two resistant, two moderately resistant and two susceptible lines, were identified and used as parents in this study (Table 4.1). The genotypes were selected based on their average performance during laboratory and field screening for resistance to the bruchids (see Chapter three) and seed availability. Two bean landraces, KK35 and KK73, were resistant, while KK25 and KK03 were moderately resistant, and two improved genotypes, Nagaga and Maluwa, were selected on the basis of their susceptibility to bruchid infestation. The six genotypes constituted the parental material that was used in a full diallel mating scheme. They were of similar maturity dates; medium-to large-seeded; different seed colour, and growth habit (Table 4.1). The six parental lines were of the Andean origin. Some lines that showed high resistance in both field and laboratory tests did not yield adequate seed for inclusion in the diallel study. As a result some genotypes with lower ranking for resistance but with adequate seed were included.

Table 4.1: Varietal characteristics of the six parents used in the 6 x 6 diallel mating

Genotype	Varietal characteristics					
	Seed size [†]	Seed colour	Growth habit	Flower colour	D50F [§]	Resistance rating [‡]
KK35	Medium	Red speckled	Determinate bush	Pink	37	R
KK25	Medium	Dark red	Indeterminate bush	Pink	36	MR
KK73	Large	Red speckled	Determinate bush	White	39	R
KK03	Medium	Red speckled	Determinate bush	White	39	MR
Nagaga	Large	Khakhi	Determinate bush	White	37	S
Maluwa	Large	Red speckled	Determinate bush	White	36	S

[‡] R=resistant; MR=moderately resistant; S=susceptible

[†]Seed size rating (Schoonhoven and Voysest, 1991): Medium (26-38g 100⁻¹ seeds); Large (>38 g 100⁻¹ seeds); [§]D50F=days to 50% flower

4.2.2 Field procedures and diallel mating scheme

Six dry bean parental genotypes were planted on 15 January 2006 at Chitedze Research Station. The genotypes were planted in rows that were 5 m long, 30 cm apart and one seed per station. Pollination was done by hand, where flowers were

emasculated before pollen shedding. Pollination was carried out following crossing procedures outlined in the CIAT handbook (CIAT, 1987). A 6 x 6 diallel cross with reciprocals (Griffing, 1956; Method 1) was done, where 30 F1 reciprocal crosses were generated (Table 4.2). The F1 generation seeds were then advanced by self-pollination to the F2 generation in a greenhouse at Agricultural Research and Extension Trust (ARET). The F2 seeds were harvested and were bulked for each of the 30 crosses (i.e., 30 bulk samples, one for each population). Forty-five seeds from the F2 generation seeds were randomly selected from each cross and were subjected to *A. obtectus* infestation in three replications of 15 seeds each in the laboratory. Simultaneously, 30 seeds, which were randomly selected from each of the 30 F2 genotypes, were planted at Kakuyu Farm, 32 km west of Chitedze Agricultural Research Station to generate seeds for the segregation analysis. The F2 generation seeds were planted in rows of 15 m long, 60 cm apart and 30 cm between planting stations (within rows). The F3 generation seeds were harvested individually from the 30 F2 plants in each of the 30 populations. Four populations failed to produce adequate seed for the laboratory screening, consequently only seed harvested from 26 populations was evaluated for resistance to *A. obtectus*. Thus, a total of 780 seed samples, 30 from each of the 26 populations, were subjected to the no-choice test for resistance to damage by bruchids in the laboratory. The F1 generation seeds were not tested for bruchid resistance because the seeds were not adequate for laboratory screening.

Table 4.2: Reciprocal crosses generated in a 6 x 6 diallel mating scheme

Parents	Nagaga	Maluwa	KK35	KK03	KK25	KK73
Nagaga	Nag/Nag [†]	Nagaga/Mal	Nag/KK35	Nag/KK03	Nag/KK25	Nag/KK73
Maluwa	Mal*/Nag	Mal/Mal	Mal/KK35	Mal/KK03	Mal/KK25	Mal/KK73
KK35	KK35/Nag	KK35/Mal	KK35/KK35	KK35/KK03	KK35/KK25	KK35/KK73
KK03	KK03/Nag	KK03/Mal	KK03/KK35	KK03/KK03	KK03/KK25	KK03/KK73
KK25	KK25/Nag	KK25/Mal	KK25/KK35	KK25/KK03	KK25/KK25	KK25/KK73
KK73	KK73/Nag	KK73/Mal	KK73/KK35	KK73/KK03	KK73/KK25	KK73/KK73

[†]Nag=Nagaga; *Mal=Maluwa

4.2.3 Resistance testing of the F2 and F3 generation seed

F2 generation seed samples were inspected for evidence of prior infestation and were found to be clean since they were grown in a greenhouse. However, the F2 seeds together with the F3 seeds that were grown in the field, were put in a deep freezer at 20°C (Horber, 1989) for 5 d to destroy any insects or eggs that could have been present in the seed. Seed samples were later removed from the deep freezer and placed in a

controlled temperature and humidity (CTH) room at 25-30°C and 70±5% relative humidity for 7 d for conditioning. The F2 and F3 generation seeds were evaluated for resistance to bruchids in separate experiments.

Screening F2 generation: Fifteen (15) F2 generation seeds of each of the 30 F1 crosses including the six parents were weighed and put in jars. A total of seven adult bruchids (1 to 3 d old) of *A. obtectus* from the laboratory culture were introduced in each jar containing the seeds. The jars were covered with a muslin cloth and placed in the CTH room at 25-30°C and 70±5% relative humidity for 10 d to allow the insects to oviposit (lay eggs) after which they were removed. Transparent plastic bottles were used to keep the seed samples infested with the bruchids. The jars were covered to prevent the insects from escaping; hence the insects did not have any choice of genotypes for feeding and oviposition (no-choice test). The bottles containing the seeds were clearly labelled indicating the cross, replicate number, date the experiment was set and daily sieving/counting date. The experiment was laid out in the CTH room as a completely randomised design with three replications for the F2 generation seed.

Screening F3 generation: Fifteen (15) F3 generation seeds from 780 F2 individual plants were put in bottles and were placed on shelves in the CTH room at 25-30°C and 70±5% relative humidity. Samples were prepared and subjected to bruchids as described in screening F2 generation section. This experiment was laid out in the CTH room as a completely randomised design without replication. Replication was not considered in this experiment because logistically the amount of seed and number were not sufficient to carry out a replicated experiment.

In both experiments, the sieving of seed samples and daily counts of the emerged insects commenced after 28 d. The initial and final grain moisture content was measured and dry weight was then calculated. In addition, the number of damaged seeds was counted.

4.2.4 Parameters used to differentiate bruchid resistance of genotypes

In the F2 generation seed the following resistance measures were used to rate genotypes for bruchid resistance:

- (i) median development period;
- (ii) adult bruchid emergence and
- (iii) Dobie susceptibility index .

In the F3 generation seed, the following parameters were used to discriminate genotypes for bruchid resistance:

- (i) median development period;
- (ii) number of adult bruchid emergence;
- (iii) Dobie susceptibility index ,and
- (iv) number of perforated seeds.

Grain weight loss (%), median development period (d) and the Dobie (1974) susceptibility index were calculated for each sample.

Grain weight loss (%) was calculated as:

$$100 * (IGW - FGW) / IGW \text{ of sample};$$

where FGW = final grain weight; IGW = initial grain weight.

Median development period (MDP) was calculated as: the number of days from the middle of oviposition (d-5) to the first progeny emergence. The data on the number of adult bruchid emerged (ABE) and the median development period were combined to calculate the Dobie susceptibility index (Dobie, 1974) using the formula:

$$100 \times [\log_e (\text{total number of progeny emerged}) / \text{MDP}]$$

where MDP = median development period. A Dobie susceptibility index of zero was assumed where no insect progenies had emerged from the test samples during the grain incubation period.

4.2.5 Determination of combining ability estimates

General analysis of variance (ANOVA) was performed for all quantitative data of adult bruchid emergence, median development period, % grain weight loss and Dobie susceptibility index (DSI), using restricted maximum likelihood (REML) in the GenStat statistical package (Lawes Agricultural Trust, 2006). In this model, genotypes were

considered as fixed effects because few parents were used, while replication effects were regarded as random. The complete F2 diallel analysis (i.e., Griffing's (1956) Method 1) was performed for the number of adult bruchid emergence, which was significant using the following model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + 1/kE_{ijk}$$

Where; Y_{ijk} = number of adult weevil emergence;

μ = grand mean;

g_i = GCA effects for parent i;

g_j = GCA effects for parent j;

s_{ij} = specific combining ability (SCA) for the cross between parent i and j;

r_{ij} = reciprocal effects for the cross between i and j;

k = replication effects;

E_{ijk} = random error.

General combining ability (GCA) effects were calculated for each parent. Specific combining ability (SCA) and reciprocal effects were also calculated for the F2 crosses and their reciprocals, respectively. The t-test was used to test whether GCA, SCA and reciprocals were significantly different from zero. The degrees of freedom for estimable GCA effects (g_i) were $(p-1)$, where p = number of parents. The degree of freedom for SCA effects (S_{ij}) and reciprocal effects (r_{ij}) were $p(p-1)/2$ (Kang, 1994).

4.2.6 Segregation analysis

The means of the following data were tested for their significant differences from zero using the t-test in the GenStat statistical package: number of adult weevil emergence, number of perforated and unperforated seeds, number of holes in damaged seeds, median developmental period, % grain weight loss and the Dobie susceptibility index in the F3 generation seeds. Differences between reciprocal crosses were tested for significance from zero using the ratios of their variances (i.e, the F-test). The frequency distribution of the Dobie susceptibility indices (DSI) of the genotypes in each of the F3 generation seeds was plotted. The genotypes were divided into five phenotypic classes based on their resistance or susceptibility to the bruchids as follows:

Class	DSI
Resistant	0
Moderately resistant	1-5
Moderately susceptible	6-10
Susceptible	11-15
Highly susceptible	16-21

The above classification for genotype resistance/susceptibility was done for convenience of comparison. Other researchers may classify differently depending on the range of Dobie susceptibility indices obtained.

Chi-square (χ^2) analyses of the segregants were conducted to test for the partial dominance ratio for single and two gene models. Using the five resistance classes, a χ^2 test was used to test the goodness of fit of the two gene model ratio of 1:4:6:4:1, the segregation ratio for resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS), respectively. A Chi-square test for the goodness of fit for the one gene model of 1:2:1 segregation ratio was conducted by pooling the moderate classes (MR and MS) and the susceptible classes (S and HS) resulting in three broad classes, namely, resistant, intermediate and susceptible respectively. A fit was accepted when the χ^2 value was not significantly different at the 95% level ($P > 0.05$). A phenotypic correlation analysis was also performed among the following insect resistance measurements:

- (i) Dobie susceptibility index,
- (ii) adult bruchid emergence,
- (iii) median development period,
- (iv) grain weight loss (%), and
- (v) number of perforated seeds

4.2.7 Confirmation of the adequacy of the additive-dominance model

The analysis of variance of the F₂ populations and the estimation of the components of variation were conducted using the Hayman's (1954, 1957, 1958) diallel model as described by Singh and Chaudhary (2004) and Dhabholkar (1992). The assumptions

underlying use of the diallel mating design have been reviewed in detail in Chapter One (Literature review). One assumption in the diallel analysis is absence of epistasis hence variation among genotypes is partitioned into additive and dominance effects (Christie and Shattuck, 1992). Adequacy of the additive-dominance model in explaining the variation among the genotypes was tested by performing the analysis of variance of the differences between the covariances of population arrays (W_r) and the variance among the population means within an array (V_r) for the lines and the replication differences (Hayman, 1954; Dhabholkar, 1992, p234). Significant differences among W_r - V_r are generally interpreted as evidence of epistatic gene action and non-significant differences indicate non-epistatic gene action (Christie and Shattuck, 1992).

4.2.8 Estimation of components of variation and genetic parameters

The reference population is the six parents used in the study, and results only pertain to this sample; hence no generalisation can be made because the sample was small.

Using the Hayman's (1954) approach the following variances and covariances were estimated:

V_p = variance among the parents;

V_r = variance among population means within an array (i.e., crosses with that particular parent);

V_{r_m} = mean value of V_r over all arrays;

V_f = variance among the means of the arrays;

W_r = covariance between populations within the i th array, and

W_{r_m} = mean value of W_r , over all arrays.

The variance components of the F₂ population were then estimated (Singh and Chaudhary, 2004) as follows:

$$E = \text{MSE}/r;$$

$$D = V_p - E;$$

$$H_1 = 16V_r - 16W_r + 4V_p - [4(5n - 4)/n] E;$$

$$H_2 = 16V_r - 16V_m - [16(n - 1)/n] E, \text{ and}$$

$$F = 4V_p - 8W_r - [4(n-2)/n] E.$$

Where E = environmental variance; D = additive variance; H₁ = dominance variance 1; H₂ = dominance variance 2; MSE = mean square error; r = number of replicates and F = additive-dominance covariance

The standard errors (S.E) for the variance components were estimated (Singh and Chaudhary, 2004) as follows:

$$\text{S.E. of E} = [S^2 n^4 / n^5]^{1/2};$$

$$\text{S.E. of D} = [S^2 (n^5 + n^4) / n^5]^{1/2};$$

$$\text{S.E. of H}_1 = [S^2 (16 n^5 + 656 n^4 - 192 n^3 + 64 n^2) / n^5]^{1/2};$$

$$\text{S.E. of H}_2 = [S^2 (576 n^4) / n^5]^{1/2}, \text{ and}$$

$$\text{S.E. of F} = [S^2 (16n^5 + 80n^4 - 64 n^3 + 6n^2/n^5)]^{1/2}.$$

where, n = number of parents and S² = the mean sum of squares of the deviations of the observed from the expected values of the variances and covariances i.e., (observed – expected)² averaged over the three replications and the degrees of freedom for the variances (Dhabholkar, 1992, pp249-251).

From the estimates of the genetic components, the following genetic parameters were estimated (Singh and Chaudhary, 2004):

- (i) Average degree of dominance was estimated as: $[1/4(H_1/D)]^{1/2}$.
- (ii) Proportion of genes with positive and negative effects was estimated using the formula: $H_2/4H_1$.
- (iii) Proportion of dominance and recessive genes in the parents was estimated using the formula: $\frac{1/4 (4DH_1)^{1/2} + (1/2) F}{1/4 (4DH_1)^{1/2} - (1/2) F}$
- (iv) Heritability in the narrow sense was estimated using the formula: $[(1/4D)] / [(1/4D + 1/16 H_1 - 1/8F + E)]$

4.3 Results

4.3.1 Bruchid resistance in the F₂ generation seeds

The median development period and Dobie susceptibility index data were not significantly different at P=0.05; but there were highly significant (P ≤ 0.01) differences among genotypes for adult bruchid emergence and grain weight loss in the F₂

generation seeds (Table 4.3). Therefore, the genotypes were classified into resistance classes based on the number of bruchids that emerged in each population. The parental genotypes Nagaga, KK03 and KK25 displayed higher adult bruchid emergence than their crosses and reciprocals. Resistance of some of the parents such as KK35 and KK73 was better than the crosses. Nagaga/Maluwa, a susceptible x susceptible cross, gave the highest adult bruchid emergence. Although differences were not significant ($P < 0.05$) for the Dobie susceptibility data, the indices ranged between 1.9 for the most resistant (KK03/KK25) to 11.5 for the most susceptible genotype (Nagaga/Maluwa) (Table 4.3). Similarly, grain weight loss was the lowest for the most resistant and highest for the most susceptible (Table 4.3). On average bruchids took 35 d to emerge from the grain samples in all genotypes, although the period ranged from 33 d to 39 d with the longest development period being in one of the most resistant genotypes (Table 4.3).

Table 4.3: Evaluation of F2 generation seed for bruchid resistance in the no-choice test (data sorted by adult bruchid emergence)

Genotype	Type of cross†	% grain wt loss	No. adult bruchid emergence	Median development period (d)	Dobie susceptibility index
KK03/KK35	MRxR	0.5	2	37	1.9
KK73/KK03	RxMR	0.6	3	35	3.1
KK25/Maluwa	MRxS	0.7	4	39	3.6
KK35/KK25	RxMR	1.4	4	35	3.9
KK73/Maluwa	RxS	1.2	5	36	4.5
Maluwa/KK35	SxR	4.6	5	35	4.6
KK35/Maluwa	RxS	1.2	6	36	5.0
Maluwa/KK73	SxR	0.7	6	36	5.0
KK35/KK35	Parent	8.4	7	36	5.4
KK35/Nagaga	RxS	7.4	7	38	5.1
KK03/KK25	MRxMR	0.8	9	34	6.5
KK73/Nagaga	RxS	1.0	9	37	5.9
KK73/KK25	RxMR	1.7	10	35	6.6
KK25/KK03	MRxMR	3.3	11	33	7.3
Maluwa/KK03	SxMR	4.4	12	35	7.1
KK25/KK73	MRxR	2.4	14	34	7.8
KK03/Maluwa	MRxS	2.8	15	34	8.0
KK73/KK73	Parent	1.3	15	35	7.7
KK35/KK73	RxR	3.9	16	37	7.5
Maluwa/KK25	SxMR	5.9	17	34	8.3
Maluwa/Maluwa	Parent	4.9	18	35	8.2
Nagaga/KK25	SxMR	4.2	18	36	8.0
KK25/Nagaga	MRxS	7.7	19	35	8.4
KK35/KK03	RxMR	3.2	19	36	8.2
KK73/KK35	RxR	3.8	19	38	7.7
Maluwa/Nagaga	SxS	5.5	19	36	8.2
Nagaga/KK73	SxR	10.6	20	36	8.3
KK03/KK73	MRxR	6.3	21	35	8.7
KK25/KK25	Parent	1.3	27	35	9.4
Nagaga/KK35	SxR	3.0	27	35	9.4
KK03/Nagaga	MRxS	8.9	31	34	10.1
KK03/KK03	Parent	6.3	37	33	10.9
KK25/KK35	MRxR	8.7	37	34	10.6
Nagaga/KK03	SxMR	9.9	38	36	10.1
Nagaga/Nagaga	Parent	3.1	39	33	11.1
Nagaga/Maluwa	SxS	10.6	50	34	11.5
Mean		4.38	17.0	35.0	7.6
SE (mean)		0.48	1.5	0.3	0.39
F Significant		*	**	ns‡	ns‡

†MR = moderately resistant; R = resistant; S = susceptible; *,** Data significant at P ≤ 0.05 and P ≤ 0.01, respectively; ns‡ = data not significant at P=0.05

4.3.2 Combining ability estimates for adult bruchid emergence in F2 generation

Combining ability estimates were obtained for adult bruchid emergence data (Table 4.4), because this data was significant in the F2 generation (Table 4.3). There were highly significant differences among genotypes for both GCA and SCA effects. The reciprocal crosses were not significantly different at $P = 0.05$. The GCA effects accounted for 9%, reciprocal effects 10% and SCA effects 81% of the sum of squares for the crosses. There were significant ($P < 0.05$) GCA effects of 9.2, -5.0 and -4.5 for the lines Nagaga, KK35 and KK73, respectively (Table 4.5). Maluwa/Nagaga, KK25/KK35, and KK25/KK03 had significant SCA effects (Table 4.5).

Table 4.4: F2 population diallel analysis for number of adult bruchid emergence

Source of variations	df	SS	MS	F-value
GCA	5	1795.84	359.168	4.307**
SCA	15	15521.32	1034.755	12.407**
Reciprocal	15	1827.89	121.860	1.461ns [†]
Error	70	5838	83.4	

** Data significant at $P \leq 0.01$; ns[†] = data not significant at $P=0.05$

Table 4.5: Estimates of general and specific combining ability effects for adult bruchid emergence in the F2 population diallel analysis[†]

Parents	Nagaga	Maluwa	KK35	KK03	KK25	KK73
Nagaga	9.2**	9.5*	-3.9	5.2	-7.3	-7.0
Maluwa		-2.6	-3.9	-4.1	-3.4	-4.2
KK35			-5.0*	-5.1	14.4**	3.6
KK03				3.4	-9.6*	0.3
KK25					-0.4	0.1
KK73						-4.5*

[†]Diagonal entries are GCA effects and off diagonal entries represent SCA effects

*, ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

4.3.3 Bruchid resistance in the F3 generation seed

There were highly significant variations ($P < 0.001$) in all the resistance parameters in the F3 generation seed (Table 4.6). A few populations, namely, KK35/KK73, KK03/Maluwa, Maluwa/KK35, KK03/KK35 and KK35/KK25 displayed high levels of

resistance (Table 4.6). KK35/KK73 (RxR) gave the lowest percent grain weight loss indicating that it incurred less bruchid damage and correspondingly it had the highest number of unperforated seeds, and the smallest Dobie susceptibility index (Table 4.6). Maluwa/KK73 (SxR) had the highest grain weight loss and highest number of holes indicating severe damage by the bruchids, and the largest Dobie susceptibility index. Some pairs of reciprocal crosses (KK35/KK73 and KK73/KK35; KK03/Maluwa and Maluwa/KK03; KK35/KK25 and KK25/KK35; KK03/KK25 and KK25/KK03) had large reciprocal differences for % grain weight loss, number of adult bruchid emergence, Dobie susceptibility index, number of holes and number of perforated and unperforated seeds (Table 4.6).

Table 4.6: Summary data for resistance of dry bean genotypes to *Acanthosclides obtectus* in the F3 generation seed (data sorted by Dobie susceptibility index)

Population	Type of cross†	% grain wt loss	No. adult emerged	Dobie susceptibility index	No. of holes	No. of perforated Seeds	No. of unperforated seeds
KK35/KK73	RxR	1.3	3	1.1	4	1	14
KK03/Maluwa	MRxS	3.0	7	2.4	7	3	12
Maluwa/KK35	SxR	7.0	6	3.2	7	3	12
KK03/KK35	MRxR	3.1	11	4.0	12	4	11
KK35/KK25	RxMR	8.0	13	4.4	14	5	9
Maluwa/KK25	SxMR	8.7	14	4.8	17	6	9
KK25/KK03	MRxMR	6.4	18	5.0	19	7	8
Nagaga/KK25	SxMR	7.3	10	5.3	11	7	8
KK25/KK73	MRxR	5.9	15	6.1	17	7	8
Nagaga/KK03	SxMR	8.5	16	6.1	17	7	8
KK35/Maluwa	RxS	10.9	17	6.4	17	7	8
Maluwa/KK03	SxMR	7.5	16	6.6	17	7	8
KK03/Nagaga	MRxS	10.4	20	6.8	20	7	8
KK25/Nagaga	MRxS	9.9	16	6.9	17	8	7
KK35/KK03	RxMR	6.1	19	7.2	20	8	7
Nagaga/KK35	SxR	10.6	25	7.2	28	8	7
KK25/Maluwa	MRxS	9.4	19	7.3	20	8	7
KK73/KK25	RxMR	8.0	23	7.3	24	7	8
KK35/Nagaga	RxS	10.5	26	7.5	27	8	7
KK73/KK35	RxR	7.8	22	7.5	24	8	7
Nagaga/Maluwa	SxS	9.8	19	7.6	18	9	6
KK03/KK25	MRxMR	10.4	28	7.7	28	9	6
KK25/KK35	MRxS	12.3	27	7.7	28	8	7
Maluwa/Nagaga	SxS	9.1	20	7.7	20	9	6
KK73/Maluwa	RxS	9.4	26	7.9	27	9	6
Maluwa/KK73	SxR	12.4	28	8.4	29	9	6
Mean		8.2	18	6.1	19	7	8
Min.		1.3	3	1.1	4	1	6
Max.		12.4	28	8.4	29	9	14
SD		6.79	17.2	3.98	18.33	4.82	4.82
Sign.(P- value)		***	***	***	***	***	***
SE (mean)		1.33	3.3	1.6	3.5	0.95	0.95

*** Data is significant at $P \leq 0.001$

4.3.4 F3 population segregation analysis

Variances for the number of bruchid emergence and the Dobie susceptibility indices varied among the populations in the F3 generation seeds. However, there were no significant differences between reciprocal crosses in each population for the Dobie susceptibility index variance (Table 4.7). Notably, some populations, Maluwa/KK73, KK35/Maluwa and KK35/KK03, had large variance ratios for the Dobie susceptibility index variances; while Nagaga/KK25, KK25/KK73 and Nagaga/KK03 had smaller variance ratios. Although reciprocal differences were not significant in the F2 generation

seed (Table 4.4), variance ratios were significant for the number of adult bruchid emergence in four of the populations of the F3 generation seed (Table 4.8). For example, KK25/KK73, KK35/Maluwa, KK73/KK35, KK03/KK35 had significant reciprocal differences.

The difference between the means also indicated significant differences among the populations for the number of bruchid emergence and the Dobie susceptibility index in the F3 generation seeds (Tables 4.7 and 4.8). For example, the population KK35/KK73 (RxR) had the smallest Dobie susceptibility index (1.07) and the least number of bruchid emergence (3); while Maluwa/KK73 (SxS) had the largest Dobie susceptibility index value (8.6), and KK35/Nagaga had the largest number of adult bruchid emergence (103). There was also high variation for both the Dobie susceptibility index and the number of bruchid emergence among the individual plants within the populations, as evidenced by the range from zero bruchid emergence to 103 for KK35/Nagaga (RxS) (Table 4.8). Overall, there were some highly resistant individuals in each of the 26 populations. Similarly, there were also susceptible individuals in each population, even in the crosses between resistant lines (RxR) (Tables 4.7 and 4.8).

Table 4.7: Phenotypic variance and variance ratios of reciprocal populations for Dobie index susceptibility in the F3 generation seed

Population	Reci- procal	Type	Total no. of plant s	Mean	SE (±)	Min.	Max.	Variance	Vari- ance ratio (F)
KK03/Nagaga	1	MRxS	30	6.77	0.581	0	12.37	10.11	
Nagaga/KK03	2	SxMR	30	6.10	0.606	0	11.93	11.01	1.09
Maluwa/Nagaga	1	SxS	30	7.70	0.499	0	11.02	7.46	
Nagaga/Maluwa	2	SxS	30	7.56	0.438	0	11.60	5.75	1.29
KK25/Nagaga	1	MRxS	30	6.92	0.556	0	11.47	9.28	
Nagaga/KK25	2	SxMR	30	5.33	0.554	0	9.45	9.21	1.01
Nagaga/KK35	1	SxR	30	7.18	0.772	0	12.26	17.90	
KK35/Nagaga	2	RxS	30	7.54	0.664	0	13.39	13.23	1.35
Maluwa/KK35	1	SxR	30	3.25	0.545	0	10.32	8.92	
KK35/Maluwa	2	RxS	30	6.41	0.672	0	10.85	13.55	1.52
KK73/Maluwa	1	RxS	30	7.90	0.719	0	12.46	15.53	
Maluwa/KK73	2	SxR	30	8.36	0.560	0	11.94	9.41	1.65
Maluwa/KK03	1	SxMR	30	6.55	0.629	0	12.03	11.88	
KK03/Maluwa	2	MRxS	30	2.38	0.713	0	12.22	15.25	1.28
Maluwa/KK25	1	SxMR	30	4.76	0.721	0	9.71	15.60	
KK25/Maluwa	2	MRxS	30	7.27	0.597	0	11.47	10.71	1.45
KK03/KK25	1	MRxMR	30	7.70	0.747	0	12.47	16.76	
KK25/KK03	2	MRxMR	30	5.03	0.878	0	11.89	23.17	1.38
KK25/KK35	1	MRxR	30	7.66	0.730	0	12.22	16.00	
KK35/KK25	2	RxMR	30	4.37	0.782	0	12.48	18.35	1.15
KK35/KK73	1	RxR	30	1.07	0.512	0	10.61	7.86	
KK73/KK35	2	RxR	30	7.48	0.613	0	11.29	11.26	1.43
KK03/KK35	1	MRxR	30	3.96	0.747	0	11.68	16.74	
KK35/KK03	2	RxMR	30	7.22	0.608	0	10.73	11.08	1.51
KK73/KK25	1	RxMR	30	7.35	0.709	0	13.88	15.08	
KK25/KK73	2	MRxR	30	6.07	0.705	0	10.61	14.90	1.01

F - value at 29df: Critical value = 1.84 (P = 0.05); 2.33 (P < 0.01)

Table 4.8: Phenotypic variance and variance ratios of reciprocal populations for adult bruchid emergence (ABE) in the F3 generation seed

Population	Reciprocal	Type	Total no. of plants	Mean	SE (\pm)	Min.	Max.	Variance	Variance ratio (F)
KK03/Nagaga	1	MRxS	30	20	3.5	0	76	373.2	
Nagaga/KK03	2	SxMR	30	16	3.0	0	65	267.2	1.39
Maluwa/Nagaga	1	SxS	30	20	2.1	0	40	136.7	
Nagaga/Maluwa	2	SxS	30	19	2.0	0	46	125.0	1.09
KK25/Nagaga	1	MRxS	30	16	2.1	0	44	134.5	
Nagaga/KK25	2	SxMR	30	10	1.7	0	30	86.7	1.55
Nagaga/KK35	1	SxR	30	25	3.8	0	73	453.5	
KK35/Nagaga	2	RxS	30	26	4.6	0	103	635.3	1.40
Maluwa/KK35	1	SxR	30	7	1.5	0	37	67.2	
KK35/Maluwa	2	RxS	30	17	1.6	0	47	197.2	2.93**
KK73/Maluwa	1	RxS	30	26	3.3	0	61	330.5	
Maluwa/KK73	2	SxR	30	27	3.2	0	60	305.8	1.08
Maluwa/KK03	1	SxMR	30	16	2.3	0	53	161.6	
KK03/Maluwa	2	MRxS	30	7	2.8	0	72	233.3	1.44
Maluwa/KK25	1	SxMR	30	14	2.0	0	33	122.2	
KK25/Maluwa	2	MRxS	30	19	2.5	0	44	184.6	1.51
KK03/KK25	1	MRxMR	30	28	4.2	0	89	520.6	
KK25/KK03	2	MRxMR	30	18	3.3	0	57	335.6	1.55
KK25/KK35	1	MRxR	30	27	3.8	0	72	444.6	
KK35/KK25	2	RxMR	30	13	3.4	0	79	338.4	1.31
KK35/KK73	1	RxR	30	3	1.7	0	41	90.9	
KK73/KK35	2	RxR	30	22	2.5	0	52	189.8	2.08**
KK03/KK35	1	MRxR	30	11	3.1	0	67	285.7	
KK35/KK03	2	RxMR	30	19	2.2	0	37	151.3	1.88*
KK73/KK25	1	RxMR	30	23	3.9	0	77	471.4	
KK25/KK73	2	MRxR	30	15	2.3	0	41	155.8	3.02***

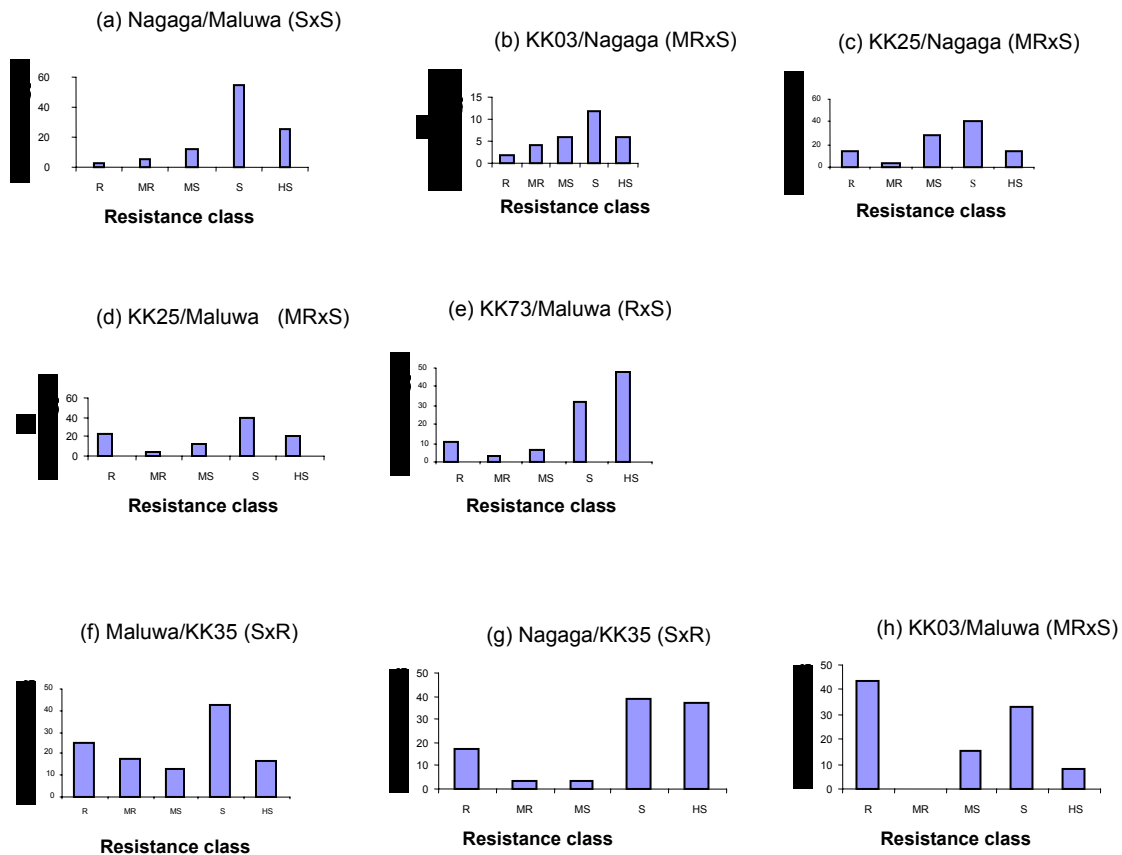
F - Value at 29df: Critical value = 1.84 (P = 0.05); 2.33 (P < 0.01);

*, **, *** Data is significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively

4.3.5 Frequency distribution of bruchid resistance of the F3 genotypes

Since there were no significant differences between reciprocal crosses for the Dobie susceptibility index (DSI), the data were pooled over reciprocals resulting in 13 populations with 60 plants each (Fig 4.2). A plot of the frequency distributions of the DSI of the genotypes in each of the 13 populations showed a pattern skewed towards susceptibility for those populations that had at least one susceptible parent (Nagaga/Maluwa, KK03/Nagaga, (KK25/Nagaga, KK25/Maluwa and KK73/Maluwa) (Fig

4.2a to Fig 4.2g, respectively). More than 80% of the plants in the susceptible x susceptible population were in the susceptible classes (Fig 4.2a). The frequency distribution of the bruchid resistance also showed some evidence for the transgressive segregation as some F3 progenies were either more resistant or more susceptible than their parents. The F2 genotypes that had transgressive segregants included KK03/KK25 (Fig 4.2i), KK25/KK35 (Fig 4.2l), KK35/KK73 (Fig 4.2m) and KK03/Maluwa (Fig 4.2h), respectively. Two populations (KK03/Maluwa and KK35/KK73) had more than 40% of their plants in the resistant class and displayed resistance almost equal to their parents. They showed a bimodal type of distribution for bruchid resistance. The MR x MR and R x MR combinations displayed a continuous distribution pattern where almost equal numbers of susceptible and resistant plants were observed in the populations.



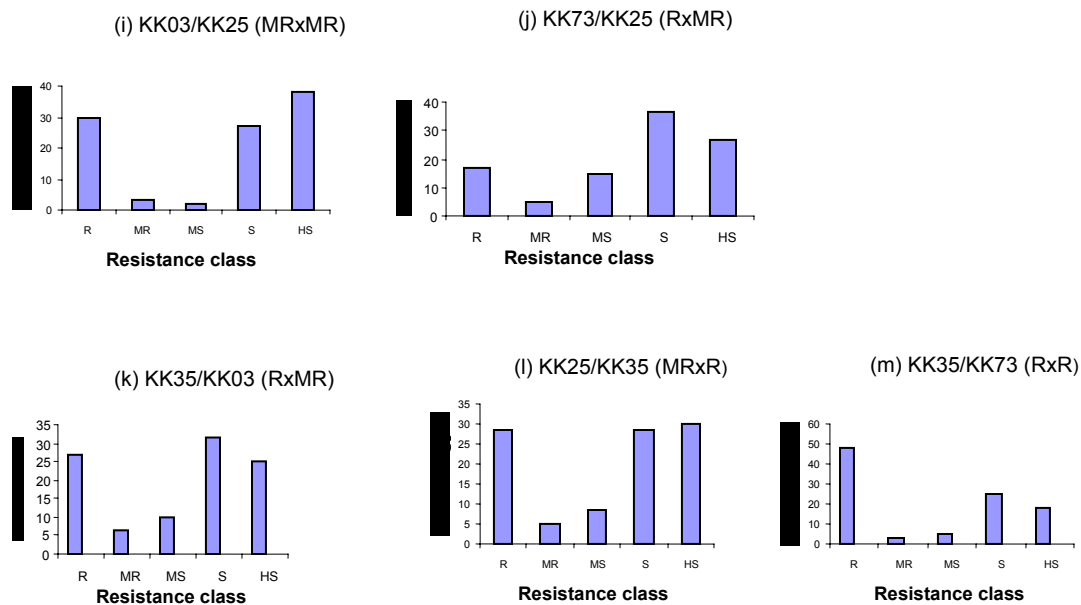


Figure 4.2: Frequency distribution for bruchid resistance of 60 F3 genotypes in each of the 13 populations (Dobie susceptibility index: R = 0; MR = 1-5; MS = 6-10; S = 11- 15; HS = 16-21)

Chi-square values showed that the frequency distribution of genotypes for resistance in eight of the 13 populations did not fit the partial dominance ratio of 1:2:1 for a single gene model (Table 4.9). In five populations the chi-square test values were not statistically significant and hence fitted the 1:2:1 partial dominance ratio.

Table 4.9: Chi-square test for goodness of fit of the 1:2:1 for a single gene model partial dominance ratio for the F3 generation seed

Population	Resistant class observed (%)			Resistance class expected (1:2:1)			χ^2	Sig.
	R	MR	S	R	MR	S		
KK03/Nagaga	8	72	20	25	50	25	21.6	***
Maluwa/Nagaga	3	72	25	25	50	25	29.0	***
KK25/Nagaga	13	72	15	25	50	25	19.4	***
Nagaga/KK35	17	46	37	25	50	25	8.6	*
Maluwa/KK35	25	58	17	25	50	25	3.8	ns
KK73/Maluwa	10	43	47	25	50	25	29.3	***
Maluwa/KK03	43	24	33	25	50	25	29.0	***
Maluwa/KK25	23	57	20	25	50	25	2.1	ns
KK03/KK25	30	32	38	25	50	25	14.2	**
KK25/KK35	28	44	28	25	50	25	1.4	ns
KK35/KK73	48	34	18	25	50	25	28.2	***
KK03/KK35	27	48	25	25	50	25	1.2	ns
KK73/KK25	17	56	27	25	50	25	3.4	ns

A chi-square test for a goodness of fit for a two gene model performed on eight of the 13 F3 populations did not conform to the 1:4:6:4:1 two gene model segregation ratio of resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible classes, respectively (Table 4.10).

Table 4.10: Chi-square test for goodness of fit of the 1:4:6:4:1 two gene model for the F3 generation seed

Population	No. of plants	Resistance class no. observed					Resistance class no. expected					χ^2	Sig
		R	MR	MS	S	HS	R	MR	MS	S	HS		
KK03/Nag†	60	5	8	14	21	12	3.75	15	22.5	15	3.75	43.0	***
Mal/Nag	60	2	3	7	33	15	3.75	15	22.5	15	3.75	76.5	***
KK25/Nag	60	8	2	17	24	9	3.75	15	22.5	15	3.75	30.1	***
Nag/KK35	60	10	2	2	23	22	3.75	15	22.5	15	3.75	133.5	***
KK73/Mal	60	15	11	8	26	10	3.75	15	22.5	15	3.75	62.6	***
Mal/KK03	60	6	2	4	19	28	3.75	15	22.5	15	3.75	189.2	***
KK03/KK25	60	14	2	8	24	12	3.75	15	22.5	15	3.75	72.2	***
KK35/KK73	60	17	3	5	17	18	3.75	15	22.5	15	3.75	124.4	***

Critical value = 9.488 at $P < 0.05$; 13.277 at $P < 0.01$; 18.467 at $P \leq 0.001$; †Nag = Nagaga; Mal = Maluwa; *** Data significant at $P \leq 0.001$

4.3.6 Adequacy of the additive-dominance model

The diallel analysis of variance of Wr-Vr for line and replication (block) differences was not significant (Table 4.11).

Table 4.11: Wr-Vr analysis of variance for adult bruchid emergence in F2 generation

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Replications	2	215888	107944	0.88	
Wr-Vr	5	597994	119599	0.98	0.478
Error	10	1226424	122642		
Total	17	2040306			

4.3.7 Estimation of the components of variation and the genetic parameters

All but one of the estimates of the genetic components were significant (Table 4.12). The additive and dominance effects were highly significant ($P < 0.01$) for adult bruchid emergence in the F2 generation. The two dominance components (H_1 and H_2) were much larger than the additive (D) and the covariance component (F). The additive-

dominance covariance (F) and the environmental variances were not significant ($P < 0.05$).

Table 4.12: Estimates of variance components for adult bruchid emergence in the F2 population diallel analysis

Variance	Definition	Estimated value
E	Environment variance	81.34 ± 49.94 ns [†]
D	Additive variance	406.846 ± 132.140 **
H ₁	Dominance variance 1	2995.917 ± 1341.79 **
H ₂	Dominance variance 2	2573.317 ± 1198.66 **
F	Additive-dominance covariance	673.43 ± 641.868 ns [†]

** Data significant at $P < 0.01$; [†]ns data not significant at $P = 0.05$.

Estimates of the genetic parameters were made in the F2 population and results are presented in Table 4.13. The average degree of dominance was in the over-dominance range. The proportion of genes with positive and negative effects was 0.22. The ratio of the total number of dominant and recessive genes in the parents was almost four-fold. The coefficient between the parental order of dominance (W_r+V_r) and the parental mean was positive. However, the coefficient correlation was not significant at $P = 0.05$. The narrow sense heritability estimate was low in the F2 population.

Table 4.13: Estimates of the genetic parameters in the F2 population for bruchid resistance

Parameter	Value
Average degree of dominance	1.4
Proportion of genes with positive and negative effects	0.22
Proportion of dominant and recessive genes in the parents	4.127
Heritability in the narrow sense	0.35
Coefficient of correlation (r) between the parental order of dominance (W_r+V_r) and parental mean (Y_r)	0.395 ($P = 0.1051$)

4.3.8 Relationships among resistance measurements in the F3 generation seed

Highly significant ($P < 0.001$) relationships were observed amongst all the measured variables for bruchid resistance in the F3 generation seeds (Table 4.14). However, there were weak correlations between grain weight loss and MDP and between ABE and MDP. Grain weight loss, which is an important economic indicator, was very strongly and positively correlated with adult bruchid emergence and the number of perforated seeds. All parameters were strongly correlated with DSI and ABE (Table 4.14), which were used to classify genotypes for resistance.

Table 4.14: Correlations among variables for bruchid resistance in the F3 generation seed

Parameter†	DSI	% wt loss	ABE	MDP	NPS
Dobie susceptibility index (DSI)					
% grain weight loss	0.75 ***				
Adult bruchid emergence (ABE)	0.85 ***	0.82 ***			
Median developmental period (MDP)	0.71 ***	0.46 ***			
Number of perforated seeds (NPS)	0.89 ***	0.77 ***	0.83 ***	0.59 ***	

†DSI = Dobie index of susceptibility;

ABE = adult bruchid emergence;

MDP = median development period;

NPS = number of perforated seeds.

*** Data significant at $P < 0.001$

4.4 Discussion

Common with most self pollinating crops, there were few F1 generation seeds available for each cross to effectively evaluate F1 crosses for bruchid resistance in the current study. Consequently, the F1 crosses were advanced to F2 and F3 generations to generate adequate number of seeds for resistance testing. Therefore, data were collected and analysed from the F2 and F3 generation seeds. Christte and Shattuck (1992) cite previous researchers who have evaluated F2 generation seed from diallel crosses in barley (Choo *et al.*, 1988); Faba beans (Kao and McVetty, 1987) and cotton (Bowman and Jones, 1984), which are self-pollinated crops.

4.4.1 Resistance of genotypes

All resistance parameters in the F3 generation seed were significant and strongly correlated with the Dobie susceptibility index and adult bruchid emergence; hence any of the resistance measurements could be used to discriminate the genotypes for bruchid resistance. The correlation of such parameters has also been reported previously (Classen *et al.*, 1990; Lale and Kolo, 1998; Appleby and Credland, 2003; Dhliwayo *et al.*, 2005). Genotypic differences were not significant for the insect development period and Dobie susceptibility index in the F2 generation seed; hence these data were not used for the genetic analysis of F2 seeds. Thus number of bruchid emergence data which was highly significant was used in the diallel analysis in F2 generation seeds. Variation of dry bean genotypes for resistance to bruchids has also been reported previously (Cardona *et al.*, 1990; Kornegay and Cardona, 1991).

4.4.2 Combining ability estimates for bruchid resistance

Significant GCA variance for the number of insect emergence indicated that additive gene action was important in determining the bruchid resistance in this germplasm. Basic knowledge of genetic make-up, the nature of gene action and combining ability are pre-requisites for the development of new varieties (Borghini and Perenzin, 1994). The combining ability estimates are useful for evaluating the potential genetic worth of lines to exploit the relevant type of gene action in a breeding programme. Parents showing high GCA effects (negative values in this case) would directly be useful in a breeding programme to improve bruchid resistance in commercial varieties that are high yielding but lack resistance to bruchids. In this study, GCA effects were significant and negative for KK35 and KK73 as parents. A negative GCA value indicated that the corresponding parent made a positive contribution to resistance (i.e., reduced the number of bruchid emergence). Nagaga had a highly positive GCA value, which indicated that it increased the level of susceptibility, i.e., increased the number of bruchid emergence. However, some crosses that involved Nagaga and Maluwa (zero GCA) had some resistant as well as susceptible segregants in the F₂ generation, indicating transgressive segregation.

Specific combining ability effects accounted for the largest portion of the variation for resistance, suggesting the preponderance of the non-additive gene action (Kenga *et al.*, 2004). Highly significant SCA variation indicated that certain crosses had higher or lower levels of resistance than expected on the basis of the GCA values of the two parents involved (Gardner and Eberhart, 1966; Baker, 1978; Cisar *et al.*, 1982). Consequently, a complex type of inheritance of resistance to bruchids would be expected. Large SCA variances have previously been reported for resistance to storage insects in other crops. Dhliwayo *et al.* (2005), in a study to assess combining ability for resistance to the maize weevil (*Sitophilus zeamais* Motsch.), reported that weevil resistance was complex and heritability was small to moderate due to the large non-additive variance. Practically, larger SCA effects imply that breeding for *A. obtectus* resistance would be difficult, because the non-additive effects cannot be fixed in a crop where the cultivar is a pure line.

Significant SCA effects were observed in three crosses, indicating the presence of non-additive gene effects. Significant and negative SCA effects were only observed for the combination KK03/KK25 (MR x MR), while KK35/KK25 (R x MR) had a highly significant

positive SCA effect. These results suggest that resistance of these progenies was higher or lower than would be expected from the average resistance of their respective parents. This implies that resistant genotypes could be produced from susceptible parents. This is supported by the fact that in the current study, the crosses between susceptible lines (i.e, SxS) also yielded some resistant genotypes (Figure 4.2a). According to Hakizimana *et al.* (2004), this could possibly be explained by the quantitative inheritance of genes. Clearly, dominant (intra-gene locus interaction) gene action was highly significant in the current study but there was no evidence for epistasis (inter-gene locus interaction), this is substantiated by the adequacy of the additive-dominance model. A frequency distribution of bruchid resistance for the F3 genotypes in each of the 13 populations studied supported the occurrence of transgressive segregation (Figures 4.2h and 4.2m).

Traditionally, the ratio of GCA and SCA mean squares has been used to assess the relative importance of GCA and SCA. However, as suggested by Kang (1994), in this study the ratio of GCA to SCA sum of squares was used to determine their relative importance; because a few parents (six) were used hence a fixed model was utilised in the F2 diallel analysis. Zhang *et al.* (2001) and Menkir and Ayodele (2005) also used the same method. Baker (1978) first suggested that the progeny performances could be predicted by the use of the ratio of combining ability variance components $[(2\sigma^2_{GCA}) / (2\sigma^2_{GCA} + \sigma^2_{SCA})]$. The closer the ratio would be to unity, the greater the predictability based on GCA alone. In the current study, the GCA to SCA ratio was 0.4, confirming that non-additive gene effects were more important than additive gene effects in controlling the inheritance of *A. obtectus* resistance in these Malawian bean landraces. Practically, this implies that the best progeny resistant to *A. obtectus* cannot simply be produced by crossing the two parents with the lowest GCA effects (most negative) alone. In addition, the predominance of the non-additive gene action indicates that resistance cannot simply be improved by selection procedures alone. In the current study, it is the significance of the GCA variance which is more important, because in beans it is the pure lines rather than the hybrid varieties that are released. Consequently, the parents KK35 and KK73, which displayed the high negative GCA, will be useful in developing new or improving the existing cultivars for bruchid resistance using backcross methods or introgression of resistance into adaptable materials that lack resistance.

4.4.3 Role of maternal inheritance or the cytoplasmic effects for resistance

Generally, the reciprocal differences were not significant for the number of bruchid emergence in the F2 diallel analysis and for the Dobie susceptibility index in the F3 generation seed, indicating the absence of maternal effects or cytoplasmic inheritance. However, four crosses out of the 13 crosses in the F3 generation seed had large reciprocal differences for the number of bruchid emergence, suggesting that maternal effects or cytoplasmic genes had some influence in these populations. Reciprocal pairs have similar nuclear genetic contribution, and any difference in the performance of reciprocal pairs will be attributed to maternal effects (Kearsey and Pooni, 1996). Maternal effects are not desirable because their presence reduce the response to selection (Roach and Wulff, 1987). However, it is further reported that the influence of maternal effects on selection depends on the type of maternal effect involved. Roach and Wulff (1987) further reported three different classes of maternal effects: cytoplasmic genetic, endosperm nuclear and maternal phenotypic. Koller (1962) reported that if the trait is completely maternally controlled, cytoplasmic maternal effects can inflate the amount of genetic variance and slow the selection response. Maternal effects for the control of bruchid resistance in dry beans have not been reported in previous studies, but they have been reported for other traits in dry beans. Leleji *et al.* (1972) reported reciprocal differences for protein content in dry beans in F2. Kornegay and Cardona (1991) reported maternal effects for seed size in the F1 crosses in dry beans. However, significant maternal effects for weevil (*Sitophilus zeamais*) resistance have been reported in maize (Derera *et al.*, 2001a; Dhliwayo *et al.*, 2005). This is not surprising in view of the genetic and tissue composition of the maize kernel, which is different from that of legumes such as dry beans. The 3n endosperm of a maize kernel has a 2n gene complement from the mother plant whereas the pericarp that forms the first line of defense against a weevil attack is entirely maternal tissue. Thus, in cereals such as maize the gene dosage of the caryopsis is in favour of the maternal parent, which explains the importance of reciprocal differences since the weevils feed on the endosperm. In beans the 3n endosperm disappears during seed development; thus bruchids feed on the cotyledons (2n) which have an equal gene dosage from both parents. The seed coat, which is entirely maternal tissue, was significant in influencing resistance to bruchids in dry beans (see Chapter 3). This could therefore partly explain the reciprocal differences that were displayed by the four crosses (Table 4.8) in the F3 generation seed. A recent study by Somta *et al.* (2007) to understand mode of

inheritance of seed resistance to *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.) in mungbean (*Vigna radiata* L. Wilczek) also showed that resistance was controlled by maternal plant genotype. However, in this present study, the seed coat alone was not enough to explain the resistance in the parent lines, therefore, cotyledons were also important in the model (see Chapter 3). There is no evidence in the literature that has supported the importance of embryo in conferring weevil resistance even in cereals (Widstrom and McMillan, 1992).

4.4.4 Transgressive segregation

Transgressive segregation for *A. obtectus* resistance in dry beans has not been reported in the literature. With respect to crop improvement, transgressive segregation represents a potential source of novel genetic variation. The most appropriate explanation for the transgressive segregation observed is that of complementary gene action with additive gene effects dispersed in the parents (Rick and Smith, 1953; Xu *et al.*, 1998). The non-significance of the chi-square for single- and two-gene models plus transgressive segregation observed in F3 generation seeds, suggests that more than two genes are involved in controlling bruchid resistance. The continuous distribution pattern observed in some of the F3 populations further supports this argument, and suggests a quantitative mode of inheritance of bruchid resistance. The occurrence of transgressive segregation for resistance indicates that in some of these populations there would be potential for improving resistance in dry beans by selection where desirable genes from both sets of parents could be incorporated.

4.4.5 Gene action and the genetic parameters

The additive and dominance effects were highly significant for adult bruchid emergence and were adequate in explaining the resistance. The analysis of the W_r-V_r for the lines was not significant, confirming that epistasis was not important for resistance. The average degree of dominance of 1.4 implies that the action of genes was in the over-dominance range. Thus, although both additive and dominance action governed the expression of resistance, the dominance component was relatively more important. The low heritability in the narrow sense further reinforced the usefulness of dominance effects in explaining bruchid resistance, which suggests that breeding for bruchid resistance cannot be easy; because the dominance effects are not fixable in self-pollinating crop like beans. The genetic components of variations were high compared to

the environmental variance because this was a laboratory experiment performed under controlled conditions, and the errors of measurement were minimised. The proportion of genes with positive (u) and negative effects (v) of 0.22 (i.e., $u \times v$), which is very close to the expected maximum of 0.25, when $u=v=0.5$, suggests that genes with increasing or decreasing effects on resistance are probably symmetrically distributed among the parental lines used in the diallel set. The estimated number of dominant genes was four-fold higher than the number of recessive genes which is significantly greater than unity, indicating a higher frequency of dominant alleles in the parents. Although not significant at $P=0.05$, the positive values of F could also suggest preponderance of dominant genes. The correlation coefficient between the parental order of dominance (W_r+V_r) and the parental mean for the number of bruchid emergence was not significant, hence it can be concluded that the dominant genes in the parental lines were equally positive and negative i.e., some dominant genes influenced resistance while others increased susceptibility in controlling bruchid resistance. Inheritance and gene action for bruchid resistance in dry beans have scarcely been reported in the literature.

4.5 Summary and conclusions

Conclusions from this study relate to the six parental lines used in the diallel mating scheme because the sample size was small as only a few lines were used. Resistance was controlled by both additive and dominance gene action, but there was preponderance of dominance gene action. Generally, over-dominance gene action was important in F_2 generation, but partial dominance was also evident in five of the 13 populations evaluated in the F_3 generation. The additive-dominance model was adequate to explain the variation among genotypes indicating that epistatic gene effects were not important in controlling bruchid resistance. There was evidence for the role of maternal effects or cytoplasmic genes in the inheritance of bruchid resistance, because reciprocal differences were significant for the number of adult bruchid emergence in four crosses involving KK35, KK73, KK25 and Maluwa in the F_3 generation, although reciprocal differences were not significant at $P=0.05$ for number of bruchid emergence in the F_2 , and Dobie susceptibility index data in the F_3 generations. However, it is not clear why some pairs of reciprocal crosses showed maternal effects or reciprocal differences while others did not. Results of the study clearly show a quantitative mode of inheritance as evidenced by existence of the transgressive segregation and continuous distribution of bruchid resistance in F_3 generation seeds. Transgressive

segregation for *A. obtectus* resistance confirmed that more than one gene was involved in conditioning resistance. Lines KK35 and KK73, which displayed significant negative GCA effects for bruchid emergence, would be valuable sources in breeding resistance in dry beans.

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Chapter Five General Overview

5.1 Introduction

This chapter provides an overview of the study, re-stating the main research objectives. It summarises the main findings. Limitations, challenges and implications of the findings and directions for future research (recommendations) are outlined.

The study had the following general objectives:

- (v) Determine farmers' perceptions of the importance of damage by *Acanthoscelides obtectus* Say (bean weevil) and *Zabrotes subfasciatus* Boheman (Mexican bean weevil) to the dry bean both in the field and storage;
- (vi) Solicit farmers' views on what they consider important traits in a bean cultivar and their implication for breeding;
- (vii) Identify effective and adaptable sources of resistance to the two economically important bruchid species, using Malawian dry bean landraces, so that farmers can grow bean varieties that are resistant to bruchids, are well adapted to their agro-ecologies and have the preferred varietal traits;
- (viii) Determine the mode of gene action and inheritance of *A. obtectus* resistance in Malawian dry bean landraces.

5.2 Summary of the major findings

5.2.1 Smallholder farmers' perceptions of bean bruchid damage and varietal preference

- By involving farmers, it has been established that bruchids were important storage pests for dry beans in Malawi, causing economic losses and destroying the food value of stored beans.
- Farmers preferred bean varieties with large-seeded sizes and good flavour.

- Local landraces were preferred to new improved bean varieties on the basis of their excellent taste and yield stability even though improved cultivars have high yield advantages which farmers appreciated.
- Farmers had a seed colour preference: dark red and red speckled seed colours were preferred to green, yellow or black seeds.
- An appraisal of the effectiveness of selected indigenous bruchid control methods used by smallholder farmers showed that some methods, such as using bean plant ash, were effective in controlling the bruchids. However, it was shown clearly that the use of these plant botanicals in combination (as a mixture) was more effective than applying them as single treatments.

5.2.2 Dry bean germplasm screening for sources of bruchid resistance

- Effective and adaptable sources of bruchid resistance were identified in Malawian dry bean landraces: KK35, KK73 and KK90 and other bean genotypes such as KK69, KK02 and KK03 showed moderate resistance when infested both in the laboratory and in the field.
- Generally, laboratory testing cannot reliably predict field resistance in the genotypes, as genotype ranking for progeny emergence after field infestation and laboratory infestation were not consistent. This could be explained by the fact that two different resistance mechanisms operate; that is pod wall represents first line of defence in the field whereas the seed coat is the first line of defence when shelled seed was tested in the laboratory. Farmers normally store shelled seed hence the laboratory tests would be more representative of the seed store situation.
- Results of the study showed that plant morpho-physiological traits such as flower colour, pod colour, days to flowering and days to physiological maturity did not influence bruchid attacks on the host plants in the field.
- There was strong evidence that the seed coat, with its constitutive elements (chemical and physical properties), was in part responsible for conferring resistance against *A. obtectus* in the Malawian bean landraces. A decortication

study confirmed this observation as removal of the seed coat made resistant genotypes susceptible. In sharp contrast, the removal of the seed coat increased the resistance of bean seeds to *Z. subfasciatus*.

- Biochemical analysis shows no evidence of the presence of arcelin in the Malawian resistant landraces. These results confirm earlier findings that arcelin is found only in wild bean accessions and not in the cultivated beans.
- All of the improved bean cultivars, released commercially for production, were highly susceptible to *A. obtectus* infestation. The frequency distribution patterns for bruchid resistance strongly indicate that breeders have in the past indirectly selected bean varieties that are more susceptible to bruchids, which is confirmed by large grain losses (38%) in storage. Although not tested in the current study, some previous studies suggested that bruchid resistance was negatively correlated to yield.

5.2.3 Genetic analysis of resistance to *A. obtectus* of the Malawian dry bean germplasm

- Specific combining ability (SCA) and general combining ability (GCA) effects were important for the genotypic variation in resistance to *A. obtectus*.
- Reciprocal effects were not significant in the F₂ generation seed but were significant in some reciprocal crosses (KK35/Maluwa and Maluwa/KK35; KK73/KK25 and KK25/KK73; KK35/KK73 and KK73/KK35; KK35/Maluwa and Maluwa/KK35) in the F₃ generation seed, indicating that maternal influence or cytoplasmic inheritance was also important in determining resistance to *A. obtectus*.
- SCA effects were responsible for 81% of the variation in bruchid resistance, indicating the predominance of the non-additive gene action for determining resistance to bruchids in beans.
- A chi-square test for goodness of fit for a single-gene model showed that segregation in five of the 13 F₂ populations conformed to a 1:2:1 single gene partial dominance ratio, indicating the importance of partial dominant gene action in these populations.

- A chi-square test for goodness of fit for a two-gene model, performed on 8 of the 13 populations, did not conform to the 1:4:6:4:1 two-gene model segregation ratio of resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible resistant classes, respectively.
- The additive-dominance model explained the variation among genotypes and confirmed that epistatic effects were not important in controlling bruchid resistance.
- The average degree of dominance was in the partial dominance range in five populations and in the over-dominance range in eight populations.
- A frequency distribution of bruchid resistance in the F2 genotypes showed strong evidence of transgressive segregation as some populations had genotypes that were either more resistant or more susceptible than their parents.

5.3 Breeding implications

- The participatory appraisal study has clearly shown that it is important for breeders to realise that other than yield, characteristics such as seed size, seed colour, cooking time and flavour are also important; farmers use them to weigh up the value of new varieties. This underscores the need to involve farmers in variety development so that the adoption of newly-developed varieties is enhanced. Effective crop improvement programmes entail the final products being adopted by farmers. However, developing a variety with all the desirable traits may be a challenging task as some traits may be correlated negatively with yield.
- SCA effects accounted for 81% of the phenotypic variation for bruchid resistance, implying that non-additive gene action was important. From a breeding perspective, it means that breeding for bruchid resistance would be difficult (Hakizimana *et al.*, 2004), because non-additive gene action cannot be fixed in a crop such as the dry bean, which is a pure line. In addition, the association of the potential lines (KK35 and KK73) with cytoplasmic inheritance poses more

challenges for the breeder as the presence of maternal effects reduces the response to selection.

This probably elucidates why, up until now, attempts to develop bean varieties resistant to the bruchid have not been successful. However, the identification of resistant Malawian bean landraces that displayed a highly negative and significant GCA for resistance (KK35 and KK73) will be exploited in a breeding programme to develop new cultivars or improve resistance in the susceptible but agronomically superior commercial bean varieties currently used by farmers in Malawi.

- The transgressive segregation for *A. obtectus* resistance, reported for the first time in this study, implies that in some populations the segregants can be used to improve bruchid resistance.
- The high susceptibility levels of all the improved varieties imply that breeders did not select for bruchid resistance in the past, but rather concentrated on agronomic traits such as yield and breeding for disease resistance. It is necessary for breeders to screen all potential bean lines for bruchid resistance before they are released to farmers. Routine screening of all breeding lines should be initiated and rapid screening methods developed. Determination of DSI is time consuming as such there is need to develop rapid methods for measuring bruchid resistance.
- Laboratory and field infestations of beans by bruchids did not show a consistent performance. In general, some genotypes that were found to be resistant under no-choice tests in the laboratory were susceptible under free-choice tests in the field. This means that when screening bean genotypes for bruchid resistance, both laboratory and field infestation methods should be used to validate the results before deriving any meaningful conclusions. Pod wall in the field and the shelled bean in storage offer different challenges to bruchid attack suggesting that different defence mechanisms exist hence should be considered by a breeder when breeding for bruchid resistance. Importantly, validation of resistance should be performed in on-farm storage where insect pressure and conditions are different compared to glass jar experiments.

- Over 90% of the bean germplasm collected had medium- to large-sized seeds. Small-seeded bean varieties are not preferred and would not easily be accepted. Bean improvement programmes in Malawi must therefore target bean varieties that are large-seeded (Andean type) to enhance adoption. Resistance to bruchids should therefore be bred in the medium- to large-seeded bean varieties. The identified resistant sources (KK35 and KK90) were medium and large-seeded, respectively; hence they have a high utility for use in breeding programmes. In any case, seed size was not correlated with resistance in the current study, suggesting that selection for bruchid resistance will not affect seed size.

5.4 Challenges in breeding for bruchid resistance in dry beans

There are a number of challenges that affect the effective breeding and subsequently delivery of bruchid-resistant varieties for use by small-scale farmers. These are outlined as follows:

- Relative to disease resistance, few cultivars have been developed that are resistant to storage pests. This is partly due to the nature of resistance to insects and to the relatively late interest shown in developing bruchid-resistant cultivars of dry beans. Resistance to storage pests is often only partial (Dent, 2000), consequently development of resistance to insects has taken second place to that of resistance to pathogens, which is easier to improve. In most cases, disease resistance is highly heritable and selection is effective. The involvement of complete resistance to diseases which is controlled by one or few genes has largely been appealing to breeders and farmers.
- The major challenge in screening for bruchid resistance, as experienced in this study, was the problem of limited seed. This is a serious problem when conducting such laboratory feeding trials as previously reported (Kornegay *et al.*, 1993). The destructive nature of the test material after the experiment leaves the breeder with very little seed for future work or not enough to repeat the experiment where there is a need to do so. Laboratory screening of genotypes for bruchid resistance needs to be planned carefully to ensure that there is enough test material and that the insects are available as and when required.

Sustaining laboratory insect cultures for timely use in feeding trials can be a challenge. This and other factors could have discouraged scientists from breeding for resistance to storage insects. Some non-destructive methods for evaluating resistance in early generations should be found.

- Breeders become discouraged from characterising germplasm for storage pest resistance due to time constraints, the costs associated with controlled screening experiments and the practical difficulties of evaluating the large number of entries a breeding programme would require, especially in the early generation of plant breeding. Thus, screening for resistance has to be delayed and conducted on the late generations after much of the variability has been lost.
- Complications presented by low heritability due in part to the preponderance of non-additive genetic variance and the inconsistency of test results as shown in this study would also discourage breeders to engage in breeding for bruchid resistance in beans. There is therefore a need to develop sound evaluation techniques that are highly repeatable so as to increase the heritability of resistance in breeding populations.

5.5 Directions for future research

- Molecular techniques such as marker-assisted selection could be a good strategy for bruchid-resistant cultivar development. The ability to identify quickly and effectively which early generation plants carry the desired gene for resistance will expedite evaluation and save generations of laboratory and field testing. The use of marker-assisted selection (MAS) is non-destructive of seed, as leaf samples are taken at the seedling stage. Future studies should therefore evaluate the possibility of using MAS.
- The identification of biochemical mechanisms of resistance in landraces is important as this is the first finding of high resistance to bruchids especially against *A. obtectus* in commercial varieties. This should be followed by further studies on genetic bases of the resistance in all the identified sources.

- An inheritance study for resistance to *Z. subfasciatus* should be investigated in future studies to establish whether or not the inheritance mechanism, which was determined in the current study, would be the same as for *A. obtectus*.
- This study has shown that the seed coat has been responsible for conferring resistance, particularly to *A. obtectus*. Future research should investigate the chemical composition of the seed coat to establish the presence of such compounds as phytohemagglutinin or lectin and levels of phenolic compounds in the seed coat, which have been reported to confer resistance in seeds. The biochemical analysis results from the current study seem to indicate the presence of lectin in some of the genotypes. A more detailed study should be conducted to confirm the role of lectins in conferring resistance.
- Most of the indigenous bruchid control methods that farmers use in Malawi were less effective than the use of chemicals, which unfortunately are beyond the reach of most farmers in remote areas of the country. An integrated strategy for bruchid management should be pursued by combining indigenous bruchid control methods with the identified host plant resistance. This would be the most effective approach to managing storage insects in order to reduce grain losses in rural stores in Malawi.

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