SCREENING OF ELITE COWPEA GENOTYPES FOR BRUCHID (*Callosobruchus rhodensianus*) RESISTANCE

By

MOKGOBA MURIEL MOGALA

MINI-DISSERTATION

SUBMITTED IN (PARTIAL) FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE IN AGRICULTURE (AGRONOMY)

IN THE

FACULTY OF SCIENCE AND AGRICULTURE

(SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO

SUPERVISOR: PROF J.N.A. ASIWE

2023

DECLARATION

I, MOGALA MM, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Agronomy) has not previously been submitted by me for a degree at this or any other university, that is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Mogala MM (Ms)	05 April 2023
Surname, Initials (title)	Date

TABLE OF CONTENTS

Contents Page

DECLARATION II

TABLE OF CONTENTS III

ACKNOWLEDGEMENTS VI

LIST OF TABLES VII

LIST OF FIGURES VIII

LIST OF APPENDICES IX

ABSTRACT X

CHAPTER 1: GENERAL INTRODUCTION 1

1.1. Background 1

1.2. Problem Statement 2

1.3. Rationale 3

1.4. Purpose of the Study 4

  1.4.1. Aim 4
  1.4.2. Objective 4
  1.4.3. Hypothesis 4

CHAPTER 2: LITERATURE REVIEW 5

2.1. The History of Cowpea 5

2.2. Cowpea Usages 5

2.3. Production Constraints of Cowpea 6

2.4. The Effects of Cowpea Insects 6

2.5. Origin, Distribution, And Biology of Bruchid 7

2.6. The Effects of Bruchid on Cowpea 7

2.7. Management Strategies Against *Callosobruchus rhodensianus* 8

2.8. The Defence of Cowpea Host Plant to Bruchid 8

2.9. Types of Plant Resistance 9

2.10. Traits Affecting Bruchid Development 9

CHAPTER 3: METHODOLOGY AND ANALYTICAL PROCEDURE........................ 10

3.1. Description of the Study 10

3.2. Bruchid Screening Procedure 10

3.3. Data Collection 12

3.4. Data Analysis 13

CHAPTER 4: RESULTS AND DISCUSSION... 14

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS 23

REFERENCES... 24

DEDICATION

I dedicate this work to my beloved parents Mr. Bernard and Mrs. Christina Mogala, and siblings.

ACKNOWLEDGEMENTS

I would like to convey my greatest gratitude to the Almighty God for walking with me throughout this journey and for providing me with knowledge, wisdom, and strength to carry out this study. Secondly, I would like to thank my supervisor Prof. J.N.A. Asiwe for his support, guidance, motivation, and patience. Thanks to my laboratory technician, Ms Mashapa for her assistance and to National Research Foundation (NRF) for financial assistance. Finally, I would like to thank Dr Thusago, and Swafo SM for their support and contributions.

LIST OF TABLES

Page

Table 1 Elite cowpea genotypes screened for bruchid resistance 10

Table 2 Percentage bruchid adult emergence on elite cowpea genotypes 16

Table 3 Percentage of seed damaged 17

Table 4 Total development time of bruchid on elite cowpea genotypes 19

LIST OF FIGURES

		Page
Figure 1	Susceptible black-eyed cowpea seeds used for bruchid culture	11
Figure 2	Mean number of eggs laid on elite cowpea genotypes by bruchid	15
Figure 3	Mean number adult bruchid emerged (%) on elite cowpea genotypes	16
Figure 4	Seeds (%) damaged by bruchid	17
Figure 5	Mean total development time of bruchid on elite cowpea genotypes	18
Figure 6	Mean development time of bruchid on cowpea genotypes	20
Figure 7	Mean number of days to insect emergence on cowpea genotypes	21
Figure 8	Mean percentage seed weight loss of elite cowpea genotypes	22

LIST OF APPENDICES

		Page
Appendix 1	Analysis of variance for number of eggs	34
Appendix 2	Analysis of variance for percentage adult emergence	35
Appendix 3	Analysis of variance for percentage seed damaged	36
Appendix 4	Analysis of variance for total development time	37
Appendix 5	Analysis of variance for mean development time	38
Appendix 6	Analysis of variance for number of days to insect emergence	39
Appendix 7	Analysis of variance for percentage seed weight loss	40
Appendix 8	Analysis of variance for initial seed weight	41
Appendix 9	Analysis of variance for residual seed weight	42

ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) plays a crucial role in in the health of both humans and animals by providing a rich source of protein especially for those in rural developing areas. However, its production is limited because of lack of seeds due to cowpea bruchid investation. Cowpea bruchid *Callosobruchus rhodensianus* (F.) (Coleoptera: Chrysomelidae) is one of the major pests of stored cowpea seeds, and it can render the seeds useless if not managed. There are various measures to control this pest, one of which is the host plant resistance which is regarded as the most efficient sustainable measure. The objective was to screen ten elite cowpea genotypes obtained from the University of Limpopo germplasm collection for bruchid resistance. The experiment was carried out at the University of Limpopo Plant Production Laboratory. Four pairs of bruchids were infested into batches of ten seeds of each genotype using a completely randomized design (CRD) with six replications and data were collected for 60 days. The variables measured included initial seed weight (g) (ISW), residual seed weight (g) (RSW), seed weight loss (%) (SWL), number of eggs (NE), seed damage (%) (SD), adult emergence (%) (AE), number of days to insect emergence (days) (NIE), total development time (days) (TDT) and mean development time (days) (MDT). Results show that significant differences ($P<0.05$) were observed for ISW, RSW, SWL, and MDT; and the findings of this study further indicated a deviation in adult emergence, with a total development time of 39.50-46.50 days. AYT205A, AYT205B, AYT208B, AYT210B, AYT211A, AYT211B, AYT302A, AYT310B and CB-24J'burg exhibited longer developmental periods, which implies that they are promising lines and can be recommended to smallholder farmers with limited access to high-quality storage facilities. It is essential that further research work be done on these genotypes on the mechanisms of resistance and to identify factors responsible for bruchid vulnerability on cowpea.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

Cowpea (*Vigna unguiculata* (L.) Walp) is the provider and cheapest source of plant protein (Asiwe 2022), particularly in rural developing areas where it may be difficult to afford other supplies of proteins. This abundant protein contributes largely to human health and diet (Akyaw *et al*., 2014). Cowpea is an economically valuable crop grown in semi-arid areas of Africa mainly as a food crop for human and animal feed (Xiong *et al*., 2016). Given that it contains lysine, a crucial amino acid that is missing from most cereal grains, cowpeas are a natural complement to cereals (Jayathilake *et al*., 2018). Even though it plays such a major role in agriculture and in the lives of both people and animals, the lack of improved varieties with insect pest resistance and high yield as well as viable seeds for planting had led to the low yield potential of cowpea in South Africa (Asiwe *et al*., 2020 a & b).

Asiwe *et al*., (2020 a & b) further reported that factors such as drought, weeds, insect pests, and diseases also contributed to limited production. Insect pests such as cowpea aphids, pod suckers, blister beetle, and cowpea bruchid cause considerable damage to cowpea yield and quality (Asiwe and Letsoalo, 2018). The insect damage to cowpea is one of the problems in regions producing cowpea, where bruchid constitutes a major post-harvest insect pest in some parts of the tropics (Ndong *et al*., 2012). Cowpea beetle (*Callosobruchus rhodensianus*) causes damage to cowpea during storage and causes seed quality to deteriorate rendering it non-fit for human consumption, and reducing the seed’s marketability, and viability (Asiwe and Letsoalo, 2018; Asiwe, 2022).

The infestation of bruchid on cowpea seeds first appears in the field before harvesting and the infestation is transferred into the storage where the population grows rapidly causing holes in cowpea seeds, thus reducing the germination along with the market worth (Lattanzio *et al*., 2005; Asiwe and Letsoalo, 2018). The *C. rhodensianus* has four life stages. An individual female lays between 100 and 150 eggs over its existence (Asiwe and Letsoalo, 2018). The egg hatches within a week resulting in the penetration of the larvae into the seeds after about four days, where it feeds and pupates. The larval stage is the most critical phase since adult cowpea weevils do not feed on the seed. The adult punctures the seed coat before emerging from the seed, creating exit holes in it (Beck and Blumer, 2014; Asiwe and Letsoalo, 2018, Asiwe *et al*., 2020b). The damage caused by bruchid on cowpea also affects the quality of the seed making it unfit for planting or consumption by humans (Opolota *et al*., 2006; Asiwe, 2022).

A variety of management strategies are available, and this includes synthetic pesticides which are often employed to control bruchids. However, it has endangered non-targeted species, causing plenty of health and environmental issues, including insect pest resistance and residues in food (Malaikozhundan and Vinodhini, 2017; Mahmud, 2018). Other control measures include multiple bagging (Amadou *et al*., 2016), which is not sustainable in the context of commercial seed storage. The biological control option of bruchid, although environment-friendly but has low efficiency and effectiveness because the biocontrol agent *Dinarmus basalis* (Rondani) in the field is affected by insecticides and extremely low temperatures during winter. These disturb the population dynamics of the biological control agents in the field (Yamane, 2013). Given the above, there is a dire need to look for more effective, safe, and environment-friendly tactics to control cowpea bruchid (Viegar Jr., 2003; Trevisan *et al*., 2006). Such tactics include host-plant resistance which enables the screening of germplasm to identify bruchid-resistant accessions.

1.2. Problem Statement

Cowpea suffers a lot of injury by insects during storage, bruchid, the main post-harvest insect pest (Asiwe and Letsoalo, 2018). Because of pest damage and a shortage of pest-resistant genotypes, the cultivation of cowpea in South Africa is constrained. According to Negbenebor and Nura (2020), several methods can be used to keep bruchids from destroying preserved cowpea such as the use of synthetic chemicals. But these chemicals may pose a threat to the environment and food products. Other control tactics include multiple bagging (Amadou *et al*., 2016) which is not sustainable in the context of commercial seed storage, as well as the use of biocontrol agent *Dinarmus basalis* (Rondani) which is environmentally friendly but has limited efficiency and effectiveness for control of bruchids since it is affected in the field by insecticide treatments and extremely cold temperatures during the winter. These affect the population dynamics of the biological control agents in the field (Yamane, 2013). Therefore, these problems led to the development of much safer and eco-friendly methods of controlling cowpea bruchids, such as host plant resistance (HPR) screening.

The host plant resistance strategy is important because it is readily adopted by farmers, and it compactible with other control methods. Although HPR is long-lasting, it is limited in its application due to the lack of resistant genotypes that can be used to establish adaptable and durable resistance (Asiwe and Letsoalo, 2018). Antibiosis, antixenosis, and tolerance are modalities of crop resistance (Painter, 1951), employed by crops to defend against insect pests. Antibiosis is a modality of resistance that interferes with the developmental process of the pest either by slowing down its growth, fecundity or increasing their mortality (Painter, 1951; Smith, 2005), whereas antixenosis, also known as non-preference, is a trait that host plant possesses to discourage the insects from using it for oviposition, shelter or feeding (Painter, 1951). Tolerance is known to minimize the detrimental impacts of insects on the overall viability without interfering with the physiology or behaviour of the insect pest (Jackai and Singh, 1988, Panda and Khush, 1995; Smith, 2005). The scope of this study was only limited to the tolerance mode of resistance (Jackai and Singh, 1988; Asiwe and Letsoalo, 2018).

Many improved elite cowpea genotypes have been developed at the University of Limpopo, but these genotypes have not been screened for bruchid resistance. According to Messina and Renwick (1985) and Chanbang *et al*., (2008), the suitability of seeds for oviposition can be determined by their physical properties, which may or may not be related to the seed's antibiotic nature. The selected genotypes for this study differ in terms of texture, size, and shape, therefore, screening these elite genotypes is important to ascertain their resistance to cowpea bruchid. The screening will also be essential in saving farmers' cost of production since some preservation methods like chemical means can be expensive and can also pose a threat to the environment.

1.3. Rationale

Cowpea is a significant grain legume crop. Many African countries use this crop as a green vegetable as well as a source of dietary protein. Its use as food is aimed at improving food security and reducing malnutrition, especially in rural regions (Kpoviessi *et al*., 2019; Asiwe *et al*., 2020 a & b). It does not only provide food but can form a symbiotic relationship with soil microorganisms to fix atmospheric nitrogen (Belane *et al*., 2011), thus reducing the farmer's dependence on nitrogen synthetic fertilizers. Cowpeas provide a source of income for millions of people in Sub-Saharan

Africa, thus ensuring a reduction in food insecurities (Asiwe and Maimela 2020, Asiwe and Nkuna, 2021). It also reduces malnutrition among poor farmers and families that are not resourceful (Kamara *et al*., 2018; Asiwe *et al*., 2020b).

Although cowpea plays a significant part in the livelihoods of most farmers, bruchids pose a serious threat to the quality and viability of seeds while in storage (Ndong *et al*., 2012). The threat posed by bruchids to food security and nutrition is severe, given the fact that varieties that escaped damage by field insect pests such as aphids, defoliators, and pod-sucking bugs could be vulnerable to bruchid damage in storage. Bruchid is considered a major post-harvest insect pest, and its presence can cause about 60% yield loss (Asiwe and Letsoalo, 2018), and this can lead to serious economic loss to traders and consumers (Ogunkanmi *et al*., 2018). Therefore, screening of elite cowpea genotypes for bruchid resistance will provide information about their response to bruchid infestation which will provide an informed decision for their deployment in the development of new germplasm. In addition, promising genotypes will be identified and recommended for farmer's cultivation. This will reduce their costs in the use of Gastoxin or fumigant to protect their seeds in storage and increase their potential to make a profit.

1.4. Purpose of the Study

1.4.1. Aim

Characterization of elite cowpea genotypes for bruchid (*C. rhodensianus*) resistance.

1.4.2. Objective

To screen ten elite cowpea genotypes for bruchid resistance.

1.4.3. Hypothesis

The resistance of 10 elite cowpea genotypes to bruchid does not differ.

CHAPTER 2: LITERATURE REVIEW

2.1. The History of Cowpea

Cowpea (*Vigna unguiculata*) is an indigenous crop utilised largely for direct consumption, animal feeds, and as a cover crop around the world. It is an annual leguminous crop from the Fabaceae or Leguminosae Family (Dumet *et al.,* 2008). According to Singh, (2005) cowpea has a lot of diversity throughout Africa and Asia; however, the exact origin of cowpea has been a source of debate. Cowpeas in Asia were found to be more diverse and morphologically distinct than those in Africa, according to early findings. As a result, the cowpea's origin was assumed to be in both Asia and Africa. However, the lack of wild cowpea as promising progenitors in Asia has raised doubts about its Asian origin. As a result, this led to all current evidence suggesting Southern Africa as the place of origin of cowpea, despite the challenges of pinpointing exactly where in Africa the crop was initially introduced. Most studies have recently shown that the highest genotype of wild species of cowpea have been discovered in Botswana, Namibia, Mozambique, Zimbabwe, as well as South Africa (Padulosi, 1987; Pandulosi *et al*., 1990).

2.2. Cowpea Usages

Cowpea contributes significantly to human health and nutrition. It provides animal feed, along with green manure and cover crops that help to sustain soil fertility (Alemu *et al*., 2016). It is very important in the agricultural system because of its proficiency to fix atmospheric nitrogen, which can counteract the nitrogen loss caused by cereals. Cowpea can grow well even in poor soils and can as well help in suppressing weed growth. The crop's tolerance to drought makes it a securing food genus in tropical environment (Bilatu *et al*., 2012; Alemu *et al*., 2016; Belay *et al*., 2017). Farmers frequently sell cowpea grains and leaves in local markets, making cowpea a valuable source of income (Alemu *et al*., 2016). The crop is one of the most extensively cultivated leguminous plants in almost every regional marketplace, particularly within Africa. Purchasing of cowpea, according to Ngalamu *et al*. (2015), gives both rural and urban people, notably women, the opportunity to earn some money. Despite its significance, the production of cowpea in South Africa is insufficient due to possible restrictions.

2.3. Production Constraints of Cowpea

Cowpea yields are low, particularly in Africa, with grain productivity of about 500 kg/ha (FAOSTAT, 2016). Legumes have historically received little interest from research and breeding programs due to their status as "orphan crops" (Ojiewo *et al*., 2018). Drought and other biotic strains such as weeds, diseases, along with insects are some of the constituents contributing to low production of cowpea (Saka *et al*., 2018), with insect pest infestation as one of the most significant issues in the production of this crop. Cowpea in Sub-Saharan Africa contributes for almost 70% of annual output, although it is impeded by post-harvest losses caused by storage insect pests (IITA, 2010). South African farmers, particularly the smallholder farmers have several difficulties while growing legumes like cowpea, one of which is low yield. Farmers' involvement in any breeding effort has been indicated as a factor in recognition and adoption of new enhanced cultivars (Franzel *et al*., 1995), as their requirements and expectations are likely to be realized. According to Asiwe *et al*. (2020 a & b), research on cowpea production has been ignored for the past three decades in South Africa and this was due to lack of improved varieties and limited breeding works which was because of insufficient funding. Other factors that contributed to the lack of cowpea production in South Africa were the lack of veritable seeds for planting as well as minimal returns to farmers which might have resulted due to crop failure.

2.4. The Effects of Cowpea Insects

Cowpea has a diverse pest population, with the pest species adapted to every section of the plant. While the pest level of various insects may range from nation to nation, deficits observed imply that any one major cowpea pest might trigger substantial economic loss if left unmanaged (Jackai and Daoust, 1986). Reduced yields of the African cowpea crop mainly result due to insects, affecting every tissue component and stage of development of the plant (Jackai and Daoust, 1986). Insects cause a severe damage on cowpea, in the field and after harvest once seeds are preserved. The production loss of cowpea triggered by insects is dependent on location, year, and cultivar; and can reach up to 95% (Carlos, 2000). Aphids are the main pests during a developmental stage in the field while bruchids are regarded as the major storage pests. Stored cowpeas in West Africa suffer major losses due to Bruchids (*Callosobruchus maculatus*) and infestation of these insects start at a low level in the field, whereby its population continues to escalate during storage until the cowpea is entirely damaged (Profit, 1997). According to Ntoukam *et al*., (2000), *Bruchidius atrolineatus* is another bruchid pest of cowpea mostly responsible for losses during harvest, and which do not multiply in storage.

2.5. Origin, Distribution, And Biology of Bruchid

The beetle *Callosobruchus rhodensianus* is a member of the Chrysomelidae family (Kergoat *et al*., 2008). This pest is found all over the world and the species is believed to have evolved in Africa and then expanded to areas around the globe (Beck and Blumer, 2014). Cowpea beetle (*Callosobruchus rhodensianus*) is a major insect of legumes in the field as well as storage. Crop infestation begins in the farm (Prevett, 1961), with nearly all of the loss occurring in storage. Bruchid insect has four life stages, and an individual female lays roughly 100 eggs during its lifetime. When an egg hatches, the larva bores into the seed and pupates. Before emerging, the adult punctures the seed coat, allowing it to emerge from the seed (Beck and Blumer, 2014). On cowpeas, bruchid is the most harmful, causing yield reductions of up to 90% (Caswell, 1981). Cowpea bruchid populations can increase exponentially, resulting in severe losses in seed weight, viability, and crop revenue of a susceptible (Singh, 1977; Southgate,1979; Beck and Blumer, 2014).

2.6. The Effects of Bruchid on Cowpea

In storage, cowpea is highly vulnerable to many kinds of Bruchidae insects. Every 3-4 weeks, a female bruchid can multiply twenty times and within 2-3 months, harvested cowpea grains with a light infection may have a high outbreak (Carlos, 2000). Perforation by the pests results in significant quantitative and qualitative losses, limiting the utility and thus, rendering the seeds unsuitable for sowing or human utilization (Ali *et al*., 2004). *Callosobruchus rhodensianus* is the main grain storage pest and losses and deterioration of quality caused by this insect make it difficult for developing countries to attain food security. Poor seed germination, holes in the seeds, nutrient losses, molds, insect-fragment contamination, and excreta are some of the damages caused by this insect pest (Ileke, 2011). The use of synthetic chemical insecticides to protect the seeds has resulted in the poisoning of the cowpea seed, people as well as the environment (FAO, 1992). This has prompted the quest for alternative agricultural and environmental management strategies, such as the usage of resistant cultivars.

2.7. Management Strategies Against *Callosobruchus rhodensianus*

In West Africa and other tropical countries, cowpea is considered a vital source of food (Adedire *et al*., 2011) and due to bruchid damage, a large amount of cowpea yield is lost. Several pest management approaches have been proposed to control this insect pest because of their devastating effects on the quality and quantity of cowpea seeds. Sanitation, cultural, mechanical, biological, and chemical control approaches, are some of the control strategies adopted by farmers to control bruchid pests (Cissokho *et al*., 2015). Chemical techniques of management are the most common, however, they are not environmentally friendly and sustainable (Baouaa *et al*., 2012). Phosphine fumigation, for example, is a widespread strategy, however, it has significant impacts on product safety and pollution problems (Tripathy, 2016). Chemical insecticides to control bruchid in the field, according to some writers, are not only dangerous to individual wellbeing and the ecosystem, but they are ineffective and can contribute to pest resistance (Malaikozhundan and Vinodhini, 2017; Mahmud, 2018). Therefore, incorporating genetically based resistance to infestations, on the other hand, would be a cost-effective and practical control method (Appleby and Credland, 2003).

2.8. The Defence of Cowpea Host Plant to Bruchid

Host plant resistance is the inherent plant traits that determine the amount of injury caused by insect pest (Painter, 1951). It has to do with plants' proficiency to resist pest outbreaks, as well as their ability to recover from pest damage (Kogan, 1994). Insect pest infestations, as well as the extent of their damage in cowpea fields, differ from one region to the next and are dependent on the developmental stage of the plant. Synthetic chemicals, cultural practices, biological practices, and the use of biopesticides are some of the common strategies used to control insect pests in cowpea, with the host plant resistance strategy seen as the highly sustainable way to eliminate the impacts of pests on cowpea (Ofuya and Lale, 2001; Fatokun, 2002). The host plant resistance strategy is important because farmers readily adopt it and it compactible with other control methods (Asiwe and Letsoalo, 2018). It allows a plant to prevent insect pests from selecting a host plant for establishing, egg-laying, and feeding, and even if they do, it interferes with insect pests' biology by disrupting their development and growth, lowering their survival rates (Mookiah *et al*., 2021).

2.9. Types of Plant Resistance

A wide variety of insect predators attack plants daily, which can have a significant impact on plant fitness. To defend against, endure, or prevent insect herbivory, plants utilize a variety of tactics (Painter, 1951; Smith, 2005). Antibiosis, antixenosis (or non-preference), and tolerance are three types of plant resistance. Antibiosis modality restricts a pest's biology by reducing the growth and reproduction rate of insect pests (Painter, 1951; Smith, 2005); whereas antixenosis is a trait that the host plant possesses to discourage insects from using it for oviposition, shelter or feeding (Painter, 1951). Tolerance, on the other hand, is considered a more long-term pest management method because it only minimizes the detrimental impacts of insects on the overall viability without interfering with the physiology or behavior of the insect pest (Jackai and Singh, 1988; Panda and Khush, 1995). The scope of this study was only limited to the tolerance mode of resistance.

2.10. Traits Affecting Bruchid Development

The seed in which seed beetle grow and the predators that harm them are just two of the various ecological components that affect the seed beetle's performance and survival. Seed beetles use the seed coat as host to lay eggs, and the egg hatches to give rise to a larva which then penetrates within the seed to continue their development (Toquenaga and Fujii, 1991, Murdock and Shade, 1991). Seed chemical characteristics like nutritional quality and defence chemicals are also known to influence seed beetle performance (Moreira *et al*., 2015). Seed physical parameters also have a big impact on seed beetle performance. Seed size is proportional to the overall number of resources available to seed beetle larvae throughout development (Oliveira *et al*., 2015; Cuny *et al*., 2017). This is especially significant when there are larvae emerging on the same seed, resulting in resource competition (Kaplan and Denno, 2007). Because bruchids frequently lay multiple eggs per seed, adult weight, lifespan, as well as growth phase are all affected by this competition of resources (Oliveira *et al*., 2015; Cuny *et al*., 2017).

CHAPTER 3: METHODOLOGY AND ANALYTICAL PROCEDURE

3.1. Description of the Study

The study was conducted at the Department of Plant Production Laboratory at the University of Limpopo, South Africa (23°53’10’’S, 29°44’15’’E) to characterize 10 elite cowpea genotypes for bruchid (*Callosobruchus rhodensianus*) resistance (Table 1). According to Messina and Renwick (1985) and Chanbang *et al*. (2008), the eligibility of seeds for oviposition can be evaluated by their physical characteristics, and the selected genotypes for this study differ in terms of texture which is one of the features influencing the bruchid insect preference for oviposition (Table 1).

Table 1: Cowpea genotypes screened for bruchid resistance.

Genotype number	Genotype name	Texture
101	AYT208$_B$	Rough
102	AYT211$_B$	Rough
103	AYT210$_B$	Smooth
104	AYT205$_A$	Rough
105	AYT305$_B$	Smooth
106	CB-24J’burg	Rough
107	AYT205$_B$	Rough
108	AYT211$_A$	Rough
109	AYT310$_B$	Rough
110	AYT302$_A$	Rough

3.2. Bruchid Screening Procedure

The screening process was carried out following the procedure described by Asiwe and Letsoalo (2018) with some adjustments. The treatments were laid out in a Completely Randomized Design (CRD), with six (6) replications. Bruchid culture was established using the already existing bruchid pests at the University of Limpopo Research Farm and these were reared using susceptible black-eyed cowpea seeds in an oven set at 28°C to obtain sufficient bruchids to be used in the study. The resistance of bruchids was tested on 10 elite cowpea seeds obtained from the University of Limpopo germplasm collection. To eliminate any existing eggs or larvae that have been in the seeds and to ensure that the moisture content of the seeds is uniform, the seeds were oven-dried at 50°C for 24 hours before infestation (Amusa *et al*., 2014; Miesho *et al*., 2018). From each variety, 10 seeds were placed into Petri dishes, where newly emerged bruchids (4 males and 4 females) were introduced using a hairbrush. Male and female bruchids were characterized by the color of the plate near the end of the abdomen. Females have dark stripes on the sides of the expanded plate that covers the tip of the abdomen and are dark brown or nearly black in appearance, whilst males are light brown with a smaller plate and no stripes (Beck and Blumer, 2014). Bruchids were left for four (4) days at 28°C to allow for mating and oviposition to take place. Four days after the eggs were laid, the bruchids were removed and the number of eggs laid on each seed was counted. After egg counting, the excess eggs were scraped off using a razor blade and only one (1) egg was randomly selected and left on each seed to allow for uniform infestation. The treatments were observed daily for the emergence and the emerged adults were counted daily until there were no more adults emerging.

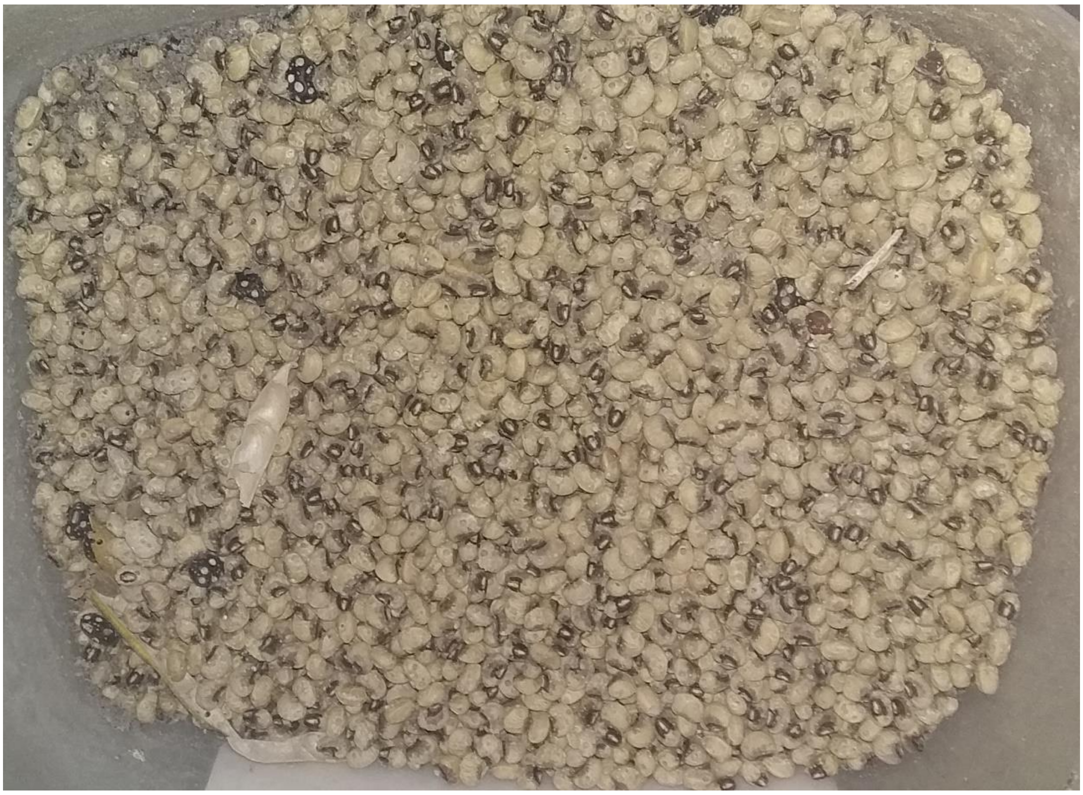

Figure 1: Susceptible black-eyed cowpea seeds used for bruchid culture.

3.3. Data Collection

Data were collected for 60 days on the following parameters from the infested samples.

3.3.1. Initial seed weight (ISW) (g)

- The weight of the seeds before the experiment.

3.3.2. Residual seed weight (RSW) (g)

- The weight of the samples after the experiment.

3.3.3. Seed weight loss (SWL) (%) = $\frac{initial\ seed\ weight - residual\ seed\ weight}{Initial\ seed\ weight}$ × 100 (Girish *et al*., 1975).

3.3.4. Number of eggs laid (NE)

- This was measured by counting the number of eggs on seeds four days after the infestation of bruchid.

3.3.5. Number of adults emerged (AE)

- Was measured by counting the number of insects that emerged.

3.3.6. Adult emergence (AE) (%) = $\frac{Number\ of\ adults\ emerged}{number\ of\ eggs\ laid}$ × 100

3.3.7. Total development time (TDT) (days)

Refers to the number of days from the start of infestation and when adult bruchids will stop emerging from the seeds. This was measured by counting the number of days from the start of infestation to the day there are no more adults emerging.

3.3.8. Mean development time (MDT) (days)

- Mean developmental time (T) is the time taken by adults to emerge. It was estimated according to Asiwe and Letsoalo (2018).

3.3.9. Number of damaged seeds (DS)

- Was measured by counting seeds having holes from an adult emergence.

3.4. Data Analysis

Data were analyzed using Genstat Version 20, and analysis of variance (ANOVA) was used to compare F-values of the performances of the genotypes. Means showing significant differences were separated using Duncan Multiple Range Test at $P<0.05$.

CHAPTER 4: RESULTS AND DISCUSSION

This study exposed ten elite cowpea genotypes to *C. rhodensianus* for 60 days in the laboratory to identify new cowpea genotypes with bruchid resistance that could be used to improve the productivity of elite cowpea around Limpopo province and South Africa as a whole.

4.1. The Mean Number of Eggs on 10 Elite Cowpea Genotypes

There was no significant difference in the mean number of eggs of the cowpea genotypes used in this study (Appendix 1). However, the results of this study showed that more eggs were laid on AYT211B (49.00) followed by AYT210B (47.33), AYT205B (43.00) and AYT205A (42.50) whereas the minimum mean number of eggs was shown on CB-24J'burg (27.50) followed by AYT302A (27.83) (Figure 4.1). The number of eggs was measured to indicate the preference of bruchid for oviposition on these genotypes, and genotypes with greater oviposition rates indicate that they were more preferred by the bruchid for oviposition. The types of resistance were not researched in this study, however, several studies showed that qualities such as seed texture, colour, shape, or thickness may determine the oviposition rate of bruchid. According to Majhi and Mogali (2020), cowpeas with greater seed size provide more surface area for oviposition, resulting in more bruchid eggs on seeds with greater size than on smaller ones.

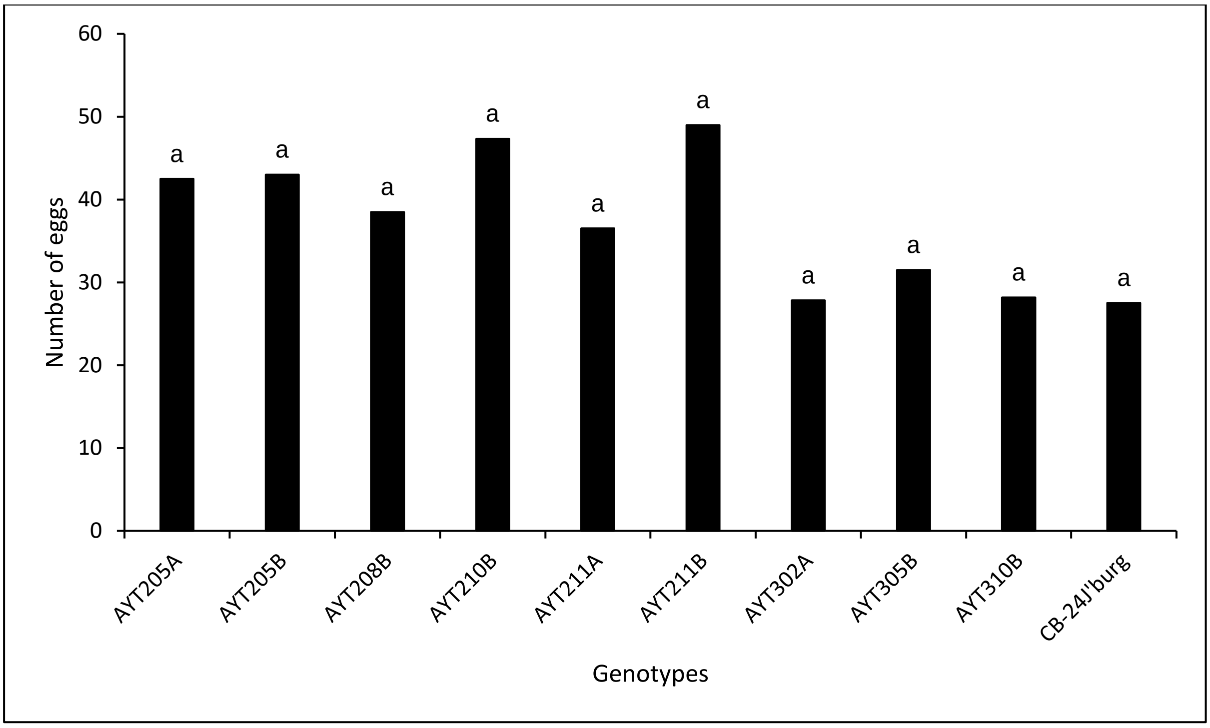

Figure 4.1: Mean number of eggs laid on elite cowpea genotypes by bruchid insect.

The findings of this study agreed with those of Kebe *et al*. (2020), who investigated the affinity of *C. maculatus* for oviposition on three varieties of cowpea and found that oviposition was the same in all three varieties. Previous research on the genotypes of *V. unguiculata* that are *C. maculatus*-susceptible and resistant, which have identical physical features such as color, texture, and size, did not demonstrate any changes in oviposition (Sales *et al*., 2005).

4.2. The Percentage of Adult Emergence

In this study, the emergence of adults was monitored daily after oviposition and there was no significant difference among the genotypes (Appendix 2). The highest percentage emergence was observed on AYT302A with 31.54% and the least percentage was recorded on AYT211B followed by AYT205A with 19.25% and 19.77%, respectively. This indicates that genotypes with the highest emergence percentage were seen as suitable hosts for bruchids as this would mean that the genotypes were able to provide food for bruchid development and survival. Jackai and Asante (2003), described adult emergence as one of the major indicators for the classification of resistant or susceptible seeds to insect infection.

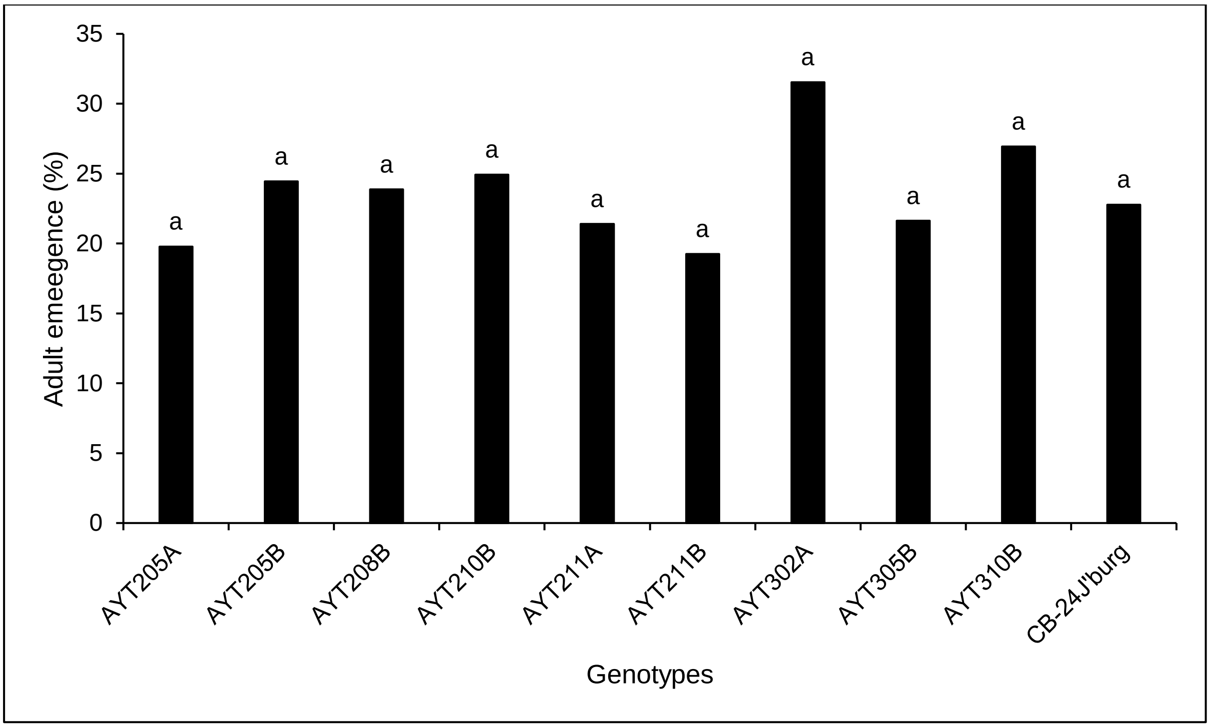

Figure 4.2: Percentage of adult bruchid (%) emerged on elite cowpea genotypes

According to Bondade and Deshpande (2021), the mean number of adults emerged within a range of 13.44-32.21 were classified as moderately resistant. This would mean that in terms of adult emergence, the genotypes used in the present study are moderately resistant to *C. rhodensianus* as they fall within the range (Table 2).

Table 2: Percentage bruchid adult emergence on elite cowpea genotypes

PAE (%)	Number of genotypes
10-20	2
21-30	7
31-40	1

The resistance may be due to antibiosis and tolerance as suggested by many past studies (Painter, 1951). Even though that may be the case, further work is needed on the mechanism of these genotypes. Studies have shown that the consumption of seed components by larvae, such as digestive enzyme inhibitors and chitin-binding proteins which attach to the peritrophic matrix of the larvae, can impede nutritional digestion and absorption, resulting in a decrease in the emergence of adults (Ventury *et al*., 2022).

4.3. Percentage of Damaged Seed

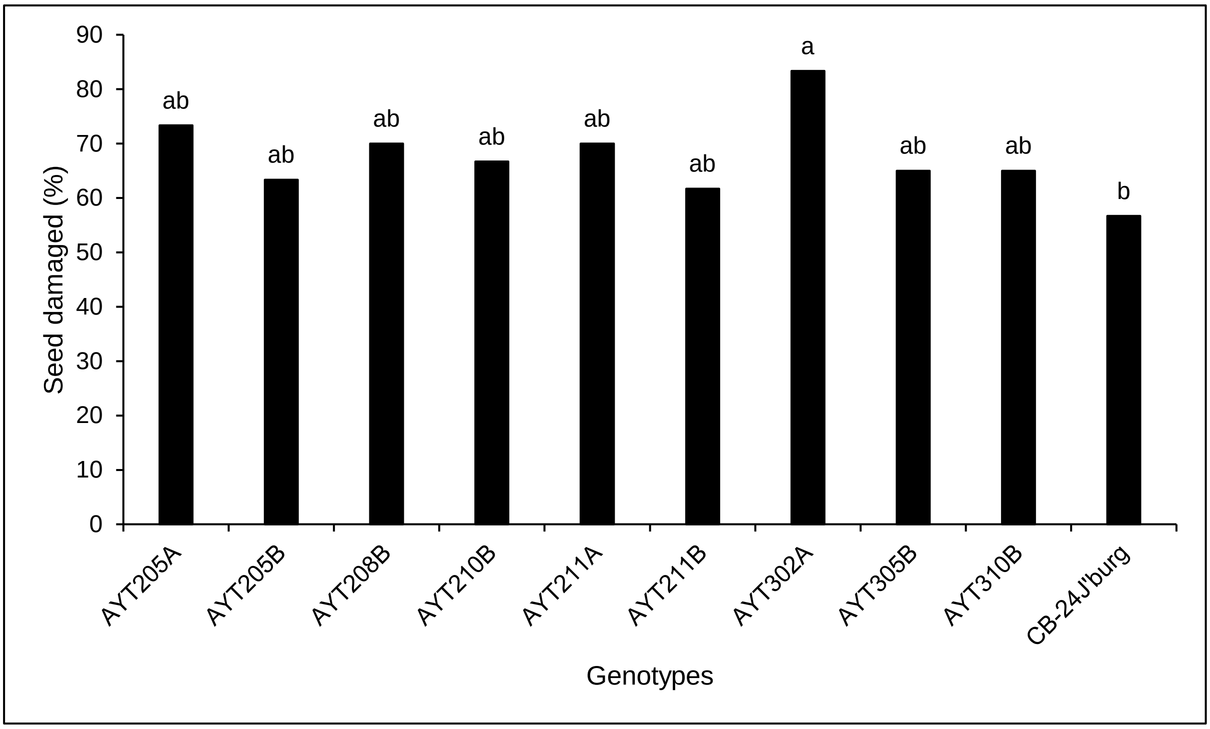

Figure 4.3: Percentage of seed damaged on elite cowpea genotypes

There was no variation observed at P <0.05 in the percentage seed damaged (Appendix 3). The holes on the seeds from which the adult bruchids emerged were used as an indication of damage. The percentage of seed damage ranged from 56.67% to 83.33% with the lowest percentage observed on CB-24J'burg (56.67%), and the highest on AYT302A (83.33%) (Figure 4.3). In this study, genotypes with the highest percentage of seed damage are vulnerable to *C. rhodensianus* whereas the resistant genotypes have the lowest seed damage percentage. More damage was observed on all these genotypes indicating that these genotypes were susceptible to *C. rhodensianus* (Table 3).

Table 3: Percentage of seed damaged.

SD (%)	Number of genotypes
50-60	1
61-70	7
71-80	1
81-90	1

According to Kpoviessi *et al*. (2019), larvae that establish within grains, consume them from within, and then emerge to form pores on the seeds once fully formed are what harm stored seeds. Due to the highest percentage of seed damage observed, this would imply that there were no physical or chemical barriers in the seeds of the genotypes used in this study, which might impede larval entry and lead to fewer holes (Lephale *et al*., 2012). The results of the present study confirmed those reported by Miesho *et al*. (2018), who observed high damage which was indicated by the presence of holes in the genotypes on susceptible varieties and less on resistant varieties. The same results were recorded by Tripathi *et al*. (2012). There is a need for further breeding work to discover and introduce new sources of genetic traits with resistance to *C. rhodensianus* into these genotypes to achieve increased productivity and enhanced consistency in future genotypes (Gore *et al*., 2016).

4.4. Total Development Time

The average total development period (days) of *C. rhodensianus* on ten elite cowpea genotypes did not differ significantly from one another (Appendix 4). However, AYT305B (39.50 days) had the lowest total developmental mean value while the highest was 46.50 days observed on AYT211A (Figure 4.4). In accordance with this study, cultivars with a long developmental period are regarded to be resistant, whereas those with a short period are vulnerable to *C. rhodensianus*.

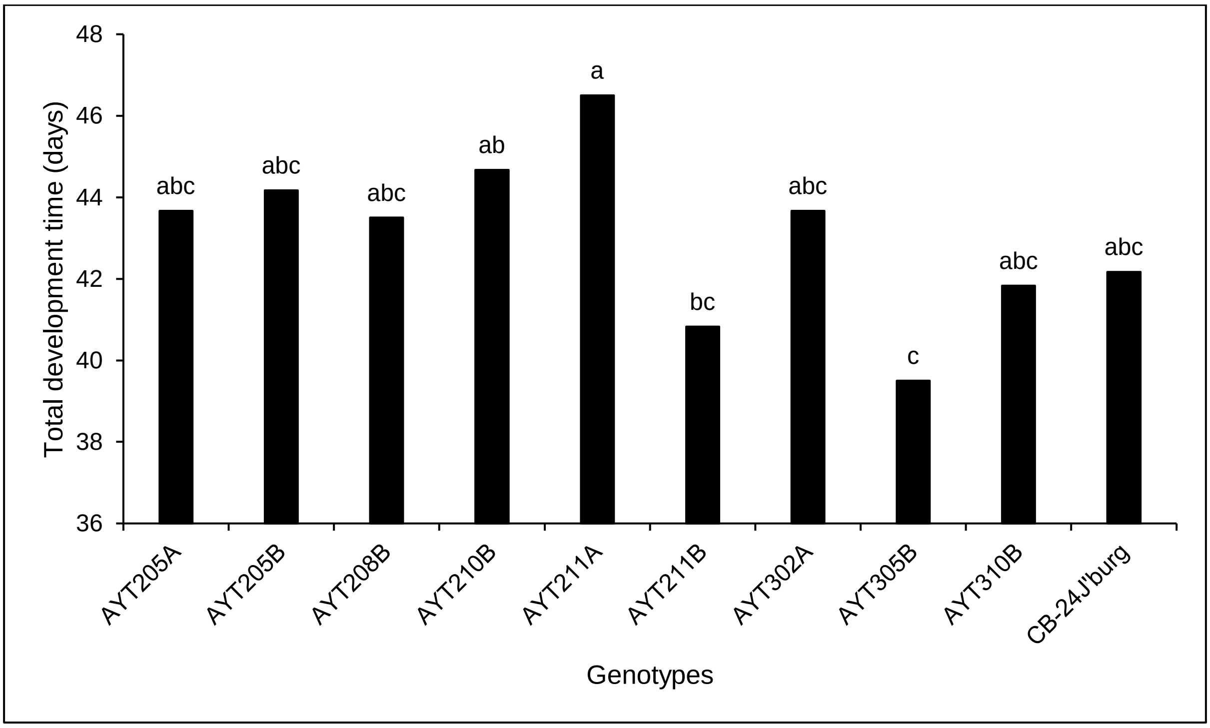

Figure 4.4: Mean total development time of bruchid in cowpea genotypes

Asiwe and Letsoalo (2018), reported that varieties with a total development time over 40 days which was noticed on IT845-2246 are regarded as moderately resistant varieties. AYT205A, AYT205B, AYT208B, AYT210B, AYT211A, AYT211B, AYT302A, AYT310B, and CB-24J’burg had mean values over 40 days, which indicated moderate resistance in terms of overall development time. The prolonged period between the egg and adult phase as well as the reduced adult emergence in most cases may be associated with the antibiosis mode of resistance (Smith and Clement, 2012), as it interferes with the developmental process of the pest either by slowing down its growth, fecundity or increasing its mortality (Painter, 1951; Smith, 2005).

Table 4: Frequency distribution of total development time of bruchid on cowpea genotypes

TDT (days)	Number of genotypes
30-40	1
41-50	9

In this case, as the study concentrated on the tolerance mode of resistance, the shortage or lack of food to support the development of larvae could have been the reason for the delayed emergence of adults or the longest development period. The findings of this study complemented that of Tripathi *et al*. (2015) who observed that resistant variants of cowpea took longer time to mature than susceptible types. The longest development period renders these genotypes promising and they can be recommended to farmers as this indicates that the food available in these genotypes is not favourable to support the development of bruchid larva.

4.5. Mean Development Period

The mean development time was obtained by counting the time in days it took the insect to emerge and the findings showed that there were significant differences among the elite cowpea genotypes (Appendix 5). AYT305B recorded the shortest development period indicating that the genotype was able to meet the feed requirements of bruchid to support its development, whereas the longest development period was recorded on AYT208B (11.33 days) followed by AYT210B (10.17days) (Figure 4.5). In this study, resistant genotypes were those that took longer days to develop whereas those with shorter days were susceptible to cowpea bruchid because were able to support all the food requirements needed by this insect, hence the larvae were able to develop faster.

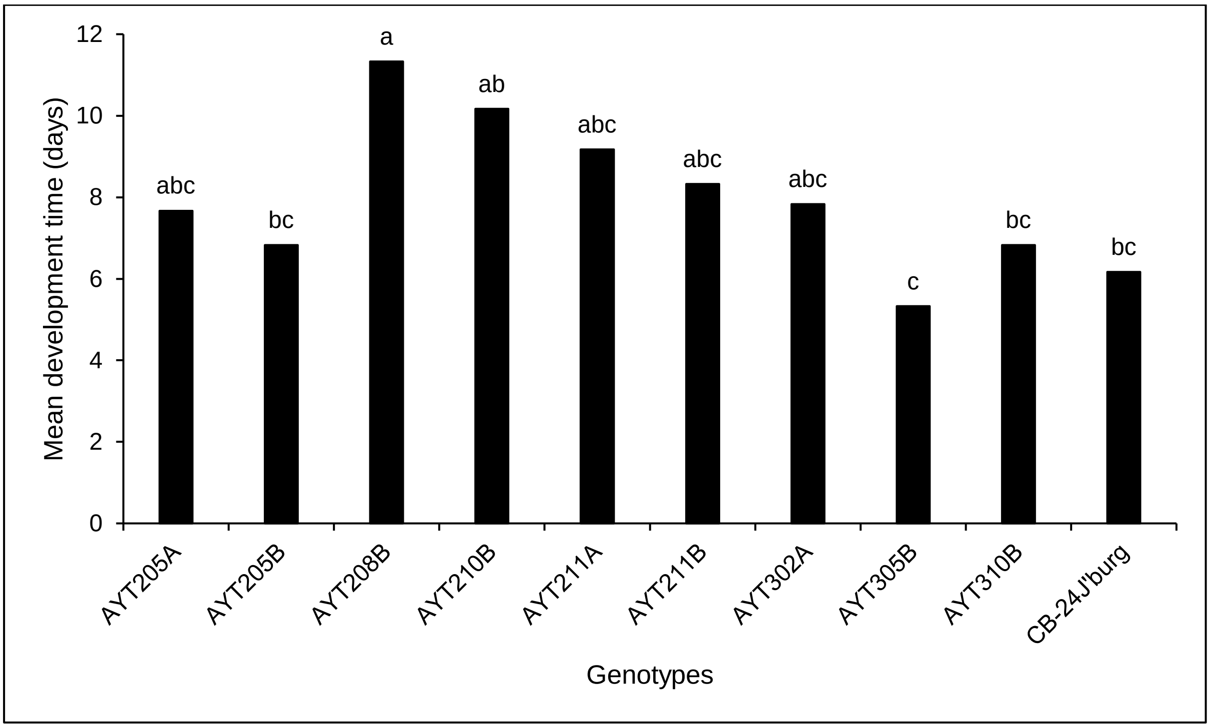

Figure 4.5: Mean development time of bruchid on cowpea genotypes

According to Amusa *et al*. (2018), the more crucial trait for assessing resistance in cowpea is mean development time (MDT). The extended MDT suggests the possibility of anti-nutritional elements, which may have influenced the slow growth of the insect pest when it is feeding on the resistant genotypes due to the antibiosis impact (Majhi and Mogali, 2020). Divya *et al*. (2012) and Jadhav *et al*. (2012) also reported a similar observation.

4.6. Number of Days to Insect Emergence

The results of number of days to insect emergence showed that there was no significant difference among the 10 elite cowpea genotypes (Appendix 6). The least mean number of days to insect emergence was recorded on AYT208B followed by AYT211B with 32.33 days and 32.50 days, respectively, whereas AYT211A recorded the highest mean score of 37.50 days followed by AYT205B with 37.33 days (Figure 4.6).

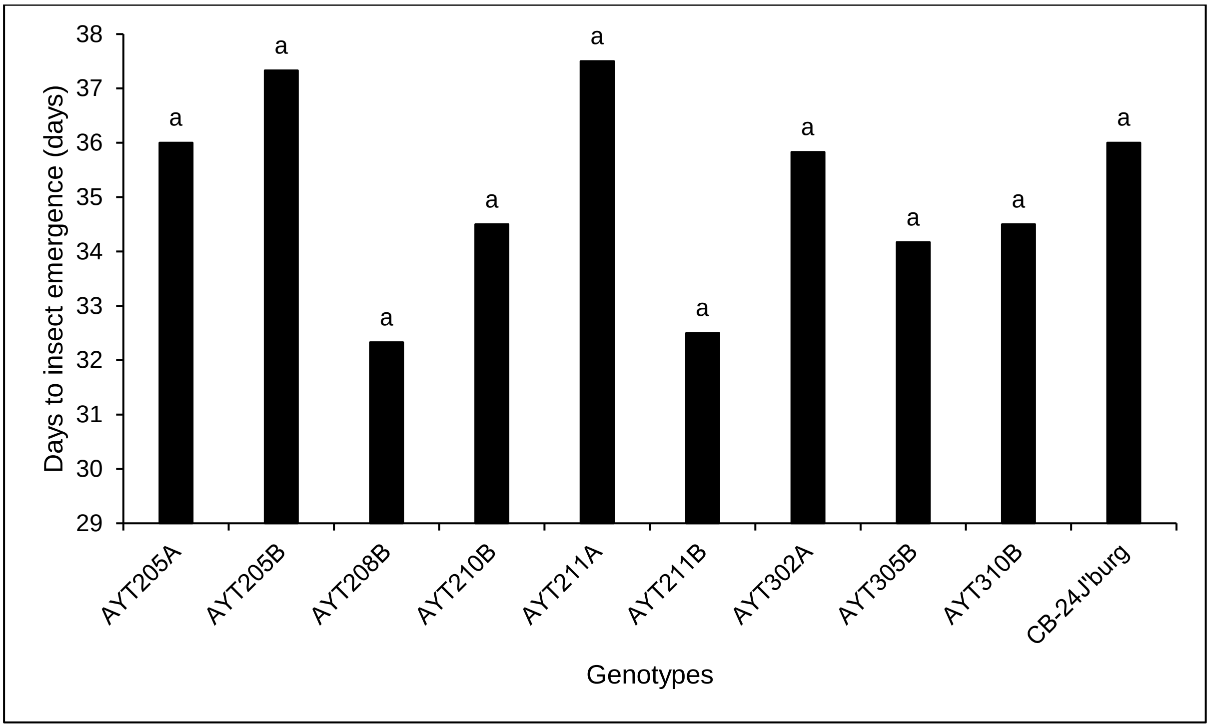

Figure 4.5: Mean number of days to insect emergence (days) on cowpea genotypes

The results of this study conform with previous experiments conducted at IITA, Nigeria, that revealed that insects that took about 25 to 30 days to emerge are moderately resistant varieties and this was observed in IT845-2246 (Adjadi *et al*., 1985). This implies that there is a greater possibility for these ten genotypes to be recommended to smallholder farmers who lack access to highly improved storage facilities because this shows that the food present in these genotypes is not suitable to support the growth of bruchid larva.

4.6. Seed Weight Loss

According to the results of our research, there was a highly significant difference (P<.001) (Appendix 7) in the means of the cowpea genotypes used in terms of seed weight loss. The AYT210B genotype had the least weight loss than all other cowpea genotypes. ATY210B recorded the lowest weight loss with 4.07%, followed by AYT205A, AYT205B, CB-24J’burg, and AYT211B (7.20%, 12.60%, 15.43%, and 15.57%, respectively) as shown in (Figure 4.6). The greatest loss was observed on AYT208B (33.90%) followed by AYT310B and AYT305B with 33.35% and 30.78%, respectively. In most of the time, the seed weight loss is likely associated with the damage caused by bruchid on the seeds as they exit the seeds by puncturing the seed coat, whereby in this study the weight loss was associated with insect feeding which occurred from inside the seed by the larvae.

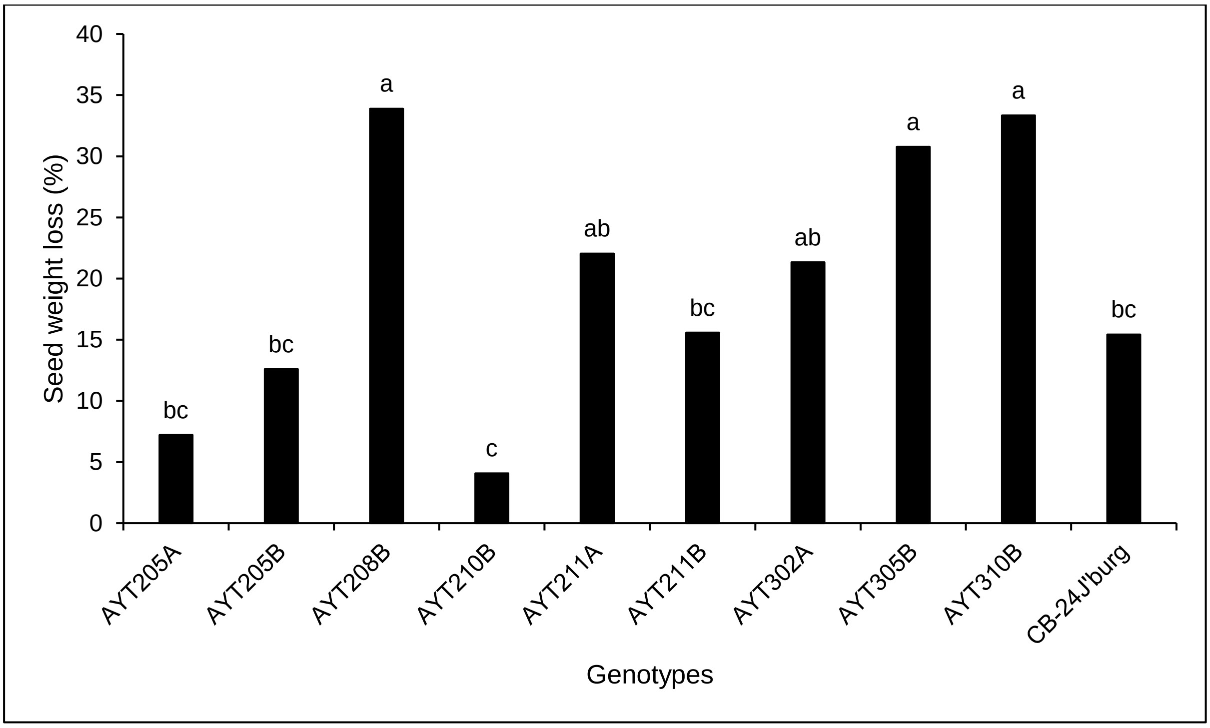

Figure 4.6: Mean percentage weight loss (%) of elite cowpea genotypes

As reported by Miesho *et al*. (2018), seed damage and a low insect growth index may account for the loss in seed weight caused by bruchid. The more the weight loss, the more the insect larvae see the genotype as a suitable host to meet its nutritional needs, hence a lot of the endosperm were consumed. Several studies have shown that seed damage and insect emergence are in most cases the reason for greater seed weight loss caused by cowpea weevil, but that was not the case in the present study as the results showed no relationship between the variables. The current outcomes are corroborated by Tripathi *et al*. (2012), who investigated cowpea response to *C. chinensis* in various crops and discovered that seed weight loss is linked to food consumption. Seed damage, adult emergence, and seed weight loss are the most accurate predictors of cowpea resistance to bruchid (Jackai and Asante, 2003).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The screening procedure was successfully performed, and the susceptibility level of the ten elite cowpea genotypes was identified. The findings of this study showed that there was a significant difference between means of initial seed weight, residual seed weight, seed weight loss, and mean development time and no significant difference was observed in the number of eggs, percentage seed damage, adult emergence, number of days to insect emergence and total development time. The null hypothesis was accepted because the response of the ten elite cowpea genotypes to cowpea bruchid resistance was the same. The ten elite cowpea genotypes used in this study were moderately resistant in terms of adult emergence, the number of days to insect emergence, mean development time, and total development time.

The least percentage weight loss was observed on AYT210B (4.07%), followed by AYT205A (7.20%). An extended days were recorded for the number of days to insect emergence as well as the mean development time for all ten genotypes. The prolonged insect developmental periods were observed on AYT205A, AYT205B, AYT208B, AYT210B, AYT211A, AYT211B, AYT302A, AYT310B and CB-24J’burg in terms of total development time. The genotypes recorded longer developmental periods and low seed weight loss is an indication that they can be stored for longer periods, therefore, these can be recommended to smallholder farmers who lack access to high-quality storage facilities. This is important because will improve the availability of elite cowpea genotypes in South Africa.

Although these genotypes can be stored for a longer period without being attacked, it is essential that sustainable control measures to control bruchid be followed to eliminate losses or damage to cowpea bruchid. For safety measures and to improve the availability of elite cowpea genotypes, it is important that these genotypes be recommended for breeding programs to improve their resistance level against *C. rhodensianus* through hybridization (crossing). The procedure is important because it will not only cut the farmer’s cost of production but reduce their reliance on insecticides and fumigants which may pose health risks to farmers. It is also important that further work be done on the mechanism of resistance because although the study concentrated on tolerance modality, the characteristics of antibiosis were exhibited.

REFERENCES

ADEDIRE, C.O., OBEMBE, O.O., AKINKUROLELE, R.O. and ODULEYE, O. 2011. Response of *Callosobruchus maculatus* (Coleoptera: Chysomelidae: Bruchidae) to extracts of cashew kernels. *Journal of Plant Disease and Protection*, 118: 75-79.

ADJADI, O., SINGH, B.E. and SINGH, S.R. 1985. Inheritance of bruchid resistance in cowpea 1. *Crop science*, 25:740-742.

AKYAW, M.O., BAIDOO, P.K. and MOCHIAH, M.B. 2014. The effect of time harvest on the damage caused to cowpea by storage bettle, *Callosobruchus maculatus* (FAB.) (Coleoptera: Bruchidae). *Global Advanced Research Journal of Agricultural Science*, 3: 351-355.

ALEMU, M., ASFAW, Z., WOLDU, Z., FENTA, B. A. and MEDVECKY, B. 2016. Cowpea (*Vigna unguiculata* (L.) Walp.) (Fabaceae) landrace diversity in Northern Ethiopia. *International Journal of Biodiversity and Conservation*, 8: 297–309.

ALI, S.M., MAHGOUB, S.M., HAMED, M.S. and GHARIB, M.S.A. 2004. Infestation potential of *Callosobrunchus chinensis* and *C. maculatus* on certain broad bean seed varieties. *Egyptian Journal of Agricultural Research*, 82: 1127-1135.

AMADOU, L., BAOUA, I.B., BARIBUTSA, D., WILLIAMS, S.B. and MURDOCK, L.L., 2016. Triple bag hermetic technology for controlling a bruchid (*Spermophagus sp.)* (Coleoptera, Chrysomelidae) in stored *Hibiscus sabdariffa* grain. *Journal of Stored Products Research*, 69: 22-25.

AMUSA, O.D., OGUNKANMI, L.A., ADETUNBI, J.A., AKINYOSOYE, S.T., BOLARINWA, K.A. and OGUNDIPE, O.T. 2014. Assessment of Bruchid (*Callosobruchus maculatus*) Tolerance of Some Elite Cowpea (*Vigna unguiculata*) Varieties. *Journal of Agriculture and Sustainability*, 6: 164-178.

APPLEBY, J.H. and CREDLAND, P.F. 2003. Variation in responses to susceptible and resistant cowpeas among West African populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Journal of Economic Entomology*, 96: 489–502.

ASIWE J. N. A. and NKUNA M. K. 2021. Performance of cowpea (*Vigna unguiculata*) varieties under cowpea-maize strip intercropping and phosphorus fertilisation in Limpopo Province, South Africa. *Research On Crops Journal*, 22: 798-806.

ASIWE, J.N.A. 2022. Advanced Breeding Approaches for Developing Cowpea Varieties in Dryland Areas of Limpopo Province, South Africa. *Legumes*.

ASIWE, J.N.A. and LETSOALO, I.M. 2018. Response of introduced cowpea breeding lines to bruchid (*Callosobruchus rhodesianus*) infestation in South Africa. Tropical Agriculture, 95: 218-228.

ASIWE, J.N.A. and LETSOALO, I.M. 2018. Response of introduced cowpea breeding lines to bruchid (*Callosobruchus rhodesianus*) infestation in South Africa. Tropical Agriculture, 95: 218-228.

ASIWE, J.N.A. and MAIMELA, K.A. 2021. Assessment of productivity variables of cowpea (*Vigna unguiculata)* varieties in cowpea-maize (*Zea mays*) strip intercropping in Limpopo Province, South Africa. *Research On Crops*, 22: 516-525.

ASIWE, J.N.A., OLUWATAYO, I.B. and ASIWE, D.N. 2020a. Enhancing food security, nutrition, and production efficiency of high-yielding grain legumes in selected rural communities of Limpopo Province, South Africa. *Production Guide, Training of Farmers and Cowpea Processing, and Capacity Building. WRC Report No. TT*, 829: 20.

ASIWE, J.N.A., OLUWATAYO, I.B. and ASIWE, D.N. 2020b**.** Enhancing food security, nutrition, and production efficiency of high-yielding grain legumes in selected rural communities of Limpopo Province, South Africa. *Production Guide, Training of Farmers and Cowpea Processing, and Capacity Building. WRC Report No. TT*, 829: 62.

BAOUAA, I.B., MARGAMB, V., AMADOUA, L. and MURDOCK, L.L. 2012. Performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. *Journal of Stored Products Research*, 51: 81–85.

BECK, C. W. and BLUMER, L.S. 2014. A handbook on bean beetles, *Callosobruchus maculatus. National Science Foundation*.

BECK, C. W. and Blumer, L.S. 2014. A handbook on bean beetles, *Callosobruchus maculatus.*

BECK, C. W. and BLUMER, L.S. 2014. A handbook on bean beetles, *Callosobruchus maculatus. National Science Foundation*.

BELANE, A.K., ASIWE, J.N.A. and DAKORA, F.D. 2011. Assessment of N_2 fixation in 32 cowpea (*Vigna unguiculata* L. Walp) genotypes grown in the field at Taung in South Africa, using 15N natural abundance. *African Journal of Biotechnology*, 10: 11450-11458.

BELAY, F., GEBRESLASIE, A. and MERESA, H. 2017. Agronomic performance evaluation of cowpea (*Vigna unguiculata* (L.) Walp) varieties in Abergelle District, Northern Ethiopia. *Journal of Plant Breeding and Crop Science*, 9: 139–143.

BELAY, F., GEBRESLASIE, A. and MERESA, H., 2017. Agronomic performance evaluation of cowpea [*Vigna unguiculata* (L.) Walp] varieties in Abergelle District, Northern Ethiopia. *Journal of Plant Breeding and Crop Science*, 9: 139-143.

BILATU, A., BINYAM, K., SOLOMON, Z., ESKINDER, A. and FEREDE, A. 2012. Animal feed potential and adaptability of some cowpea (*Vigna unguiculata*) varieties in Northwest lowlands of Ethiopia. *Wudpecker Journal of Agricultural Research*, 1: 478–483.

BONDADE, V. and DESHPANDE, S.K. 2021. Bruchid Resistant Studies using Stabilized Derivatives (F 7 Families) of Interspecific Cross Cowpea x Rice Bean. *Legume Research: An International Journal*, 44.

CARLOS, G. 2000. Post-Harvest Operation, Food and Agriculture Organisation of United Nations, Rome, Italy.

CASWELL, G.H. 1970. storage of cowpea in the northern states of Nigeria. *Samaru research bulletin*.

CHANBANG, Y., ARTHUR, F.H., WILDE, G.E. and SUBRAMANYAM, B.H. 2008. Methodology for assessing rice varieties for resistance to the lesser grain borer, *Rhyzopertha dominica*. *Journal of insect science*, 8: 1-5.

CISSOKHO, P.S., GUEYE, M.T., SOW, E.H. and DIARRA, K. 2015. Substances inertes et plantes à effet insecticide utilisées dans la lutte contre les insectes ravageurs des céréales et légumineuses au Sénégal et en Afrique de l‟Ouest. *International Journal of Biological and Chemical Science*, 9: 1644-1653.

CUNY, M.A., SHLICHTA, G.J. and BENREY, B. 2017. The large seed size of domesticated lima beans mitigates intraspecific competition among seed beetle larvae. *Frontiers in Ecology and Evolution*, *5*: 145.

DIVYA, P., KANAKA DURGA, K., SUNIL, N., RAJASRI, M. and UDAYABABU, P. 2012. Screening of horse gram accessions against pulse beetle, *Callosobruchus chinensis. Indian Journal of Plant Protection*, 40: 280-286.

DOBIE, P. 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera Curculionidae). *Journal of Stored Products Research*, 10:183-197.

DONGRE T.K., PAWAR S.E., THAKARE R.G. and HARWALKAR M.R. 1996. Identification of resistant sources to cowpea weevil (*Callosobruchus maculatus* (F.) in Vigna sp. and inheritance of their resistance in black gram (*Vigna mungo* var. *mungo*). *Journal of Stored Product Research*, 32: 201-204.

DUMET, D., ADELEKE, R., and B., FALOYE. 2008. Regeneration guidelines: cowpea. In: DULLOO, M.E., THORMANN, I., JORGE, M.A., and J., HANSON editors. Crop-specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme, Rome, Italy, 8.

FAOSTAT. 2016. Food and agriculture organization of the United Nations Statistics Division. http://faostat3.fao. org/download/Q/QC/E

FATOKUN, C.A., 2002. Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *Vigna vexillata*. *Challenges and opportunities for enhancing sustainable cowpea production*, 52-61.

FOOD AGRICULTURAL ORGANIZATION. 1992. Pesticide Residues in Food. Report number 116: Rome.

FRANZEL, S., HITIMANA, L. and AKYEAMPONG, E., 1995. Farmer participation in on-station tree species selection for agroforestry: a case study from Burundi. *Experimental Agriculture*, 31: 27-38.

GIRISH, G.K., KUMAR, A. and JAIN, S.K. 1975. Part II assessment of the quality loss in wheat damaged by Trogoderma granarium everts during storage. *Bulletin Grain Technology*, 13: 26-32.

GORE, P.G., TRIPATHI, K., CHAUHAN, S.K., SINGH, M., BISHT, I.S. and BHALLA, S. 2016. Searching for resistance in wild Lens species against pulse beetle, *Callosobruchus chinensis* (L.). *Legume Research-An International Journal*, 39: 630-636.

ILEKE, K.D. 2011. Effect of *Sitophilus zeamais* Motschulsky and *Sitophilus oryzae* (L) [Coleoptera: Curculionidae] infestation on grain quality of wheat (*Triticum aestivum*). *Journal of Physical and Biological Sciences*, 4: 7-12.

INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE. 2010. Ibadan, Nigeria. Fifth World Cowpea Conference 2010 held between 27th September to 1st October, Palm Beach Hotel, Saly, Senegal.

JACKAI, L.E. and DAOUST, R.A. 1986. Insect pests of cowpeas. *Annual review of entomology*, 31: 95-119.

JACKAI, L.E.N. and ASANTE, S.K. 2003. A case for the standardization of protocols used in screening cowpea, *Vigna unguiculata* for resistance to *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). *Journal of Stored Products Research*, 39: 251-263.

JACKAI, L.E.N. and DAOUST, R.A. 1986. Insect pests of cowpeas. *Annual Review of Entomology*, 31: 95-119.

JACKAI, L.E.N. and SINGH, S.R. 1988. Screening techniques for host plant resistance to cowpea insect pests. *Tropical Grain Legume Bulletin*, 35: 2-18.

JADHAV, D.R., MALLIKARJUNA, N. and RANGA RAO, G.V. 2012. *Callosbruchus maculatus* resistance in some wild relatives and interspecific derivatives of Pigeonpea. *Indian Journal of Plant Protection*, 40: 40-44.

JAYATHILAKE, C., VISVANATHAN, R., DEEN, A., BANGAMUWAGE, R., JAYAWARDANA, B.C., NAMMI, S. and LIYANAGE, R., 2018. Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture*, 98: 4793-4806.

KAMARA, A.Y., OMOIGUI, L.O., KAMAI, N., EWANSIHA, S.U. and AJEIGBE, H.A. 2018. Improving cultivation of cowpea in West Africa, 1-18.

KAPLAN, I. and DENNO, R.F. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology letters*, 10: 977-994.

KEBE, K., ALVAREZ, N. and ESPINDOLA, A. 2020. Oviposition choice and larval development of the seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) on three cowpea varieties. *Journal of Stored Products Research*, 86: 101578.

KERGOAT, G.J., DELOBEL, A., LE RU, B. and SILVAIN, J.F. 2008. Seed beetles in the age of the molecule: recent advances on systematics and host-plant association patterns. *Research on Chrysomelidae*, 1: 59-86.

KOGAN, M. 1994. Plant resistance in pest management. In Metcalf RL, Luckmann WH (eds), *Introduction to insect pest management*, 3rd edition. Wiley, New York.

KPOVIESSI, D., AGBAHOUNGBA, S., AGOYI, E.E., CHOUGOUROU, D.C. and ASSOGBADJO, A.E. 2019. Resistance of cowpea bruchid (*Callosobruchus maculatus* Fab.): knowledge level on the genetic advances. *Journal of Plant Breeding and Crop Science*, 11: 185-195.

LATTANZIO, V., TERZANO, R., CICCO, N., CARDINALI, A., DI VENERE, D. and LINSALATA, V. 2005. Seed coat tannins and bruchid resistance in stored cowpea seeds. *Journal of Science Food and Agriculture*, 85: 839-846.

LEACH J.E., CRUZ C.M.V., BAI J. and LEUNG H. 2001. Pathogen fitness penalty as a predictor of the durability of disease resistance genes. Annual Review of Phytopathology, 39: 187-224.

LEPHALE, S., ADDO-BEDIAKO, A. and AYODELE, V. 2012. Susceptibility of seven cowpea cultivars (*Vigna unguiculatus*) to cowpea beetle (*Callosobruchus Maculatus*). *Agricultural Science Research Journals*,2: 65–69.

LETSOALO, I.M. 2015. *Evaluation of introduced cowpea breeding lines for Aphid (Aphis craccivora) and bruchid (Callosobruchus rhodensiansus) resistance in South Africa* (Doctoral dissertation, University of Limpopo).

MAHMUD, H. 2018. Effect of pulse beetle, *Callosobruchus Chinensis* L. on oviposition and damage in some important genotypes of pulse crops in Bangladesh. *Biomedical Journal of Scientific and Technical Research*, 2: 1–5.

MAJHI, P.K. and MOGALI, S.C. 2020. Characterization and Selection of Bruchid [*Callosobruchus maculatus* (F.)] Tolerant Greengram [*Vigna radiata* (L.) Wilczek] Genotypes. *Indian Journal of Agricultural Research*, 54: 679-688.

MALAIKOZHUNDAN, B. and VINODHINI, J. 2017. Biological control of the pulse beetle, *Callosobruchus maculatus* in stored grains using the entomopathogenic bacteria, *Bacillus thuringiensis*. *Microbial Pathogenesis*, 114: 139–146.

MESSINA, F.J. and RENWICK, J.A.A. 1985. Resistance to *Callosibruchus maculatus* (Coleoptera: Bruchidae) in selected cowpea lines. *Environmental Entomology*, 14: 868-872.

MIESHO, W.B., GEBREMEDHIN, H.M., MSISKA, U.M., MOHAMMED, K.E., MALINGA, G.M., SADIK, K., ODONG, T.L., RUBAIHAYO, P. and KYAMANYWA, S. 2018. New sources of cowpea genotype resistance to cowpea bruchid *Collosobruchus maculatus* (F.) in Uganda. *International Journal of Agronomy and Agricultural Research*, 12: 39-52.

MOOKIAH, S., SIVASUBRAMANIAM, B., THANGARAJ, T. and GOVINDARAJ, S. 2021. Host Plant Resistance. In *Molecular Approaches for Sustainable Insect Pest Management*. Springer, Singapore, 1-56.

MOREIRA, X., ABDALA-ROBERTS, L., HERNANDEZ-CUMPLIDO, J., RASMANN, S., KENYON, S.G. and BENREY, B. 2015. Plant species variation in bottom-up effects across three trophic levels: a test of traits and mechanisms. *Ecological Entomology*, 40: 676-686.

MURDOCK, L.L. and SHADE, R.E. 1991. Eradication of cowpea weevil (Coleoptera: Bruchidae) in cowpeas by solar heating. *American Entomologist*, 37: 228-231.

NDONG, A., KEBE, K., THIAW, C. and DIOME, T.M.S. 2012. Genetic Distribution of the Cowpea (*Vigna Unguiculata* (L.) Walp) Bruchid (*Callosobruchus Maculatus* F., Coleoptera, Bruchidae) Populations in Different Agro-Ecological Areas of West Africa. *Journal of Animal Science Advances*, 2: 616-630.

NEGBENEBOR, H.E. and NURA, S. 2020. Bio-efficacy of Some Plants Ethanolic Extracts Against Cowpea Weevil (*Callosobruchus maculatus* Fabricius) Infestation of Stored Cowpea Seeds. *Asian Journal of Biotechnology*, 12: 16-21.

NGALAMU, T., ODRA, J. and TONGUN, N. 2015. Cowpea production handbook. IFS/AGRA, Afristar Publishing House.

NTOUKAM, G., MURDOIK, L.L. and SHADE, R.E. 2000. Managing insect pests of cowpea in storage Bean/cowpea midcourse 2000 Research meeting, Senegal.

OFUYA, T.I. and LALE, N.E.S., 2001. Pests of stored cereals and pulses in Nigeria: biology, ecology, and control. *Pests of stored cereals and pulses in Nigeria: biology, ecology, and control.*

OJIEWO, C., RUBYOGO, J.C., WESONGA, J., BISHAW, Z., ABANG, M.M. and GELALCHA, S. 2018. Mainstreaming efficient legume seed systems in Eastern Africa: challenges, opportunities, and contributions towards improved livelihoods. Food and Agriculture Organization of the United Nations.

OLIVEIRA, S.O.D., RODRIGUES, A.S., VIEIRA, J.L., ROSI-DENADAI, C.A., GUEDES, N.M.P. and GUEDES, R.N.C. 2015. Bean type modifies larval competition in Zabrotes subfasciatus (Chrysomelidae: Bruchinae). *Journal of economic entomology*, 108: 2098-2106.

OGUNKANMI, L.A., OGUNDIPE, O.T., AMUSA, O.D., BOLARINWA, B. and AKINDELE, O.O. 2018. Comparative evaluation of cold and container storage preservation efficiencies on cowpea grains against *Callosobruchus maculatus* Fab.

OPOLOTA, H.N., AGONAB, A., KYAMANYWA, S., MBATAC, G.N. and ADIPALA, E. 2006. Integrated field management of cowpea pests using selected synthetic and botanical pesticides. *Crop Protection*, 25:1145–1152.

PADULOSI, S. 1987. Plant exploration and germplasm collection in Zimbabwe. *IITA Genetic Resources Unit Exploration Report*. IITA, Ibadan, Nigeria.

PADULOSI, S., LAGHETTI, G. and P., PERRINO. 1990. Collecting in Swaziland and Zimbabwe. *FAO/IBPGR Plant Genetic Resources Newsletter*, 78: 38.

PAINTER, R.H. 1951. Insect resistance in crop plants. *University of Kansas Press*, Lawrence, KS.

PANDA, N. and KHUSH, G.S. 1995. *Host plant resistance to insects*. CAB international.

PREVETT, P.F. 1961. Field infestation of cowpea (*Vigna unguiculata*) pods by beetles of the families Bruchidae and Curculionidae in Northern Nigeria. *Bulletin of Entomological Research*, 52: 635-645.

PROFIT, M. 1997. Bruchid Research at Royal Holloway, University of London.

SAKA, J.O., AGBELEYE, O.A., AYOOLA, O.T., LAWAL, B.O., ADETUMBI, J.A. and OLOYEDE-KAMIYO, Q.O. 2018. Assessment of varietal diversity and production systems of cowpea (*Vigna unguiculata* (L.) Walp.) in southwest Nigeria, *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 119: 43-52.

SALES, M.P., ANDRADE, L.B.S., ARY, M.B., MIRANDA, M.R.A., TEIXEIRA, F.M., OLIVEIRA, A.S., FERNANDES, K.V.S. and XAVIER-FILHO, J. 2005. Performance of bean bruchids *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae) reared on resistant (IT81D-1045) and susceptible (Epace 10) *Vigna unguiculata* seeds: relationship with trypsin inhibitor and vicilin excretion. *Comparative biochemistry and physiology Part A: molecular & integrative physiology*, 142: 422-426.

SINGH, B.B. 2005. Cowpea [*Vigna unguiculata* (L.) Walp.]. *Genetic resources, chromosome engineering, and crop improvement*, 1: 117-162.

SINGH, S.R. 1977. Cowpea cultivars resistant to insect pests in world germplasm collection. *Trop Grain Legume Bull*.

SMITH, C.M. 2005. *Plant resistance to arthropods: molecular and conventional approaches*. Dordrecht: Springer Netherlands.

SMITH, C.M. 2005. *Plant resistance to arthropods: molecular and conventional approaches*. Dordrecht: Springer Netherlands.

SMITH, C.M. and CLEMENT, S.L. 2012. Molecular bases of plant resistance to arthropods. *Annual review of entomology*, 57: 309-328.

SOUTHGATE, B.J. 1979. Biology of the Bruchidae. *Annual review of entomology*, 24: 449-473.

TOQUENAGA, Y. and FUJII, K. 1991. Contest and scramble competitions in two bruchid species, *Callosobruchus analis* and *C. phaseoli* (Coleoptera: Bruchidae) II. Larval competition experiment. *Population Ecology*, 33: 129-139.

TREVISAN, M.T.S., PFUNDSTEN, B., HAUBNER, R., WURTELE, G., SPIEGELHALDER, B., BARTSCH, H. and OWEN, R.W. 2006. Characterization of alkyl phenols in cashew (Anacardium occidentale) products and an assay of their antioxidant capacity. *Food and Chemical Toxicology*, 44: 188-197.

TRIPATHI, K. 2012. Differential reaction of cowpea genotypes to pulse beetle under artificial seed infestation and biochemical basis of resistance. *Indian Agricultural Research Institute, New Delhi*.

TRIPATHI, K., CHAUHAN, S.K., GORE, P.G., PRASAD, T.V., SRINIVASAN, K. and BHALLA, S. 2015. Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse-beetle, *Callosobruchus chinensis* (L.). *Legume Research*, 38: 675-680.

TRIPATHY, S.K. 2016. Bruchid resistance in food legumes-an overview. *Research Journal of Biotechnology*, 11: 97-105

VENTURY, K.E., FERREIRA, S.R., DE MOURA ROCHA, M., DO AMARAL GRAVINA, G., DA SILVA FERREIRA, A.T., PERALES, J., FERNANDES, K.V.S. and OLIVEIRA, A.E.A. 2022. Performance of cowpea weevil *Callosobruchus maculatus* (F.) infesting seeds of different *Vigna unguiculata* (L.) Walpers genotypes: The association between bruchid resistance and chitin-binding proteins. *Journal of Stored Products Research*, 95: 101925.

VIEGAS JUNIOR, C. 2003. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Química Nova*, *26*: 390-400.

XIONG, H., SHI, A., MOU, B., QIN, J., MOTES, D., LU, W., MA, J., WENG, Y., YANG, W. and WU, D. 2016. Genetic diversity and population structure of cowpea (*Vigna unguiculata* L. Walp). *PloS one*, 11: e0160941.

YAMANE, T. 2013. Biorational control methods for protection of stored grain legumes against bruchid beetles. *Agricultural Sciences*, 4: 762-766.

LIST OF APPENDICES

Appendix 1: Analysis of variance for number of eggs (NE)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	3610.5	401.2	0.64	0.762
Residual	50	31584.5	631.7		
Total	59	35195.0			

Appendix 2: Analysis of variance for adult emergence (AE %)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	718.1	79.8	0.62	0.778
Residual	50	6477.9	129.6		
Total	59	7196.0			

Appendix 3: Analysis of variance for seed damaged (% SD)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	2875.0	319.4	0.88	0.545
Residual	50	18050.0	361.0		
Total	59	20925.0			

Appendix 4: Analysis of variance for total development time (TDT in days)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	219.02	24.34	1.78	0.096
Residual	50	683.83	13.68		
Total	59	902.85			

Appendix 5: Analysis of variance for mean developmental time (MDT in days)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	183.600	20.400	2.09	0.048
Residual	50	488.333	9.767		
Total	59	671.933			

Appendix 6: Analysis of variance for number of days to insect emergence (NIE)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	173.40	19.27	1.32	0.249
Residual	50	728.33	14.57		
Total	59	901.73			

Appendix 7: Analysis of variance for seed weight loss (% SWL)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	6035.5	670.6	4.85	<.001
Residual	50	6916.0	138.3		
Total	59	12951.4			

Appendix 8: Analysis of variance for initial seed weight (g ISW)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	7.9147	0.8794	6.75	<.001
Residual	50	6.5143	0.1303		
Total	59	14.4290			

Appendix 9: Analysis of variance for residual seed weight (g RSW)

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotype	9	4.45350	0.49483	11.50	<.001
Residual	50	2.15192	0.04304		
Total	59	6.60542			