EVALUATION OF INTRODUCED COWPEA BREEDING LINES FOR APHID (APHIS CRACCIVORA) AND BRUCHID (CALLOSOBRUCHUS RHODENSIANSUS) RESISTANCE IN SOUTH AFRICA

by

ISAAC MOTSOENG LETSOALO

MINI-DISSERTATION

Submitted in partial fulfilment of the requirements of the degree of

Master of Science

in

Agriculture (Agronomy),

In the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental sciences)

at the

UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR: PROF J.A.N. ASIWE

TABLE OF CONTENTS

		Page
TITLE	PAGE	i
TABLE	OF CONTENTS	ii-iv
DECL	ARATION	٧
DEDIC	CATION	vi
ACKN	OWLEDGEMENTS	vii
LIST (OF TABLES	viii
LISTO	F FIGURES	ix
LIST (OF APPENDICES	Х
ABSTI	RACT	xi-xiii
CHAP	TER 1: GENERAL INTRODUCTION	1
1.1	Background	1
1.2	Problem statement	2
1.3	Motivation of the study	2
1.4	Purpose of the study	3
	1.4.1 Aim	3
	1.4.2 Objectives	3
1.5	Hypotheses	3
CHAP	TER 2: LITERATURE REVIEW	4
2.1	Distribution and origin of cowpea	4
2.2	Importance and utilisation of cowpea	4
2.3	Constraints in cowpea production	5
2.4	Major insect pest of cowpea	5
2.5	Biology and ecology of cowpea aphid	6
2.6	Effect of aphids on cowpea (Vigna unguiculata)	6
2.7	Mode of aphid feeding	7
2.8	Damages and losses caused by cowpea aphids (Aphis craccivora)	7
2.9	Aphid borne mosaic virus	8
2.10	Resistance of cowpea lines against cowpea aphids	8
2.11	Cowpea beetle (Callosobruchus rhodensiansus)	9

2.12	Biolog	y and ecc	ology of cowpea weevil (Callosobruchus rhodensiansus)	9
2.13	Effect	of bruchio	ds on cowpea (<i>Vigna unguiculata</i>)	10
2.14	Effect	of aphid a	and bruchid on cowpea yield	10
2.15	Mode	of bruchic	d feeding	11
2.16	Resist	ance of c	owpea lines against bruchids	11
2.17	Dama	ge and lo	sses caused by bruchids	12
2.18	Host-p	olant resis	tance to insect pests of cowpea	12
2.19	Contro	ol of insec	t pests of cowpea	12
2.20	Host-p	olant resis	tance to insect pests of cowpea	12
CHAP ⁻	TER 3: MA	TERIALS	S AND METHODS	13
3.1	Projec	t and stud	dy sites description	13
3.2	Field 6	experimer	nt for aphid screening	13
	3.2.1	Data co	llection from aphid screening	14
3.3	Labora	atory expe	eriment for bruchid screening	14
	3.3.1	Data co	llection from bruchid screening	15
3.4	Data a	analysis		15
CHAP ⁻	TER 4: RE	SULTS A	ND DISCUSSION	16
4.1	Field 6	experimer	nt results (Aphid screening)	16
	4.1.1	Aphid in	cidence of different cowpea lines	16
		4.1.1.1	Rating for aphid score	17
		4.1.1.2	Effect of aphids on plant height	18
		4.1.1.3	Effect of aphids on plant vigour	19
		4.1.1.4	Cowpea phenology (50% flowering)	20
		4.1.1.5	Canopy height	21
		4.1.1.6	Canopy width	22
		4.1.1.7	Pod weight	24
		4.1.1.8	Grain yield	25
4.2	Labora	atory expe	eriment results (Bruchid screening)	28
		4.2.1	Percentage adult emergence of bruchids	28
		4.2.2	Number of days to insect emergence (NIE)	30
		4.2.3	Mean developmental time (MDT)	31
		4.2.4	Total developmental time (TDT)	32
		4.2.5	Percentage of damaged seeds (%DS)	34

4	1.2.6	Percentage seed weight loss (%SWL)	37
CHAPTER 5: CON	CLUSIO	NS AND RECOMMENDATIONS	40
REFERENCES			41

DECLARATION

I declare that the mini-dissertation hereby submitted to the	ne University of Limpopo for
the degree of Master of Science (MSc) in Agriculture (Ag	gronomy) has not previously
been submitted by me for a degree at this or any other	university; that it is my work
in design and execution, and that all material contain	ned herein has been duly
acknowledged.	
Letsoalo, IM (Mr)	Date

DEDICATION

I dedicated this work to my beloved brothers and sisters and to my honourable parents Mr. Andries and Mrs. Monicca Letsoalo; I have perceived their love in ensuring and providing courage to fulfil my dreams every step of the way.

ACKNOWLEDGEMENTS

I will like to thank my saviour and protector the Almighty God, for giving me knowledge, understanding and wisdom throughout the study and for providing me with abilities and strength to complete this research. Without him I would be like a puppy left in the bush not knowing where to go.

Special appreciation goes to my supervisor Prof. J.A.N. Asiwe for his fatherly assistance, patience, motivation and support. Without his sacrifices and contribution this study would not have been successful. Thanks to my technicians Mr. F.H Nndwambi, Mr. K.C. Phefadu and Mrs. P.M. Kgopa for their technical assistance and all the lecturers who contributed to this study. More thanks go to Department of Agriculture Forestry and Fisheries (DAFF) for financial assistance that made this study easy and possible.

Thanks to Maphosa T.M, Moropa P.B, Gololo M.N. and Mokou M.B. and other colleagues for their help with field and laboratory work. May God bless them at all times. To all my friends, I say thanks for being with me during happy and bad times.

LIST OF TABLES

		Page
Table 1	Plant height of cowpea lines after infestation	18
Table 2	Rating of plant vigour to aphid infestation of different cowpea lines	19
Table 3	Canopy height of cowpea lines for aphid infestation at maturity	22
Table 4	Canopy width of cowpea lines for aphid infestation at maturity	23
Table 5	Effect of aphid infestation on pod weight of cowpea lines	24
Table 6	Grain yield of cowpea lines after aphid attack	26
Table 7	Percentage adult emergence after infestation	28
Table 8	Number of days to bruchid emergence after infestation	31
Table 9	Total developmental time of bruchid in the endosperm of seeds	33
Table 10	Percentage of damaged seed by cowpea beetle (Bruchid)	35
Table 11	Seed weight loss after bruchid damage in storage	37

LIST OF FIGURES

		Page
Figure 1	Aphid incidence of cowpea lines	16
Figure 2	Frequency distribution of aphid severity score	17
Figure 3	Frequency distribution of the best 10 lines compared with control check	19
Figure 4	Best 10 lines in plant vigour compared with the control check (Vita 7)	20
Figure 5	Frequency distribution of the number of days to 50% flowering	21
Figure 6	Top 10 best lines compared to the control check (Vita 7) in canopy height	22
Figure 7	Canopy width of lines attacked by aphids towards maturity	23
Figure 8	Frequency distribution of canopy width (cm) at maturity stage	24
Figure 9	Frequency distribution of pod weight (g) of the lines screened	25
Figure 10	Frequency distribution of best 10 lines in terms of grain yield (g)	26
Figure 11	Frequency distribution of grain yield of cowpea lines after aphid attack	27
Figure 12	Percentage levels of adult emergence	29
Figure 13	Cowpea lines with low level of percentage adult emergence	30
Figure 14	Mean developmental time of emerged bruchid	32
Figure 15	Frequency distribution of Total developmental time of cowpea bruchid	33
Figure 16	The best ten cowpea lines with long developmental time	34
Figure 17	Frequency distribution of percentage damaged seeds (%DS)	35
Figure 18	Top ten cowpea lines with the lowest percentage of damaged seeds	36
Figure 19	Relationship between percentage adult emergence and damaged seed	36
Figure 20	Top 10 cowpea breeding lines with the lowest % seed weight loss	38
Figure 21	Percentage seed weight loss after bruchid attack	39
Figure 22	Relationship between percentage of damaged seed and seed weight loss	39

LIST OF APPENDICES

		Pag
Analysis of Variance	e (ANOVA) tables for field study	45
Appendix 1	Analyses of variance for aphid incidence (%) of cowpea lines	45
Appendix 2	Analyses of variance for aphid score	46
Appendix 3	Analyses of variance for plant vigour	47
Appendix 4	Analyses of variance for plant height (cm)	48
Appendix 5	Analyses of variance for number of days to 50% flowering	49
Appendix 6	Analyses of variance for canopy height at crop maturity (cm)	50
Appendix 7	Analyses of variance for canopy width (cm)	51
Appendix 8	Analyses of variance for pod weight (g)	52
Appendix 9	Analyses of variance for grain yield (g)	53
Analysis of Varianc	e (ANOVA) for laboratory study	54
Appendix 10	Analyses of variance for percentage adult emergence (%AE)	54
Appendix 11	Analyses of variance for number of days to insect emergence (NIE)	55
Appendix 12	Analyses of variance for mean Developmental Time (MDT)	56
Appendix 13	Analyses of variance for Total Developmental Time (TDT)	57
Appendix 14	Analyses of variance for percentage of damaged seeds (%DS)	58
Appendix 15	Analyses of variance for percentage seed weight loss (%SWL)	59

ABSTRACT

Cowpea is an important grain legume in South Africa after groundnuts and dry beans (Asiwe, 2009). It is important because it serves as a source of nutrients for human and as fodder for animals and its ability to fix atmospheric nitrogen enhances soil fertility. Production of cowpea (Vigna unguiculata [L.] Walp) in South Africa is limited by lack of improved cowpea varieties. Therefore, new introductions of cowpea breeding lines were made and evaluated for their adaptation. These introduced breeding lines have not been screened for cowpea aphid resistance in South Africa. The objective was to evaluate the response of introduced cowpea breeding lines from IITA, Nigeria and Texas A&M University to aphid infestation. Ninety seven elite cowpea breeding lines were evaluated in a field trial during 2013-14 growing season at the University of Limpopo Experimental Farm, Mankweng, Limpopo Province. The experimental design was lattice design with three replications and local line Vita 7 was the control. Three weeks prior to planting test lines, spreader rows were planted in areas which would surround test lines to ensure aphid spread. There were highly significant differences among the parameters measured such as aphid incidence, aphid score, plant vigour, plant height, canopy width, number of days to 50% flowering, pod weight and grain yield. Eighty six (89%) of the 97 lines exhibited 100% aphid incidence indicating that lines were uniformly infested. Control was susceptible to aphid as indicated by aphid and plant vigour scores. Only 22 lines had aphid score of 1 to 4 (resistant), but 32 lines were medium strength to strong (vigorous). Plant biomass (r = -0.71), plant height at 3WAI (r = -0.75), plant vigour (r = -0.84) were strong and negatively correlated to aphid score. Canopy height at crop maturity (r = -0.66) and canopy width (r = -0.55) were medium to strong and negatively correlated with aphid score. Pod weight (r = -0.34) and grain yield (r= -0.32) were weak and negatively correlated with aphid score. The results from this study indicated that the cowpea breeding lines introduced responded differently to aphid infestation and aphid damage reduced the performance of the lines. Cowpea lines with better aphid severity, promising plant vigour, high canopy height and grain yield were selected to meet the needs of the poor masses.

Additionally, the identified 22 resistant lines and 32 lines with promising plant vigour will be subjected to more intensive evaluation to validate the results obtained from this study.

Another major damaging pest of cowpea [Vigna unguiculata (L.) Walp] is Callosobruchus rhodensiansus, usually known as bruchid or cowpea beetle. In this study, different cowpea lines were evaluated for their resistance. These introduced cowpea breeding lines have not been evaluated for cowpea bruchid resistance in South Africa. The objective was to evaluate the response of cowpea breeding lines from IITA, Nigeria and Texas A&M University to bruchid infestation. Ninety seven elite cowpea breeding lines were evaluated in laboratory during the 2014 growing season at the University of Limpopo F block, Department of Plant Production, Limpopo Province. The experimental design was randomized complete block design with three replications. Prior to infestation, seeds of the cowpea lines were placed in petri dishes and kept in an oven at 50°c for 3 hours to kill any pre-existing larva in the seeds. Each petri dish containing 10 seeds was infested with freshly emerged bruchid to lay eggs for 24 hrs. Egg count was done with the aid of a magnifying lens 3 days after infestation. For uniform infestation, only one egg was left on each seed and excess removed using a razor blade. The following variables were collected %adult emergence, number of days to insect emergence, mean developmental time, % damaged seeds and % seed weight loss. Results showed that there were highly significant differences among the variables measured. Number of days to insect emergence (r = -0.29), Mean developmental time (r = 0.36), total developmental time (r = -0.08), % seed weight loss (r = 0.38) were weakly correlated to % adult emergence. Meanwhile, percentage of damaged seed showed strong correlation (r = 0.86) with % adult emergence. The results of this study indicated that the breeding lines responded differently to bruchid infestation. The identified resistant lines IT95K-1491, IT98K-692, TX08-30-6, IT86D-1010 and IT98K-692 will be subjected to more intensive evaluation to validate the results obtained from this study. Cowpea lines with long developmental time, low level of damaged seeds and low seed weight loss will then be introduced to small holder farmers in South Africa as they will have the possibility of being stored for long period in storage. The findings further suggest that the level of resistance in these lines can provide reasonable protection against bruchids during storage.

Key words: Aphid infestation, breeding lines, cowpea aphid, bruchid infestation, cowpea bruchid

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Cowpea [Vigna unguiculata (L.) Walp] is the third most important grain legume in South Africa after groundnut and dry beans (Asiwe, 2009). It is a dicotyledonous plant and a multi-functional crop in a sense that it is eaten as green leaves, green pods and green seeds (Singh et al., 1996). It is a natural supplement to cereals in family diet due to its high protein content of 23-25% (Giga, 2001). It is therefore a major source of cheap vegetable protein for rural dwellers in South Africa. In general, production and productivity of cowpea in South Africa is low due to lack of improved cowpea lines and good quality seeds for planting (Singh and Singh, 1990). Others include drought, biotic stress that include weeds, diseases and insect pests (Asiwe et al., 2009).

The insect pest spectrum of cowpea is wide and practically every stage of the crop is affected (Jackai and Daoust, 1986). Aphids are one of the serious insect pests in South Africa that limit production of cowpea (Asiwe, 2009). Bruchid (*Callosobruchus rhodensiansus*) is another threatening insect pest responsible for the damage of cowpea grains in storage and it is a serious post-harvest pest (Oluwafemi *et al.*, 2013). In storage, *Callosobruchus rhodensiansus*, also called cowpea beetle is considered as one of the most important insect pest of cowpea both in Africa and Asia (Jackai and Daoust, 1986). Few resistant cowpea lines that escape the damage of seedling and post-flowering pest spectrum are vulnerable to bruchid damage in the storage (Asiwe *et al.*, 2009). For this reason, it is crucial to screen cowpea lines for resistance to this pest to enhance good yield and quality seeds for both planting and consumption.

1.2 Problem statement

In South Africa, one of the problems facing cowpea production is that there are no improved cowpea breeding lines for farmers to plant. The popular lines commonly used are Glenda and Bechuana white which are made available to farmers since late 60s for animal feed. The Agricultural Research Council (ARC) has introduced some cowpea germ-plasm from IITA, Nigeria and the majority has not been screened against the above mentioned insect pests. Little work has been done on the response of introduced breeding lines to aphid and bruchid resistance in South Africa. In addition, lack of improved cowpea lines and limited breeding work occasioned by insufficient funding has resulted in low productivity and low rate of adoption of improved cowpea lines by farmers.

1.3 Motivation of the study

Food security may not be fully realized if food crops that constitute people's dietary needs are not produced in sufficient quantity. Cowpea is one of the important grain legume crops in South Africa that can meet the dietary needs of the poor masses. The production of this crop in South Africa is constrained by insect pests, notably cowpea aphids and bruchids. Total yield loss due to these two pests is possible under severe attacks, if no resistant line is grown. No cowpea lines grown in South Africa have been quantified for resistance to these two pests of cowpea. The ARC has introduced several improved breeding lines and currently over 50 lines have been introduced through AgriLIFE, A&M University, Texas but none of these lines has been screened for aphid and bruchid resistance. This study will firstly evaluate and select the promising resistant cowpea lines for possible recommendation for on-farm trials and for adaptive breeding program. Secondly, it will provide useful information on the reaction of the breeding lines to aphid and bruchid infestation which currently is not available in South Africa.

The results of this study will benefit small-holder farmers in South Africa by planting lines that are resistant to aphid and bruchid infestation as it will minimize the cost of production needed for the control the insects. This will invariably increase the profitability of farmers. Another expected outcome (long-term) of the study is that it will enhance the productivity as well as increasing their family

income and employment for all in the value chain. In addition, training of more experts will improve the human capital development of University of Limpopo and South Africa in general.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to screen cowpea breeding lines introduced from IITA, Nigeria and A&M University Texas, USA for two insect pest resistances in South Africa.

1.4.2 Objectives

The objectives of the study were to evaluate introduced cowpea breeding lines for aphid and bruchid resistance in South Africa

1.5 Hypotheses

- i) The introduced cowpea breeding lines do not differ in their resistance to cowpea aphids in South Africa.
- ii) The introduced cowpea breeding lines do not differ in their resistance to cowpea bruchids (beetles) in South Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 Distribution and origin of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most critical human food and was been used as a crop plant since Neolithic times (DAFF, 2011). The production of cowpea has spread to East and Central Africa, India, Asia, South and Central America (Saria, 2010). It is originated in savannah region of west and Central Africa (Jackai and Daoust, 1986). Some literature indicated that cowpea was introduced from Africa to the Indian subcontinent approximately 2 000 to 3 500 years ago, at the same time as the introduction of sorghum and millet, while others state that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia (DAFF, 2011). Cowpea is believed to have originated from West Africa by some workers, because both wild and cultivated species thrive in the region (Jackai and Daoust, 1986). Cowpea plays a major role in the family diet as it is utilised in different ways.

2.2 Importance and utilisation of cowpea

Cowpea is an important crop grown worldwide (Singh *et al.*, 1996). It is used as a vegetable using both the spreading types and grain types. Singh *et al.* (1996) revealed that utilisation of cowpea grains as a vegetable provides an inexpensive source of protein in the human diet. Apart from that, cowpea is also grown as a dual-purpose crop because the green pods are used as a vegetable and the haulms utilised for livestock fodder during the dry season when food is scarce (Asiwe, 2009). DAFF (2011) stressed that cowpea is a very palatable crop, highly nutritious and relatively free of metabolites. Cowpea meals can be served with various popular maize meals such as custard, bread, pap and rice in South Africa. Besides its importance and utilisation, cowpea production in South Africa is low due to possible constraints that are to be outlined.

2.3 Constraints in cowpea production

Production of cowpea in South Africa has recently experienced some major draw backs which caused major reduction in cowpea yield from farmers (Asiwe *et al.*, 2009). Research on production of cowpea has been neglected in South Africa in the last three decades (Asiwe, 2009). This is caused by lack of improved cowpea breeding lines, lack of knowledge on noble agronomic practices such as conservation agriculture, organic farming and integrated pest management. Other constraints that hamper the production of cowpea include no good quality seed for planting and poor marginal returns to farmers further worsen the limitations to cowpea production in South Africa. The poor marginal returns are normally obtained because of crop failure. Other constraints include abiotic and biotic stress such parasitic weeds (such as *striga* and *alectra*), drought, floods and insect pests (Asiwe, 2009). The major constraints to cowpea production have been discussed in several studies (Singh *et al.*, 1996).

2.4 Major insect pests of cowpea

There are many different types of insect pests that affect cowpea to such an extent where low yields are attained due to heavy infestations. These types of cowpea insect pest include seedling insect pests such as Leaf hoppers, bud thrips and aphids, flowering and post flowering insect pests such as foliage beetles, flower thrips, maruca pod borer, pod-sucking bugs (*Anaplocnemis curvipes*, *Riptortus dentipes*, *Clavigralla tomentoscollis*, *Clavigralla shadabi* and *Nezara viridula*) and storage pests (*Callosobruchus spp*). According to Singh *et al.* (1996) the insect pest spectrum of cowpea is wide and practically, every stage of cowpea is attacked by insect pests. Apart from this, there are also post-flowering pest of cowpea which include storage beetles such as cowpea beetle (*C. rhodensiansus*, *C. analis*, *C. chinensis and C. maculatus*) as stressed by Devereau *et al.* (2002) and Oluwafemi *et al.* (2013). It is crucial to understand the biology and ecology of insect pests for effective evaluation and management in order to measure the level of infestation of cowpea insect pests.

2.5 Biology and ecology of cowpea aphid

Cowpea aphids (*Aphis craccivora*) belong to the family homoptera: Aphididae. They are soft bodied plant lice half an inch in size and they range in colour black, white, green and brown (Githiri *et al.*, 1996). There are two types of aphids which attack cowpea and these types include; wingless and winged aphids. They produce nymphs by viviposition (Jeff-Whitworth and Ahmad, 2009). They are also known to be small sap-sucking insects because they consume the contents of the plant tissues. The biology and ecology of cowpea aphids varies depending on climate and soil. Under favourable conditions a generation may take only 13 days and adults live from 6-15 days and may produce more than 100 progeny (Singh *et al.*, 1996). The life-cycle of aphids is by asexual reproduction. They give birth to nymphs which then develop into adult since aphids are viviparous (Githiri *et al.*, 1996). The major effect of aphids on cowpea has been greatly studied.

2.6 Effect of aphids on cowpea (Vigna unguiculata)

Aphids (*A. craccivora*) are partly responsible for pre-harvest pod damage in the field and they primarily infest seedlings, although large populations also infest flower buds, flowers and pods (Githiri *et al.*, 1996). They cause direct damage to the plant by removal of its phloem tissues (sap). Apart from physical damage, aphids are believed to transmit virus to cowpea plants and transmission causes aphid borne mosaic viruses (Giga, 2001). Small and isolated populations have no major impact on cowpea yield, but large populations can cause leaf distortion, stunted growth, and poor nodulation of root systems and in some cases, result in plant mortality and low yield (Singh and Singh, 1990). Cowpea aphids also reduce photosynthetic capacity of the crop because of colonies that cover the stems and leaves during feeding and thereby shading the crop from receiving sunlight and thus reducing growth noticed by distortion of leaves (Jackai *et al.*, 2001). The mode of aphid feeding on its substrate has been greatly studied.

2.7 Mode of aphid feeding

Cowpea is mostly attacked by several insect pests of which the most serious are the sucking piercing pests (Aphids) as revealed by Jackai *et al.* (2001). The cowpea aphids feed on young, succulent terminal growth by piercing plant tissue and penetrating the phloem with needle-like mouthparts (Githiri *et al.*, 1996). They suck fluid or sap from the stem, terminal shoots, petioles, flowers, seeds and pods (Kitch *et al.*, 1999). They also inject a powerful toxin into the plant when feeding and in situations where the populations are large, stunting and plant death results. These insects are responsible for the damage of the crop during vegetative stage in the field. Heavy feeding affect the growth of the crop causing stunted growth, pod set reduction, delay of flower initiation and plant maturity (Asiwe, 2009). In many cases, aphid feeding causes significant damage to the plant and it appears that aphids are able to escape plant defences while moving their stylets intercellularly and by manipulating their host through secretion of saliva into the phloem sieve elements (Walling, 2008). These sap-sucking insects cause damages and losses in susceptible cowpea lines by modifying metabolism of the plant.

2.8 Damages and losses caused by cowpea aphids (*Aphis craccivora*)

Aphids cause a serious damage to cowpea which in most cases result in low yield. The damage that is caused by aphids is mainly due to a number of causes, including loss of plant sap and clogging of leaf surfaces (Githiri *et al.*, 1996). However, large populations can turn leaves yellow and stunted growth of shoots (Bugg *et al.*, 2008). They also cause leaf curling which is a common symptom of aphid infestation that occurs when a colony attacks the underside of a leaf, causing the leaves to desiccate. The curling behaviour provides protection for the aphid colony, but the leaf becomes useless to the plant (Asiwe, 2009). The damages of aphids on cowpea cause a disease called aphid borne mosaic virus (GRDC, 2010).

2.9 Aphid borne mosaic virus

Aphids can damage cowpea by spreading viruses or causing direct damage when feeding on plants (GRDC, 2010). Feeding damage is caused by large population of aphids, but virus transmission can occur before aphids are seen to be present. Crops affected by aphids are noticed by yellowing and leaves and thus plant death (Singh *et al.*, 1996; Kitch *et al.*, 1999; Soleymane *et al.*, 2013). Viruses are widespread in pulses and can cause significant economic losses, especially when extensive infection occurs in early crop growth (GDRC, 2010). Kitch *et al.* (1990) stressed that the aphids transmit virus through vectors (stylet-borne) and seed. Transmission through seed is increased by planting affected seed year after year. The resistance of cowpea against sap-sucking insect pests (aphids) has been greatly studied.

2.10 Resistance of cowpea lines against cowpea aphids

Breeding for aphid resistance is needed to reduce further losses from cowpea aphid outbreaks in the field (Singh *et al.*, 1996). Singh and Singh (1990) revealed that the use of resistant lines is the cheapest, most effective and sustainable way to control insect pest in the production area. Resistance to aphid attack is characterized by a lower and isolated insect population density or fewer damage symptoms on the resistant plants (Giga, 2001). This can also be seen by the development of new leaves (trifoliate) even under heavy attack and thus the crop continues to flower and form pods (Singh *et al.*, 1996). The other threatening insect pest of cowpea includes cowpea beetle (bruchid) which has been greatly studied.

2.11 Cowpea beetle (*Callosobruchus rhodensiansus*)

Cowpea weevil belongs to the family Coleoptera: Bruchidae. They are hard bodied insect that are less than 1 cm in length. They vary both in size and colour and it is a field-to-storage pest (Oluwafemi *et al.*, 2013). The female weevils are dark coloured and larger while the male bruchids are light coloured and smaller in size. It is important to understand the biology and ecology of bruchid in order to evaluate the level of resistance and susceptibility of different cowpea lines.

2.12 Biology and ecology of cowpea beetle

Cowpea beetle have the capacity to lay eggs on the seeds and when they hatch, the larvae burrow into the seed and consume the endosperm of seeds (Singh and Singh, 1990). The entire life cycle of cowpea bruchid takes about 35 days. During development, the larva feeds on the interior of the seed, consuming the tissue leaving exit holes through which it exits when it becomes an adult. The holes make it easy to recognize infested seeds and also to determine the seed resistance through seed damage index. The matured adult emerges after a larval period of 3 to 7 weeks, depending on climatic conditions (Toriola, 2010).

Female beetle can lay up to sixty eggs in its life cycle. The life cycle of cowpea beetle consist of egg, larva, pupa and adult. The eggs are small, clear, shiny and smooth and oval shaped. They are normally glued firmly to the surface of pods and seeds (Toriola, 2010). The newly laid eggs are translucent grey and inconspicuous and when they hatch, the empty eggs shells become white and clearly visible to the naked eye. Eggs hatch within 5-6 days of oviposition. Upon hatching, the larvae bite through the base of the eggs and bore into the seeds where they spend the whole life cycle feeding on the seed. The larvae pupate inside the seed and pupation takes place in the seed. Pupation takes about 7 days to complete. The adult emerged from the seed do not live longer than 12 days (Singh and Singh, 1990). During this time the females lay more than hundred eggs. The optimum temperature for egg-laying is about 30 to 35° C. The effect of bruchid on cowpea in storage has been greatly studied.

2.13 Effect of bruchids on cowpea (*Vigna unguiculata*)

Cowpea suffers heavily from insects, both in the field and when grains are stored after harvest (Joana and Gungula, 2010). According to Redden and McGuire (1983) the simplest reliable variable to use for evaluation of resistance to bruchids is the percentage of undamaged seed with zero emergence holes. In addition to yield loss in the field, cowpea also suffers considerable loss in storage due to bruchids. The cowpea bruchid not only causes a reduction in dry weight but it also reduces grain quality and seed viability making it unfit for human consumption as well as for planting and thus causing substantial reduction in market value (Singh and Singh, 1990).

Bruchids change the flavour and nutritive value of grains which reduce the marketability and acceptability of pulses (Singh and Singh, 1990). They are not easily seen in the field but they are visible after harvesting of the crop from the field (Huignard *et al.*, 1985). Shade *et al.* (1990) was of the opinion that bruchids can destroy about 80% of cowpea grains in storage when the seeds are not treated with insecticides. Singh and Singh (1990) revealed that within a storage period of 100 days, the resistant varieties show about 25% damaged seeds in contrast to 95% damage for the susceptible varieties. Bruchids are responsible for seed weight loss and quality in storage (Singh and Singh, 1990). The mode of bruchid feeding on cowpea seeds has been done in several studies.

2.14 Mode of bruchid feeding

In stored seeds, cowpea weevil (bruchid) causes irreparable damage to the endosperm and reduces nutritive value and quality of seeds (Obopile *et al.*, 2011). The damage is caused by larvae feeding inside the seed by consuming the cotyledons and when adults emerge they leave circular exit holes (Devereau *et al.*, 2002). This is a practical situation where the seeds are often seen with holes especially on susceptible cowpea lines and the damage reduces the weight and may render the seeds to be unfit for human consumption (Shade *et al.*, 1990). The resistance of cowpea against storage insect pest has been measured.

2.15 Resistance of cowpea lines against bruchids

The resistance of cowpea to bruchid in storage is measured by the delayed, staggered and lower emergence levels of insects (Singh and Singh, 1990). Recent literature revealed that grains with hard seed coat, rough texture and wrinkled shape are more resistant to bruchid infestation than the soft, smooth and fine-textured grains (Singh et al., 1996). Bruchid infestations start in the field on pods but population growth of bruchids increase following threshing when eggs can be laid directly on the seeds in storage because they are oviparous. Giga (2001) stressed that bruchid larvae feed and develop inside the seed endosperm and emerge as adults after 3-4 weeks. The adult mate and give rise to another generation in the storage. Singh and Singh (1990) screened several cowpea varieties for bruchid resistance and reveal that it is not morphological characters such as seed weight and seed colour but the nutritional value of the seeds which will determine its acceptability or consumption. It has been reported that variables such as percentage adult emergence, developmental period and percentage seed weight loss are the most reliable indicators for resistance of cowpea to damage by C. rhodensiansus (Mogbo et al., 2004). Cowpea bruchid causes significant damages and losses that are greatly discussed.

2.16 Damage and losses caused by bruchids

The cowpea weevil (bruchid) is a serious pest of stored grain legumes. According to Oluwafemi *et al.* (2013) cowpea bruchid is an insect pest capable of causing high yield loss both in quantity and quality of the seeds. Shade *et al.* (1990) estimated that the dry weight loss due to damage by *C. rodensiansus* exceed 2900 tons each year. Additionally, bruchid infestation can impact on seed quality, reduces germination ability and quality for both planting and consumption (Toriola, 2010). There are several control measures of insect pests of cowpea.

2.17 Control of insect pests of cowpea

Various control measures are adopted to suppress insect pest population which include use of synthetic insecticides (chemical control), biological control or integrated pest management (IPM), cultural practices, double bagging and host-plant resistance (HPR). The use of chemical control is the quickest method to control aphids yet it is not environmentally friendly due to adulteration of both soil and water (Asiwe, 2009). Biological control involves the use of natural enemies or predators, parasites and pathogens to control cowpea insect pests. Double bagging is a technique of integrated pest management that involves covering of crops from attack by insect pests. This technique is used to control insect pests and to minimize the use of pesticides. It is a method that can be recommended to small holder farmers that are resource poor. The other cultural practices to manage cowpea insect pest involve crop rotation, intercropping and sanitation. The use of host plant resistant is the most important method adopted because it is environmentally friendly. This is because it does not involve the use of synthetic chemicals that pose danger to crops and soil. The other control measure of insect pest of cowpea is by host plant resistance (Ego, 2011).

2.18 Host-plant resistance to insect pests of cowpea

The most economical and environmentally friendly strategy of controlling insect pests both in-field and in storage is through host-plant resistance (Babura and Mustapha, 2012). In stored grains several factors lead to the production of resistance against infestation by storage insect pests. These include hardness of seeds which is thought to make insect penetration more difficult thus providing protection. The texture of the seed coat of cowpea seed may have negative influence on the oviposition of the cowpea weevil; the quantity and quality of nutritional constituents (Ahmed *et al.*, 2007). The other strategy of reducing seed damage in cowpea is by harvesting and storing cowpea seeds in pod form. Through host plant resistance, more cowpea breeding lines can be developed by crossing resistant varieties with susceptible lines.

CHAPTER 3

MATERIALS AND METHODS

3.1 Project and study sites description

Two experiments were conducted to assess the resistance of 97 introduced cowpea breeding lines in terms of their reactions to cowpea aphids and bruchids. This project was part of bigger cowpea breeding project being executed by IITA, Nigeria and A&M Texas, USA at Ukulima Farm, near Modimolle. The aphid screening was conducted in field at the University of Limpopo experimental farm (23° 49' S, 29° 41' E), near Mankweng in the Capricorn district of the Limpopo Province. Then, Bruchid screening was conducted at F block Laboratory, Department of plant production (23° 53'10" S, 29° 44' 15"E). The climate of the area is classified as semi-arid and receives average annual rainfall ranging from 400 mm to 600 mm that falls predominantly in summer.

3.2 Field experiment for aphid screening

The land was prepared using ploughs and harrows to soften the soil. Weeding was performed using post-emergence herbicides, Basagran at the rate of 180 ml per hectare. The experiment was laid in a randomized complete block design with 3 replications. Ninety seven cowpea breeding lines introduced from IITA Nigeria and A&M Texas University, USA were used. The seeds were obtained from Ukulima Research Station (URS) and planted in 1 row of 3 m length and 0.75 m spacing. In addition, adjacent control plot was planted were aphids was sprayed.

The aphid spreader rows were planted 3 weeks before the test lines using a cowpea line IT82D-889. The essence of planting the spreader rows earlier was to attract aphid spread and to sustain aphid infestation on the test lines as soon as they germinate to ensure uniform natural infestation. The aphids were scored on scale of 1 to 9 and plant vigour on the scale of 1 to 3 as described by Obopile (2006). Plant biomass was weighed at 3 weeks after planting (WAP). Reactions of the lines were evaluated at 3 WAI by estimating the degree of aphid colonization.

3.2.1 Data collection from aphid screening

The variables collected from this study was aphid incidence (%), aphid score, plant vigour scores, plant height, canopy height and canopy width at maturity, number of days to 50% flowering, pod weight and grain yield. The number of insects was counted using a visual score of 1 to 9, where 1 =1-4 aphids, 3=5-20 aphids, 5=21-100 aphids, 7= 101-500 aphids and 9= >500 (high aphid colonisation and plant death). Plant vigour scores was calculated using scale of 1-3, where 1 denotes senescence (weak), 2 indicating medium growth and 3 denoting survival (more vigorous).

3.3 Laboratory experiment for bruchid screening

The experiment was conducted in the plant production laboratory, F-block University of Limpopo. The experiment was laid in a completely randomized design (CRD) with 3 replications. Ninety seven breeding lines obtained from IITA, Nigeria and A&M Texas University, USA was used. Prior to the infestation of the lines with bruchids, seed samples was placed in an oven at 50°C for 3 hours to kill any pre-existing bruchid eggs or larvae that might be in the seeds (from the field) and also to keep moisture level of the seeds at equilibrium. The experiment was conducted using population of *C. rhodensiansus* obtained from Ukulima Farm. The bruchids used was reared on a susceptible cowpea line (Ife brown). Ten seeds of each breeding line was selected and placed in petri dishes and then artificially infested with freshly emerged bruchids (2 males and 2 females) using camel hair brush. Bruchids was left for 2 days to allow for mating and oviposition. The number of eggs on the seeds was counted with the aid of magnifying lense 4 days after egg laying. For uniform infestation, only one egg was left on the seed and excess was carefully removed by scraping using razor blade.

3.3.1 Data collection from bruchid screening

Data collected from the infested replicates of each cowpea line was % adult emergence (number of eggs hatched/ total number of eggs laid x 100), number of days to insect emergence (days from date of infestation to date of emergence on the seed), mean developmental time (days from date of first emergence to date when bruchid stop emerging on the seed), total developmental time (Days from date of infestation to date when bruchid stop emerging), % damaged seed (number of damaged seed/ total number of seed x 100) and % weight loss (initial seed weight - final seed weight/final seed weight x 100), following the procedures of (Toriola, 2010).

3.4 Data analysis

The data collected from both studies was subjected to analyses of variance (ANOVA) for test of significance using Statistics 9.0. Variables that display significant differences were separated using Turkey's HSD test. Treatment means were considered significant when P≤ 0.05 at 5% level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Field experiment results (Aphid screening)

4.1.1 Aphid incidence on different cowpea lines

Results obtained showed that aphid incidence was highly significant (P<0.05) as shown in Appendix 1. This indicated uniform aphid incidence because most of the cowpea lines had high aphid population including control check (Vita 7). The cowpea lines varied from one another in terms of aphid colonisation on the plants because fifty eight varieties recorded aphid colonisation of 71-90% and twenty eight cowpea lines recorded 91-100% (Fig. 1).

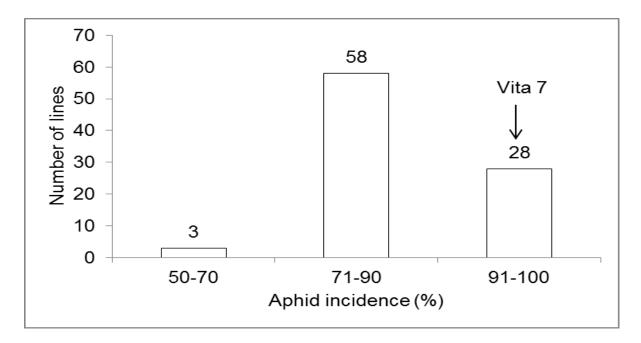


Figure 1: Aphid incidence of cowpea lines

Only three cowpea lines out of 97 lines recorded (50-70%) aphid incidence as shown Figure 1. Due to the statistical evidence, variation in spread of aphid colonies to different cowpea lines might be due to sweetness of the crop. That is, without antixenosis and antibiotic compounds in the plant tissues that distracts growth, development and functioning of aphids. The overall aphid incidence on cowpea lines showed uniform aphid spread.

4.1.1.1 Rating for aphid severity score

Among the lines screened, there were significant differences (P<0.05) in terms of aphid score as shown in Appendix 2. Among the cowpea lines screened only twenty lines showed some level of resistance and the remaining 69 susceptible as shown in Figure 2. Most of the introduced cowpea lines in the scale range of 5-9 managed to escape aphid attack while other resulted in death. The results from this study confirmed the findings of Githiri *et al.* (1996) who stated that cowpea infested by aphids result in yellowing of the leaves and thus plant death. This is indicated by rating scale 5-9 where susceptible check vita7 is included. In this scale range, most cowpea lines showed poor growth, vigour and biomass.

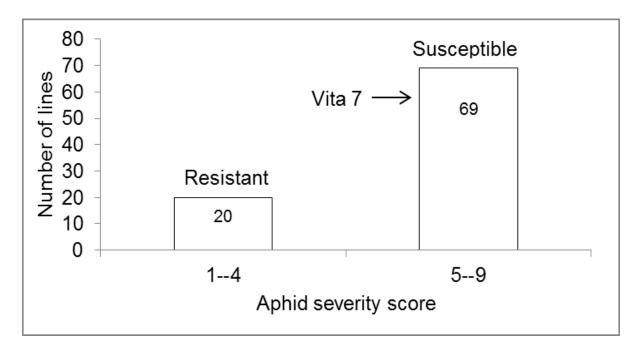


Figure 2: Frequency distribution of aphid severity score

4.1.1.2 Effect of aphids on plant height

The result obtained from the study showed that plant height of different cowpea lines was highly significant (P<0.05) indicating that certain cowpea lines resulted in less and high plant height due to severe attack by aphids (Appendix 4). Apart from this, thirty-four cowpea lines attained the highest plant height between 16-20 cm at three week after planting (3WAP) and 9 cowpea lines with the lowest plant height (6-10 cm) and forty-six lines were found to have medium plant height (11-15 cm) as shown in Table 1. This brings the fact that different cowpea breeding lines respond differently to aphid infestation as indicated in Figure 3, where there was more growth in resistant lines and less growth in susceptible lines including control check Vita 7. A cowpea line TX12-473 as shown in Figure 3 showed good performance in terms of plant height as it recorded the highest plant height compared to other lines.

Table 1: Plant height of cowpea lines after infestation

Number of lines	Plant height (cm)	
9	6-10	
46	11-15	
34	16-20	
P≤0.05	0.0000	

Some of the cowpea lines evaluated did not even reach the height of 20 cm even at maturity as shown in Table 1. These indicated that some cowpea lines resulted in slow growth due to retardation by aphids earlier during the vegetative growth stage of the crop.

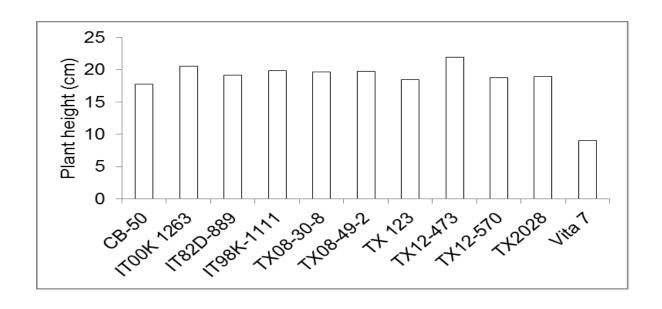


Figure 3: Frequency distribution of the best 10 lines compared with control check 4.1.1.3 Effect of aphids on plant vigour

The results obtained showed that plant vigour of different cowpea varieties was highly significant (P<0.05) as shown in Table 2 and Appendix 3. Fifty six cowpea lines out of ninety seven lines showed poor growth and performance and thirty cowpea lines with medium promising vigour and the remaining three cowpea lines out of ninety seven lines showed good performance in terms of vigour (growth) as shown in Table 2. Thus, the cowpea lines with medium to good performance it means they managed to tolerate the severity of aphid attack and apart from the poor performing lines, some turned yellow and totally knocked off by aphids in the field.

Table 2: Rating of plant vigour to aphid infestation of different cowpea lines

Number of lines	Plant vigour
56 (Low survival)	1
30 (Medium-survival)	2
3 (Vigorous)	3
P≤0.05	0.0000

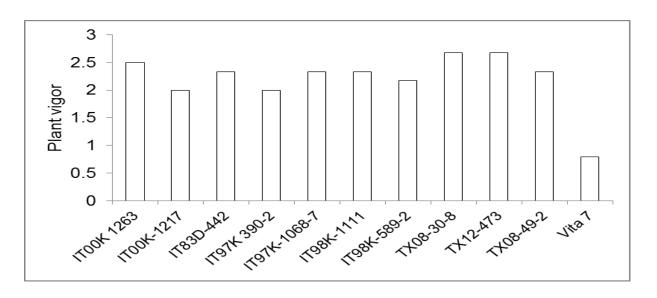


Figure 4: Best 10 lines in plant vigour compared with the control check (Vita 7)

One of the possible reliable variables to determine the resistance and susceptibility of cowpea lines is utilisation of plant vigour and aphid score. Most of the introduced lines performed better than the control check Vita 7 as shown in Fig. 4. The lines such as IT00K 1263, TX08-30-8 and TX12-473 (Fig. 4) should be improved and selected for on-farm trial to alleviate the problem of food security. This is because they were found to attain good performance even when they were attacked by aphids.

4.1.1.4 Cowpea phenology (50% flowering)

The results as shown in appendix 5 indicated that number of days to 50 % flowering was highly significant (P< 0.05). Thus heavy infestations by aphids cause cowpea lines to respond differently to their flowering stages (early and late flowering). Aphid attack resulted in delay of flowering and thus crop maturity (Fig. 5) were other cowpea lines took 86-95% to reach 50% flowering. There was a great variation in flowering of different cowpea lines. Besides this, fifty two cowpea lines showed early flowering (36-65 days), thirty three lines flowered 66-85 days and the remaining four cowpea lines took longer (86-95 days) to reach 50% flowering as compared to the other lines and this include vita 7 as susceptible check (Fig. 5). The results of this study was in agreement with the findings of Jackai and Daoust (1986) and Jackie *et al.* (2001) who stated that, the delay in flowering stage of cowpea is caused mainly by heavy attack from large aphid populations by sucking the contents of the flower and flowers buds.

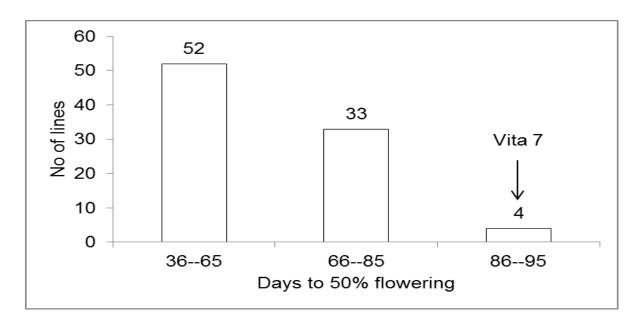


Figure 5: Frequency distribution of the number of days to 50% flowering

4.1.1.5 Canopy height

The results obtained from this study indicated significant difference (P<0.05) in canopy height at maturity stage as shown in Table 3 and Appendix 6. Twenty-nine cowpea lines attained the highest canopy height (41-60 cm), fifty-six lines recorded medium plant height (21-40cm) and three cowpea lines were found to be struggling to survive even at maturity stage (0-20 cm). These results were consistent with the findings of Singh *et al.* (1985) who reported that large population of aphid infestation result in stunted growth as shown in Table 3 whereby susceptible variety (Vita 7) were still less than 20cm even at crop maturity.

Thus, the cowpea lines tested showed significant variation in their susceptibility and resistance to the infestation by piercing sucking pests (cowpea aphids). This also explain that beside more aphid population and attack, there were cowpea lines that managed to tolerate aphids infestation and this was clearly indicated by vigorous growth (41-60 cm) towards maturity. Only four lines expressed some level of susceptibility since they did not even grow above 20 cm in height even at maturity of the crop.

Table 3: Canopy height of cowpea lines for aphid infestation at maturity

Number of lines	Canopy height(cm)	
4	0-20	
56	21-40	
29	41-60	
P≤0.05	0.0000	

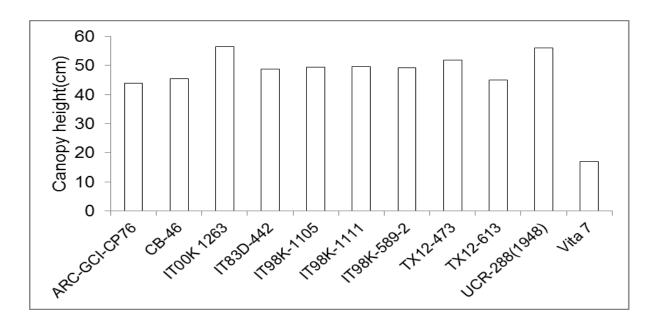


Figure 6: Top 10 best lines compared to the control check (Vita 7) in canopy height

Cowpea lines IT00K 1263 and UCR-288(1948) as shown in Figure 6 showed better canopy height. Therefore, it is of critical value to select these lines for fodder to feed animals in dormant seasons when food is insufficient.

4.1.1.6 Canopy width

Results obtained indicated significant difference (P<0.05) in canopy width of the lines as shown in Appendix 7. Three cowpea lines did not recover until maturity stage by attaining 20 cm in width and 35 cowpea lines recovered (41-60cm) as shown in Table 4. The results obtained indicate that there was more variation in canopy width of different cowpea lines.

Three of the ninety seven lines including control check Vita 7 showed small canopy width (0-20 cm) which is another basis of determining resistance and susceptibility of the lines. Six cowpea lines (61-70 cm) recorded the best canopy height while thirty five lines recorded (41-60 cm) as shown in figure 8. These also indicate that aphids have the responsibility of crops being stunted because they feed on the crop until death.

Table 4: Canopy width of cowpea lines for aphid infestation at maturity

Number of lines	Canopy width (cm)	
3	0-20	
45	21-40	
35	41-60	
6	61-70	
P≤0.05	0.0000	

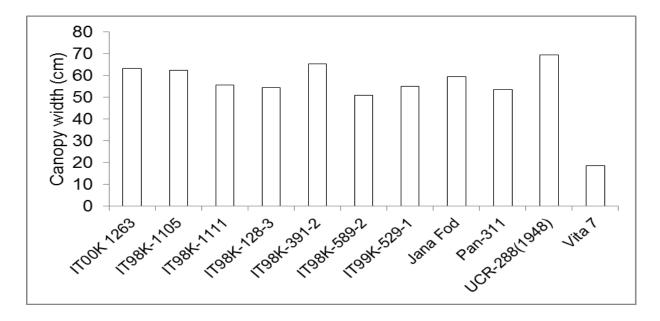


Figure 7: Canopy width of lines attacked by aphids towards maturity

As shown in Figure 7, cowpea lines respond differently when attacked by aphids. The cowpea lines with the best canopy height explain some level of resistance to aphid attack. Thus, those lines can be recommended for farmers producing livestock for animal feed.

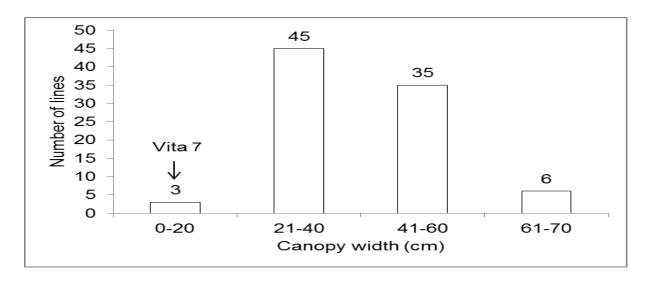


Figure 8: Frequency distribution of canopy width (cm) at maturity stage

4.1.1.7 Pod weight

Statistical evidence indicated significant differences (P<0.05) in pod weight of different cowpea breeding lines as shown in Table 5 and Appendix 8. Apart from this, out of ninety seven lines screened, forty eight cowpea lines were found to have low pod weight between (0.02-30g) including control check Vita 7 while eight lines had highest pod weight between (121-180g) and the remaining varieties were medium in pod weight (31-120g) as shown in Figure 9. These bring the fact that aphids on susceptible cowpea lines result in reduction pod weight due to their sap sucking ability. Few lines obtained more pod weight as compared to the majority.

Table 5: Effect of aphid infestation on pod weight of cowpea lines

Number of lines	Pod weight (g)
48 (including control check (Vita 7)	0.02-30
18	31-60
9	61-90
6	91-120
8	121-180
P≤0.05	0.0000

Aphid infestation had a significant effect on weight of cowpea pods as shown in figure 9. Cowpea lines such as UCR-288(1948) and IT83D-442 the highest pod weight and thus, they can be recommended to farmers because of their high yielding ability.

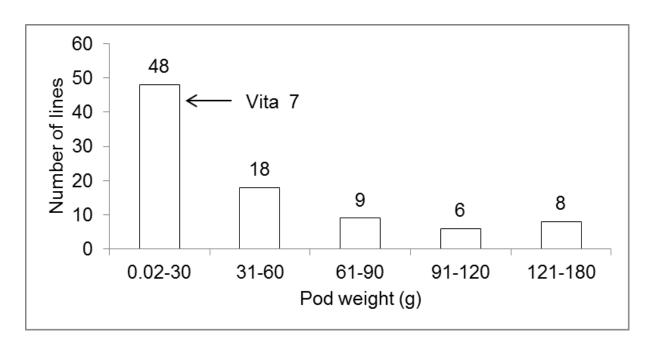


Figure 9: Frequency distribution of pod weight (g) of the lines screened

4.1.1.8 Grain yield

According to the results obtained (Table 6 and Appendix 9) there was high significant difference (P<0.05) in grain yield. This is due to the high aphid attack were in most cases susceptible cowpea lines produce pods with no seeds inside. The findings of this study agreed with that of Soleymane *et al.* (2013) who indicated that cowpea affected by aphids result in low grain yield from biotic stress such as insect pest and the study of Babura and Mustapha (2012) also supported that aphid attack reduce yield and in many cases the whole plant dies. But this study did not agree with the study of Haviz and Damarany (2006) that tested five varieties and did not find any significant difference in grain yield of cowpea infested by aphids.

Table 6: Grain yield of cowpea lines after aphid attack

Number of lines	Grain yield (g)	
64	0.10-30	
12	31-60	
7	61-90	
6	91-110	
P≤0.05	0.0000	

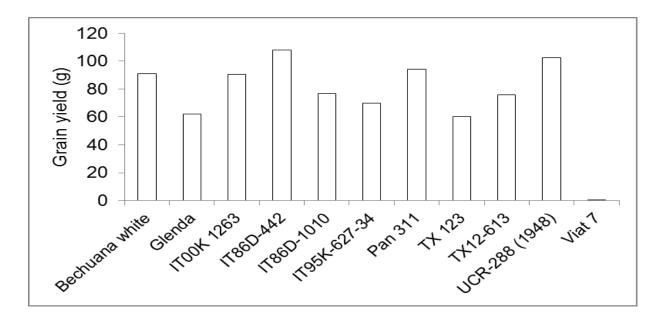


Figure 10: Frequency distribution of best 10 lines in terms of grain yield (g)

The study showed that different cowpea lines can exhibit good performance even under heavy attack by aphids (Fig. 10). The introduced cowpea lines such as IT83D-442, Pan 311 and UCR-288 (1948) as shown in (Fig 10) produced high grain yield as compared to the check (Vita 7). These lines will be selected for farmers and for further adaptive breeding where they will be crossed with better adapted lines in South Africa.

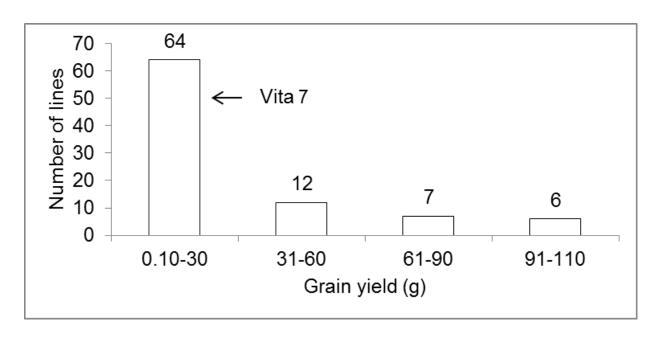


Figure 11: Frequency distribution of grain yield of cowpea lines after aphid attack

4.2. Laboratory experiment results (Bruchid screening)

4.2.1 Percentage adult emergence of bruchids

The results obtained showed that there was high significant differences (P<0.05) in terms of percentage adult emergence (Appendix 10). High weevil emergence occurred in some cowpea lines than in others which then indicate some level of resistance and susceptibility. The level of resistance and susceptibility was extrapolated by low level of emergence and delayed number of days to emerge from the seed. Twenty cowpea lines with promising resistance was identified as shown in (Table 7), where emergence level of adult bruchid recorded (29-58%) compared to 54 lines which showed medium resistance (59-88%) and 14 cowpea lines showed high susceptibility which is evidenced by high emergence level of bruchid (89-100%) that emerged from the interior of the seeds. The highest percentage of adult emerging from the seed interior implies susceptibility of the cowpea line.

Table 7: Percentage adult emergence after infestation

Number of cowpea lines	%Adult emergence	
20	29-58	
54	59-88	
14	89-100	
P≤0.05	0.0005	

Percentage of adult emergence is done based on the number of eggs laid and insect emerged from the eggs. Adult emergence was low as shown in table 10, where 20 cowpea lines exhibited (29-58%). The results of this study confirmed with the study of Singh *et al.* (1985) where less percentage adult emergence was recorded on resistant line (32.8%) such as TVu 2027 than 95% such as Ife brown as susceptible line.

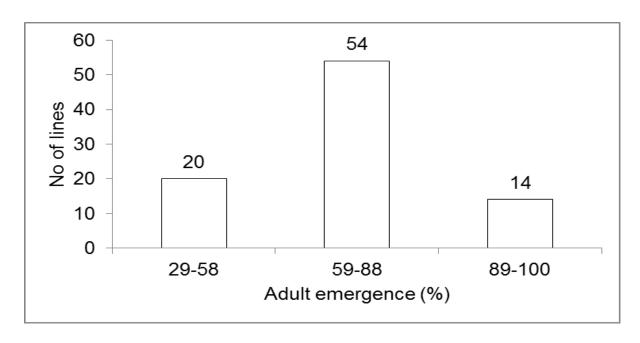


Figure 12: Percentage levels of adult emergence

The findings of this study was in agreement with the findings of Braga *et al.* (2007) who found that there was significant difference in the percentage of adult emergence from the seeds and also the findings of Mogbo *et al.* (2004) found that cowpea weevil cause significant difference in percentage adult emergence. Fourteen cowpea lines showed high adult emergence and 20 showed low adult emergences as shown in Figure 12.

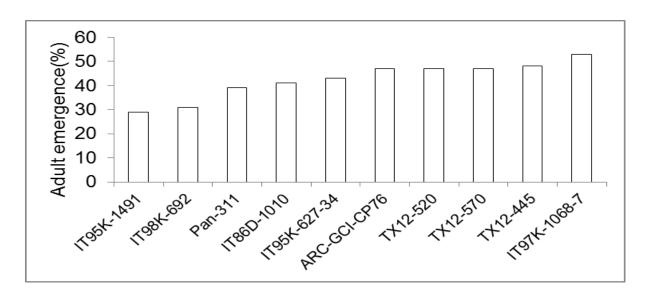


Figure 13: Cowpea lines with low level of percentage adult emergence

The results of Nagaraja (2006) confirmed the results of the present study and found the lowest percentage of adult emergence to be 32.02% as shown in (Fig. 13) on cowpea line IT95K-1491 and IT98K-692. The lowest percentage of adult emergence recorded in the present study was 29.0%. The result of the present also study confirmed the results of Adam and Baidoo (2008) who found that the greater the emergence of bruchid from cowpea grains, the heavier the infestation and the greater the susceptibility of cowpea line to attack by cowpea bruchids (beetles).

4.2.2 Number of days to insect emergence (NIE)

The statistical analyses indicated that number of days to insect emergence was significantly different (P<0.05) as shown in Appendix 11. Two cowpea lines (IT98K-491- 4 and IT00K-1060) was found to have longer number of days to emerge (56-65 days) as compared to eighty-four lines with short number of days (30-45 days) for adult insect to emerge from date of infestation as shown in Table 8. Three cowpea lines (Bechuana white, Glenda and IT98K-1105) recorded medium number of days to insect emergence (46-55 days). These explain some level of resistance and susceptibility for this notorious storage insect pest. The cowpea lines with the shortest number of days for adult emergence cannot be stored for longer periods, so they cannot be recommended for small holder farmers with poor access to quality storage facilities.

Table 8: Number of days to bruchid emergence after infestation

Number of lines	Days to insect emergence (NIE)
84	30-45
3	46-55
2	56-65
P≤0.05	0.0000

4.2.3 Mean developmental time (MDT)

There were high significance differences (P<0.05) in mean developmental time of the insect on larval development as shown in Appendix 12. This indicate that the time that insect emerge from the seed and when they cease to emerge was not the same. 62 cowpea lines was found to have the shortest developmental time (1-10 days), 22 lines recorded medium larval development (11-20 days) and 5 lines (IT86D-1010, IT98K-1105, TX08-74-1, TX12-471 and TX12-541) recorded the longest developmental time (21-36 days) of the bruchid larva within the seed endosperm as shown in Figure 14.

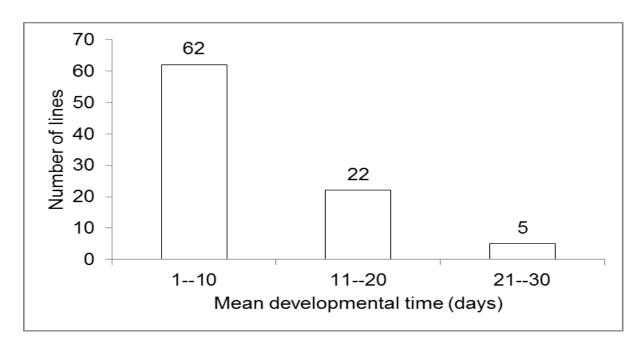


Figure 14: Mean developmental time of emerged bruchid

The results of the present study contradicted with the study of Adam and Baidoo (2008) who found no significant difference in the mean developmental time of bruchid in cowpea seeds.

4.2.4 Total Developmental Time (TDT)

The result obtained showed that there was high significant differences (P<0.05) in total developmental time (TDT) as shown in Appendix 13. Different cowpea lines responded differently to bruchid infestation in terms of larval development inside the seed. Forty-five cowpea lines exhibited longer total developmental time (50-68) as shown in Table 9 which explains some level of resistance because of delay in number of days as compared to 43 lines with shortest developmental time (37-49 days). The developmental period was found to vary significantly which ranged from 37 days to 68 days.

Table 9: Total developmental time of bruchid in the endosperm of seeds

Number of lines	TDT	
43	37-49	
45	50-68	
P≤0.05	0.0000	

The statistical evidence showed that variety TX08-74-1 exhibited longer total developmental time as compared to IT00K-1263 with the shortest developmental time as shown Figure 15. The developmental period of *C. rhodensiansus* ranged from 37-68 days. The mean different development period was significant in all cowpea lines evaluated.

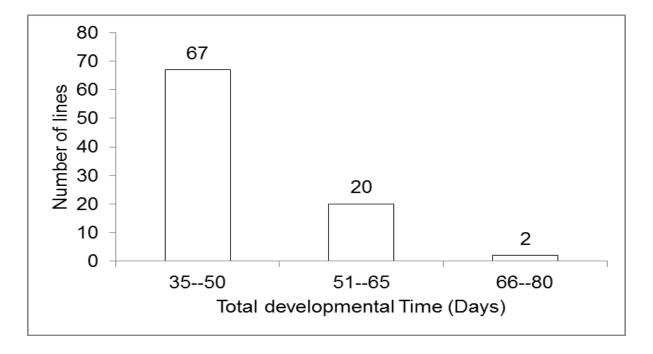


Figure 15: Frequency distribution of Total developmental time of cowpea bruchid

Total developmental times refers to the total days from time of infestation to the time when adult bruchid cease emerging from the seeds. Only two cowpea lines were found to have longer developmental time as shown in Figure 15.

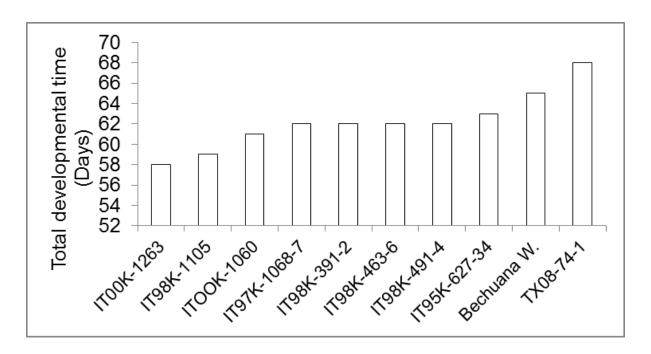


Figure 16: The best ten cowpea lines with long developmental time

TX08-74-1 recorded the longest total developmental time as compared to IT00K-1263 which took 58 days for insects to emerge. This might be due to the fact that this cowpea line (TX08-74-1) contains some compounds that that prevent larval development within the seed and the presence of a hard seed coat as shown in figure 18.

4.2.5 Percentage of damaged seeds (%DS)

The results obtained indicated that there was high significant difference (P<0.05) in percentage of damaged seeds (Appendix 14). This implies that the different cowpea lines differed in relation to the damage that was caused by bruchids. According to the result of the study, twelve cowpea lines showed the least damage from cowpea bruchid (17-39%), 26 lines recorded medium damage (40-59%) and 50 lines exhibited high percentage of damaged seeds (60-100%) as shown in table 10.

Table 10: Percentage of damaged seed by cowpea beetle (Bruchid)

Number of lines	%DS
12	17-39
26	40-59
50	60-100
P≤0.05	0.0076

Less or no damage from grains by bruchid explain the index or level of resistance due to the factors that hinder the growth, development and functioning of bruchid larva. Similarly, the percentage of damaged seeds ranged from (17-39%) in some cowpea lines with promising resistance after infestation. IT98K-692 recorded the lowest percentage of damaged seed (Fig. 18). Apart from this, the results of the current study were in agreement with the findings of the previous study of Idoko and Adesina (2012) who reported that cowpea weevil account for 90% damage to stored cowpea seeds after harvesting.

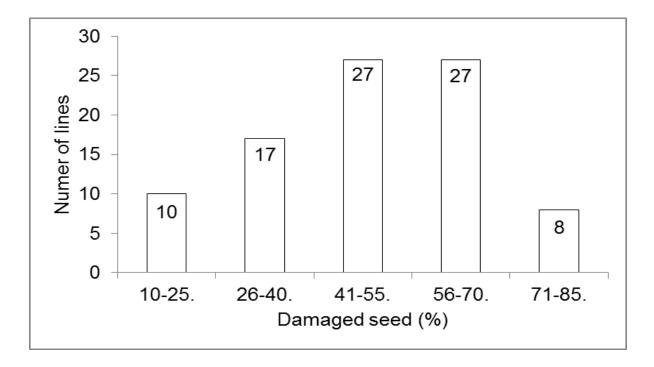


Figure 17: Frequency distribution of percentage damaged seeds (%DS)

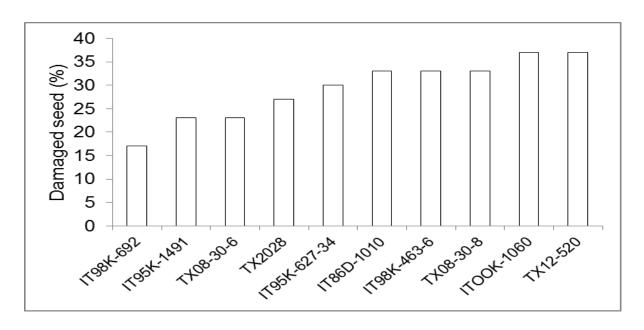
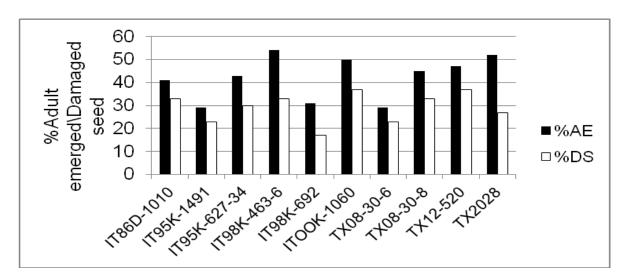


Figure 18: Top ten cowpea lines with the lowest percentage of damaged seeds



Key: %AE= Percentage adult emergence, %DS= Percentage of damaged seeds. Correlation (r = 0.86)

Figure 19: Relationship between percentage adult emergence and damaged seed

According to Figure 19, an increase in percentage adult emergence result also in increased percentage of damaged seeds and thus more seed weight loss. The cowpea line IT98K-692 recorded the lowest percentage of damaged seed and compared to the cowpea line IT00K-1060 with 78% of damaged seed.

4.2.6 Percentage seed weight loss (%SWL)

According to the results obtained, there was high significant difference (P<0.05) in percentage seed weight loss of different cowpea breeding lines as shown in Appendix 15. Thus no seed damage, no seed weight loss. Sixty-nine cowpea lines recorded low percentage seed weight loss (1-20%), fifteen lines recorded medium seed weight loss (21-30%) and 4 varieties recorded the highest seed weight loss (31-46%) as shown in table 12.

Table 11: Seed weight loss after bruchid damage in storage

Number of lines	%SWL
69	1-20
15	21-30
4	31-46
P≤0.05	0.0000

The percentage seed weight loss caused due to feeding by bruchids caused the minimum weight loss that was recorded in cowpea line IT98K-692 Figure 20. The results of the present study agreed with the study of Mogbo *et al.* (2004) who found significant difference in seed weight loss. The increase in seed weight loss of the seeds was significantly caused by an increase in insect emergence out of the seeds. Beside this, adult emerged from the seeds have the tendency to re-enter the seeds and consume the contents of the seed further which then result in more reduced seed weight.

The results of the present study also exhibited wide variation with respect to percentage seed weight loss. Therefore, this variability may be painstaking by plant breeders to improve high storability and seed quality. The results from the previous study of Badii *et al.* (2013) also confirmed the results of the present study were there was high significant difference found on percentage seed weight loss. Variables such as adult emergence, developmental period, seed damage and seed weight loss are the most reliable indicators for resistance of cowpea to damage by bruchid. These

variables indirectly assess the possibility of the damaged seed to germinate if planted. The higher the %seed weight loss the less the seed can germinate if planted.

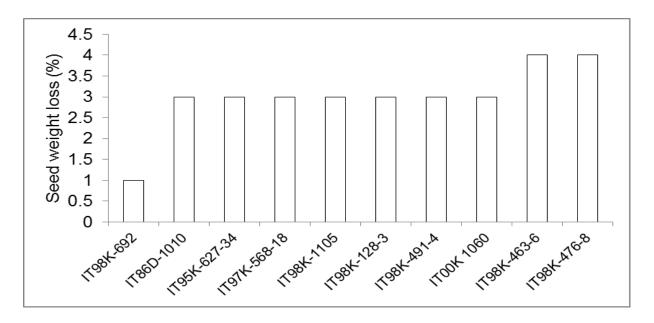


Figure 20: Top 10 cowpea breeding lines with the lowest % seed weight loss

Cowpea lines with the lowest damage by bruchid exhibited some level of tolerance. The results of the present study was in accordance with the results of Idoko and Adesina (2012) who found that percentage damaged seed was positively correlated with percentage adult emergence (r = 0.86) except seed weight loss that was weakly correlated to adult emergence (r = 0.38). Cowpea lines varied significantly with respect to percentage seed weight loss and percentage of damaged seed (Figs. 21&22). IT00K1060 suffered the highest weight loss whilst IT98K-692 with the lowest seed weight loss and percentage of damaged seed.

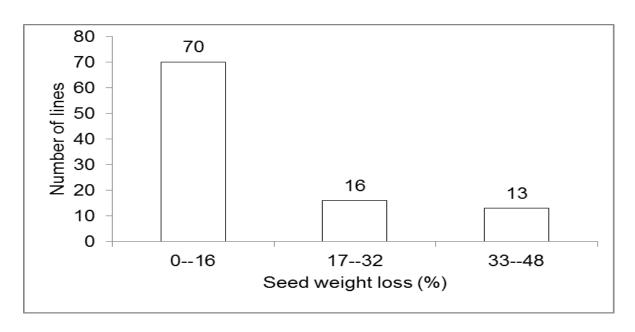


Figure 21: Percentage seed weight loss after bruchid attack

Seventy cowpea lines showed the lowest seed weight loss (0-16%), 16 lines with medium seed weight loss (17-32%) and 3 lines with the highest seed weight loss (33-48) as shown in Figure 21. The high seed weight loss is caused by high percentage of adult insect emergence from the interior of the seed. The results of the present study were in agreement with the study of Oluwafemi *et al.* (2013) where the study found significant variation in percentage seed weight loss of five cowpea lines tested. The higher the seed damage, the less the seed weight (Fig. 22)

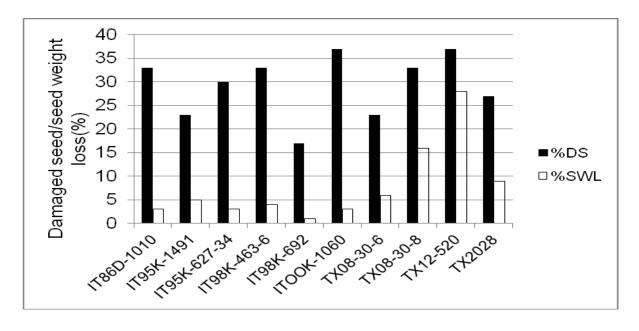


Figure 22: Relationship between percentage of damaged seed and seed weight loss

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the overall evaluation of cowpea breeding lines showed that it is beneficial to screen cowpea breeding lines for Aphid and Bruchid resistance in order to improve yield and to plant well adapted lines. This therefore suggests that it is of critical importance to evaluate cowpea lines for on-farm trials and further adaptive breeding programs to make crosses among resistant and high yielding lines to produce high yielding and insect pest resistant cowpea lines. This however will only be possible to farmers in South Africa as there will be no need to purchase insecticides and fumigants for the management of cowpea insect pests because of resistant cowpea lines that will be made available to farmers.

Cowpea lines with long larval developmental time, low level of damaged seeds and low seed weight loss will be recommended to small holder farmers in South Africa as they will have the possibility of being stored for long period in storage. Cowpea lines IT98K-692, IT95K-1491, TX08-30-6, TX2028, IT95K-627-34, IT86D-1010, IT98K-463-6, TX08-30-8, IT00K-1060 and TX12-520 were found to be the best lines recommended for small holder farmers in South Africa.

In terms of aphids, cowpea lines with better aphid resistance, promising plant vigour, high canopy height and grain yield are selected to meet the needs of the poor masses. Cowpea lines IT00K 1263, IT00K 1217, IT83D-442, IT97K 390-2, IT97K-1068-7, IT98K-1111, IT98K-589-2, TX08-30-8, TX12-473 and TX08-49-2 can be recommended for small holder farmers because of their good yield even after aphid damage. The null hypothesis of the study tested was rejected because it has been found that introduced cowpea breeding lines responded differently to aphid and bruchid resistance. In general, the study was successful in identifying cowpea lines with resistance to aphid and bruchid infestations. These lines will be useful for breeding program (backcross) in the development of new pest resistant germ-plasm. In addition, they can be recommended for cultivation by farmers and will reduce their production cost with increased profitability.

REFERENCES

ADAM, J.I. and P.K. BAIDOO. 2008. Susceptibility of Five Cowpea Varieties to Attack by *Callosobruchus maculatus*. *Journal of Ghana Science Association Vol. 10*.

AHMED, B.I. and A.U. YUSUF. 2007. Host–plant resistance: A viable non – chemical and environmentally friendly strategy of controlling stored products pests-a review. *Emirates Journal of Food and Agriculture* 19 (1): 01-12.

ASIWE, J.A.N. 2009. Needs assessment of cowpea production practices, constraints and utilization in South Africa. *African Journal of Biotechnology* 8: 5383-5388.

ASIWE, J.A.N., BELANE, A. and F.D. DAKORA. 2009. Evaluation of cowpea breeding lines for nitrogen fixation at ARC-Grain Crops Institute, Potchefstroom, South Africa.

BABURA, S.R. and Y. MUSTAPHA. 2012. Screening for host-plant resistance to infestation by aphid (*Aphid craccivora Koch*) in cowpea (*Vigna unguiculata L. Walp*). Bayero Journal of Pure and Applied Science 5 (1): 44-47.

BADII, K.B., ASANTE, S.K. and N.K. SOWLEY. 2013. Varietal Susceptibility of Cowpea (*Vigna unguiculata* L.) to the Storage beetle (*Callosobruchus maculatus*). *International Journal of Scientific and Technology Research Vol. 2.*

BRAGA, Y.F., GRANGEIRO, T.B., FREIRE, E.A., BEZERRA, J.A., ANDRADE-NETO, M. and M.A. LIMA. 2007. Insecticidal activity of 2-tridecanone against the cowpea weevil. Fortaleza: Brazil.

BUGG, R. L., COLFER, R. G., CHANEY, W. E., SMITH, H. A. and J. CANNON. 2008. Flower flies (Syrphidae) and Other Biological Control Agents for Aphids in Vegetable Crops. Oakland: University of California Agriculture and Natural Resources.

DAFF, 2011. Production guidelines of cowpea. Pretoria, South Africa.

DEVEREAU, A.D., JACKAI, L.E.N., TAYO B.O. AND J.A.N. ASIWE. 2002. Evaluation of a novel technique for screening cowpea varieties for resistance to the seed beetle *Callosobruchus maculatus*. IITA-Ibadan: Nigeria.

GIGA, D.P. 2001. Importance of Cowpea (*Vigna unguiculata*) in Africa. Post-harvest Storage Technologies for Cowpea in Southern Africa. FAO, Harare.

EGO, E.O. 2011. Management of major field insect pests and yield of cowpea under calendar and monitored application of synthetic chemicals in Asaba, Southern Nigerian. *American Journal of Scientific and Industrial Research*.

GITHIRI, S.M., AMPONG-NYARKO, K., OSIR, E.O. and P.M. KIMANI. 1996. Genetics of resistance to *Aphis craccivora* in cowpea. Nairobi: Kenya.

GRDC, 2010. Aphids and Viruses in pulse crops. Fact sheet.

HAFIZ, N.A. AND A.M. DAMARANY. 2006. Variation in the susceptibility of some cowpea (*Vigna unguiculata* (L.) Walp) genotypes to infestation with certain pests in Upper Egypt. Assiut University Bulletin Environmental Researches 9: 7-15.

HUIGNARD, J., LEOR, B., ALZOMA, I. and J.F. GERMAIN. 1985. Oviposition and development of *Callosobruchus maculatus* (Coleoptera: Bruchidae) in organic Garbanzo beans. *Journal of Economic Entomology* 96: 1952-1957.

IDOKO, J.E. and J.M. ADESINA. 2012. Infestation Level of *Callosobruchus maculatus* on Cowpea using Different Particle Size of Eugenia aromatic and Piper guineense Powders. *World Journal of Engineering, Pure and Applied Science* 2(5): 156.

JACKAI, L. E. N. and R. A. DAOUST. 1986. Insect pests of cowpeas. Annual Review of Entomology 31(1): 95 – 119.

JACKAI, L.E.N., GOUDOU, C., ASIWE, J.A.N. and B.O. TAYO. 2001. Integrated control of the cowpea aphid using seed dressing and varietal resistance. *Samaru Journal of Agricultural Research* 17: 13-23.

JEFF-WHITWORTH, R. AND A. AHMAD. 2009. Cowpea aphid. Kansas crop pest. Kansas State University.

JOANA, O.I. and D.T. GUNGULA. 2010. Preservation of cowpea (*Vigna unguiculata* (L) Walp) grains against cowpea bruchids (*Callosobruchus maculatus*) using Neem and Moringa seed oils. Department of Food Science and Technology. Federal University of Technology, Nigeria.

KITCH, L.A.W., BOTTENBERG, H. and J.L. WOLFSON. 1999. Indigenous knowledge and cowpea pest management in sub-Saharan Africa: Entomology Department, Purdue University, West Lafayette, IN 47007, USA.

MOGBO, T.C., OKEKE, T.E. and C.E. AKUNNE. 2004. Studies on the Resistance of Cowpea Seeds (*Vigna unguiculata*) to Weevil (*Callosobruchus maculatus*) Infestation. *American Journal of Zoological Research 2(2): 37-40.*

NAGARAJA, M. 2006. Evaluation of Pigeon pea and cowpea genotypes for bruchid resistance. Department of Agricultural Entomology, College of Agriculture, Dharwad.

OBOPILE, M. 2006. Economic threshold and injury levels for control of cowpea aphid, *Aphis craccivora*. (Homoptera; Aphididae) on cowpea. *African Journal of Plant Protection* 12: 111-115.

OBOPILE, M., MASIAPETO, K. and C. GWAFILA. 2011. Variation in reproductive and developmental parameters of *Callosobruchus maculatus* (F) reared on ten Botswana cowpea landraces. *African Journal of Biotechnology* 10: 13924-13928

OLUWAFEMI, D.A., ADEBAYO, L.O., KEHINDE, B. and O. OMOCHA. 2013. Evaluation of Four Cowpea Lines for Bruchid Tolerance. *Journal of Natural Sciences* Research

REDDEN, R.J. and J. McGUIRE. 1983. The genetic evaluation of bruchid resistance in seed of cowpea. *Australian Journal of Agricultural Research* 34: 707-715.

SARIA, J.E. 2010. Enhancing Cowpea (*Vigna unguiculata*) Production through Insect Pest Resistant Line in East Africa. University of Limpopo.

SHADE, R.E., FURGASON, E.S. and L.L. MOURDOCK. 1990. Detection of hidden insect infestation by feeding generated ultra-sonic signals. *American Journal of Entomology* 36: 231-234.

SINGH, B.B. and S.R. SINGH. 1990. Breeding for bruchid resistance in cowpea. *African Journal of Entomology* 46: 219-228.

SINGH, B.B., SINGH, S.R. and O. ADJADI. 1985. Bruchid Resistance in Cowpea. *African Journal of Entomology* 25: 736-739.

SINGH, B.B., ASANTE, S.K., JACKAI, L.E.N. and J.D. HUGHES. 1996. Screening for resistance to parasitic plants, virus, aphid and bruchid. *IITA annual report*, project 11 pp 24.

SOLEYMANE, A., AKEN'OVA, M.E., FATOKUN, C.A. and O.Y. ALABI. 2013. Screening for Resistance to Cowpea Aphid (*Aphis craccivora Koch*) in wild and cultivated Cowpea (*Vigna unguiculata L. Walp*) Accessions. *International Journal of Science, Environment and Technology* 12: 611-621.

TORIOLA, A.E. 2010. Laboratory evaluation of fifteen cowpea (*Vigna unguiculata*) varieties to infestation and damage by *Callosobruchus maculatus* (Coleoptera: Bruchidae). University of Agriculture, Abeokuta.

WALLING, L.L. 2008. Avoiding effective defences: Strategies employed by phloem-feeding insects. *Journal of Plant Physiology* 146: 859-866.

LIST OF APPENDICES

Analysis of Variance (ANOVA) tables for aphid field study Appendix 1: Analyses of variance for aphid incidence (%) of cowpea lines

Source of variation	DF	SS	MS	F	Р
Replication	2	1195.78			
Variety	90	19171.7	213.019	1.45	0.0150
Error	210	30754.0	146.448		
Total	302	32	91		

Appendix 2: Analyses of variance for aphid score

Source of variation	DF	SS	MS	F	Р
Rep	2	0.105	0.05258		
Variety	90	263.417	2.92685	2.73	0.0000
Error	210	225.103	1.07192		
Total	302				

Appendix 3: Analyses of variance for plant vigour

Source of variation	DF	SS	MS	F	Р
Replication	2	3.8513	1.92567		
Variety	90	63.4783	0.70531	1.99	0.0000
Error	210	74.5167	0.35484		
Total	302				

Appendix 4: Analyses of variance for plant height (cm)

Source of variation	DF	SS	MS	F	Р
Replication	2	290.40	145.202		
Variety	90	3064.52	34.050	2.94	0.0000
Error	210	2431.81	11.580		
Total	302				

Appendix 5: Analyses of variance for number of days to 50% flowering

Source of variation	DF	SS	MS	F	Р
Replication	2	102.1	51.066		
Variety	90	57161.6	635.129	1.83	0.0002
Error	210	72765.8	346.504		
Total	302				

Appendix 6: Analyses of variance for canopy height at crop maturity (cm)

Source of variation	DF	SS	MS	F	Р
Replication	2	1151.2	575.607		
Variety	90	27154.0	301.711	4.16	0.0000
Error	210	15233.4	72.540		
Total	302	32		_	

Appendix 7: Analyses of variance for canopy width (cm)

Source of variation	DF	SS	MS	F	Р
Replication	2	656.9	328.465		
Variety	88	41317.2	469.914	4.88	0.0000
Error	212	20413.5	96.290		
Total	302				

Appendix 8: Analyses of variance for pod weight (g)

Source of variation	DF	SS	MS	F	Р
Replication	2	2930	1465.14		
Variety	89	531975	5977.25	9.61	0.0000
Error	211	131306	622.30		
Total	302				

Appendix 9: Analyses of variance for grain yield (g)

Source of variation	DF	SS	MS	F	Р
Replication	2	1050	525.09		
Variety	89	226728	2547.51	8.24	0.0000
Error	211	65249	309.24		
Total	302				

2. Analysis of Variance (ANOVA) for Laboratory study

Appendix 10: Analyses of variance for percentage adult emergence (%AE)

Source of variation	DF	SS	MS	F	Р
Replication	2	577	288.478		
Variety	87	86821	997.947	1.78	0.0005
Error	201	112941	561.898		
Total	290				

Appendix 11: Analyses of variance for number of days to insect emergence (NIE)

Source of variation	DF	SS	MS	F	Р
Replication	2	251.90	125.948		
Variety	88	7745.23	88.014	2.09	0.0000
Error	201	8481.66	42.197		
Total	291				

Appendix 12: Analyses of variance for Mean Developmental Time (MDT)

Source of variation	DF	SS	MS	F	Р
Replication	2	9.78	4.8900		
Variety	87	4965.32	57.0726	1.69	0.0013
Error	201	6781.05	33.7366		
Total	290				

Appendix 13: Analyses of variance for Total Developmental Time (TDT)

Source of variation	DF	SS	MS	F	Р
Replication	2	31.4	15.684		
Variety	88	16015.6	181.996	1.64	0.0024
Error	201	22331.4	111.101		
Total	291				

Appendix 14: Analyses of variance for percentage of damaged seeds

Source of variation	DF	SS	MS	F	Р
Replication	2	1454	726.77		
Variety	87	94887	1090.65	1.53	0.0076
Error	201	143143	712.15		
Total	290				

Appendix 15: Analyses of variance for percentage seed weight loss (%SWL)

Source of variation	DF	SS	MS	F	Р
Replication	2	2848.8	1424.41		_
Variety	87	23753.0	273.02	2.11	0.0000
Error	201	25995.3	129.33		
Total	290				