PHENOTYPING OF *Striga gesnerioides* (Willd.) AND *Megalurothrips sjostedti* (Trybom) RESISTANCE IN COWPEA (*Vigna unguiculata* (L.) Walp.) POPULATION IN NORTHERN GHANA

BY

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NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

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A Thesis Submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in Partial Fulfilment of the

Requirements for the Award of Degree of

MASTER OF PHILOSOPHY IN PLANT BREEDING

BY

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NOVEMBER, 2016

DECLARATION

I, PODA Saadon Leandre, hereby declare that this submission is my own work toward the Master of Philosophy (Plant Breeding) and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of University, except where due acknowledgment has been made in the text.

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ABSTRACT

Parasitic weed *Striga gesnerioides* (Willd.) and flower Thrips (*Megalurothrips sjostedti* Trybom) are among the major constraints of cowpea (*Vigna unguiculata* (L.) Walp.) production. The single approaches used for their control appears to be highly insufficient. Host-Plant Resistance seems to have merit in efficiently and economically controlling these pests. The objectives of this study were to evaluate recombinant inbred lines developed between *Striga* and Thrips resistant parents, IT97K 499-35 and Sanzi respectively by Single Seed Descent (SSD), for *Striga* and Thrips resistance in Northern Ghana. The study also evaluated the promising *Striga gesnerioides* resistant lines for yield loss assessment. Studies involved field and pot screening under artificial inoculation. Twenty-seven (27) RILs out of the 251 RILs screened were completely resistant to *Striga gesnerioides*. The level of Thrips infestation was very low (0 to 11 flower Thrips per plot) making it difficult to rank the genotypes into the categories (resistant and susceptible). The damage index (scores) were therefore not recorded due to the total absence of flower Thrips in a good number of plots. The percentage reduction in the grain yield and dry biomass among the RILs was lower in the resistant RILs (0.55% to 3.08% and 1.11 to 7.7% respectively) than the susceptible ones (28.45% to 58.88% and 47.29% to 61.71% respectively). The negative effect of *Striga* infestation on cowpea grain yield and dry biomass can then be reduced when resistant genotypes are used.

DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who are close to our heart. I dedicate this humble work to my beloved family: Poda/Somé Léocadie, Poda Urbain, Armel, Stéphane, Thierry and Sonia. You have successfully made me the person I am becoming. You will always be remembered.

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Finally for all those who contributed silently to this great achievement, I say:

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

% Percentage

C	Degre Celsius
AFLP	Amplified Fragment Length polymorphism
AGRA	Alliance for Green Revolution in Africa
CSIR	Council for Savannah and Industrial Research
DAP	Day after planting **DF**
Degree of freedom ***et al.***	et
alia (and other people)	
FAO	Food and Agriculture Organisation
FAOSTAT	FAO statistics
Fth	Flower Thrips **HPR**
Gram per litre	
IITA	International Institute of Tropical Agriculture
IMCDA	Improved Masters for cultivar Development in Africa
INERA	Institut de l'Environnement et de la Recherche Agricole
<	Less than
0	
ANOVA	Analysis of variance

Cm Centimeter

CRI Crop Research Institute

Host plant resistance **g/l**

Kg ha -1 kilogram per hectare

KNUST Kwame Nkrumah University of Science and Technology **Lsd**

Least significant difference **m** Meter

MAB Marker assisted breeding

MABC	Marker assisted backcrossing
P	Probability
RF	Rainfall
RH	Relative Humidity
S. gesnerioides	*Striga gesnerioides*
SARI	Savannah Agricultural Research Institute
S. gesnerioides	*Striga gesnerioides*
SNP	Single nucleotide Polymorphism
SSA	Sub Saharan Africa
SSD	Single Seed Descent **µg**

microgram

QTL	Quantitative Traits Loci
RIL's	Recombinant Inbred Lines

SG1	*Striga gesnerioides* race 1
SR4	*Striga gesnerioides* race 4

CHAPTER ONE

BACKGROUND OF THE STUDY

1.1 INTRODUCTION

Cowpea is an important crop in the semi-arid tropics including parts of Asia, Africa, Southern Europe, Southern United States, Central and South America (Singh, 2005; Timko *et al*., 2007a). It is highly adapted to the warm and sparse rainfall climates of the Sahelian and Sudanian zone in Africa (Hall *et al.,* 2002; Hall, 2004). The total production area of the crop was estimated around 12 million hectares with West Africa accounting for about 10 million hectares (FAO, 2016).

Cowpea contains high-quality protein (Langyintuo *et al.,* 2003). According to Ohler *et al*. (1996), the grain and dried foliage contain about 23-25% of protein by weight. Its fodder is used for livestock feed and also to improve soil fertility by its ability to fix nitrogen in the soil. Cowpea production is affected by many constraints. Currently, cowpea yields are estimated around 300 to 500 kg ha^{-1} on farmer's field in the Savannahs of Sub Saharan Africa (SSA) while its yield potential is up to 3000 kg ha^{-1} in optimum growing conditions (Tanzubil *et al.,* 2008).

Cowpea production is influenced by both biotic and abiotic constraints. The constraints to cowpea production include weeds infestation such as *Striga gesnerioides* and *Alectra vogelii* (Parker and Riches, 1993) and low soil fertility (Asare, 2012). Other factors limiting yield include its susceptibility to several bacterial, fungal, and viral diseases and various insect pests (Singh, 2005; Timko *et al*., 2007a). The most common insect pests that cause injury to cowpea are aphids (*Aphis craccivora*), flower bud Thrips (*Megalurothrips sjostedti*), Maruca pod borer (*Maruca vitrata*), pod sucking bugs

(*Clavigralla* spp., *Riptortus* spp.), and the storage weevil *Callosobruchus maculatus* (Caswel, 1981).

The flower bud Thrips is the most economically important cowpea pest at the flower initiation and flowering stage that cause yield losses between 20% and 70% depending on infestation level (Ngakou *et al*., 2008). Nevertheless, a severe infestation can result in complete grain yield loss (Singh and Allen, 1980). The damage to cowpea flower as a result of Thrips is characterized by a distortion, a malformation of the floral parts, flower bud abscission and non-elongation of peduncles. Apart from the direct damage caused by Thrips, Ullman *et al. (*1997) reported that Thrips are vectors for a number of pathogens that they transmit mechanically from plant to plant. They are known to be vectors of some bacterial (Bailey, 1935), fungal (Farrar and Davis, 1991) and viral (Garcia *et al*., 2000) diseases. Several Thrips species, all belonging to the family of Thripidae are able to transmit plant viruses (Ullman *et al*., 1997) which are "prunus necrotic ringspot ilavirus" (Greber *et al.,* 1991), "tobacco streak ilavirus" (Sdoodee and Teakle, 1993), "soybean mosaic sobemovirus" (Hardy and Teakle, 1992), and the most important "tomato spotted wilt virus" (Marchoug *et al.*, 1991). Thrips are the only known transmitters of tospoviruses which belong to the Bunyaviridae (Ullman *et al*., 1997).

Apart from insect pests that are harmful to cowpea, parasitic plants are also a major constraint to today's agriculture with most crop species being potential hosts (Westwood *et al*., 2010). Out of about 30 *Striga* species which have been identified, *Striga gesnerioides* is the only *Striga* species that is virulent to dicots (Mohamed and Musselman, 2008). *S. gesnerioides* is a major limitation to cowpea production in Africa

(Timko *et al*., 2007b), causing considerable yield losses (Aggarwal and Ouédraogo, 1989).

The extent of the damage in cowpea is due to the close interaction between the host and the parasitic weed. Crop yield losses due to *S. gesnerioides* may be up to 70 % depending on the extent of damage and level of infestation (Alonge *et al*., 2004). On susceptible cultivars, yield losses can reach up to 100 % when *S. gesnerioides* population is over 10 emerged shoots per plant (Kamara *et al*., 2008). Omoigui *et al*. (2009) reported that yield losses caused by *Striga* in dry savannah of SSA are estimated in millions of tons annually and the prevalence of *Striga* infested soils is steadily increasing.

Methods including improved cultural practices and the use of chemicals to control *S. gesnerioides* are available but most of them are ineffective whilst others are not affordable for small-scale farmers of SSA (Singh *et al*., 1997; Timko *et al*., 2007).

In general, *S. gesnerioides* control is difficult to achieve due to the close association with its host (Lane *et al.*, 1997) and because each plant produces a large number of seeds which remain viable in soil up to 20 years. The use of resistant cultivars appears to be therefore, a generally acceptable, effective, economically sound and environmentally safe method to control this parasite (Timko *et al*., 2007).

Host plant resistance (HPR) can also be used to control Thrips and reduce or eliminate the use of environmentally toxic chemicals (Jackai and Adalla, 1997). Insect resistant cowpea varieties can thus help to sustain the productivity of cowpea by resource-poor farmers (Jackai and Adalla, 1997).

The significance of resistance and its durability for plant production in all countries especially in developing countries justifies that breeding for resistance be given top priority worldwide (Shaner, 1981).

i.

The development of resistant cowpea cultivars to multiple pests would have a significant impact on yield and food availability and nutritional status in many regions. It will positively influence seed production and yield without the use or reduce used of insecticides.

The main objective of this study was therefore to identify *Striga* and Thrips resistant lines for Thrips and *Striga* resistance in Northern Ghana

The specific objectives were to:

Evaluate the field performance of 251 Recombinant Inbred Lines (RILs) under *Striga gesnerioides* infestation ii. Evaluate the performance of 251 Recombinant Inbred Lines (RILs) under

Megalurothrips sjostedti infestation iii. Assess yield loss of promising *Striga gesnerioides* resistant lines under artificial infestation.

CHAPTER TWO

LITERATURE REVEW

2.1 Cowpea

Cowpea [*Vigna unguiculata* (L) Walp.] is one of the most important food and forage legumes in many countries of the world (Singh, 2005; Timko *et al*., 2007a). It is a

multifunctional crop, giving food to man and livestock and also used as a valuable and dependable revenue-generating commodity for farmers (Singh, 2002; Langyintuo et *al.*, 2003).

2.1.1 Taxonomy

Cowpea is a diploid (2n=22) dicotyledonous crop in the order *Fabaceae*, subfamily *Faboideae* (Syn. *Papillionoideae*), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section Catiang (Verdcourt, 1970; Maréchal *et al*., 1978; Padulosi & Ng, 1997; Timko, 2008). Based on morphological characteristics, the genus *Vigna* was divided into subgenera which are the African sub-genera *Vigna* and *Haydonia*, the most important group, the Asian sub-genus *Ceratotropis*, and the American subgenera *Sigmoidotropis* and *Lasiopron* (Timko and Singh, 2008). Several authors have concluded that, *Vigna unguiculata* sub-species *unguiculata* is divided into four groups and *includes* the cultivated groups: *unguiculata, biflora* (or *cylindrica*), *sesquipedalis*, and *textilis* (Ng and Maréchal, 1985; Singh *et al.*, 1997; Reis and Frederico, 2001).

2.1.2 Origin, Domestication and Diversity

The origin of cultivated cowpea is still not precisely known. However, most authors agree that, Asia and Africa could be the domestication sites of this crop (Angessa, 2006). The lack of wild ancestors in Asia, has led some authors to question, whether the Asian Center of origin is still valid and then Southern Africa has been considered as the most probable center of domestication because of the highest genetic diversity and the presence of the most primitive form of wild cowpea (Padulosi, 1987; 1993). The distribution of several

wild cowpea from Ethiopia to South Africa supported the idea that East and Southern Africa were the first centres of diversity while West and Central Africa were secondary centres (Baudouin and Mare'chal, 1985).

In contrast, some studies with molecular markers such as the Amplified Fragment Length Polymorphism (AFLP) profiles suggested North eastern Africa as cowpea domestication centre (Coulibaly *et al.*, 2002).

2.1.3 Floral biology

Cowpea is a self-pollinating plant with a small rate of outcrossings due to insect activities (Rachie and Roberts, 1974). Cowpea floral structure is represented by a symmetric flower style with a short beak (stigma) (Marechal *et al.*, 1978). Each flower contains ten stamens, with each stamen carrying an anther sac with pollen.

The cultivated cowpea is a monoecious species with complete and perfect flowers. (Marechal *et al*., 1978). Flower opening occurs after pollination and fertilization, which reduces chances for out-crossings from foreign pollen (Marechal *et al.*, 1978).

Cowpeas have large flower buds and this attribute facilitates emasculation during crosses. The flowers vary in color from white, cream and yellow to purple (Ng and Huges, 1998; Fatokun and Ng, 2007).

2.1.4 Cowpea production and uses

Cowpea is cultivated not only as a pulse but also as a vegetable and as a cover crop as well as a fodder. Blackeyed pea, crowder pea, southern pea, lubia, niebe, coupe and frijole are the most common names of the crop (Fatokun and Ng, 2007).

It is an important grain legume in the world especially in the African tropical savannah and Sahelian zones. In 2013 cowpea production in Africa was estimated at almost 7,782,054 tons of dry grain (FAO, 2016).

Cowpea is usually grown in association with pearl millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*) in the Sahelian zones of West Africa where 70 % of the crop is produced (Langyintuo *et al*., 2003, Asiwe, 2005). Eastern and Southern Africa and South America (particularly Peru and North Eastern Brazil) are also other important areas where cowpea is produced (Langyintuo *et al*., 2003). The largest producers and consumers of cowpea in Africa are Nigeria followed by Niger (Singh *et al*., 2002) and Burkina Faso (Abate *et al*., 2012).

In Ghana, it is one of the most cultivated legumes, mostly in the savannah and transitional zones (CRI, 2006). Cowpea yield in Ghana, was among the lowest in the world with about 310 kg ha^{-1} (Ofosu-Budu *et al*., 2007).

According to Hall *et al*. (2002) and Hall (2004), cowpea is known to be tolerant to drought compared to other legumes. Farmers are able to produce about 1,000 kg ha^{-1} of dry grain in Sahelian zones, where climatic conditions are mostly unfavorable for the crop with an average rainfall of about 181 mm (Hall and Patel, 1985). Because of its high ability to fix nitrogen. Cowpea is an important component of farming systems in low-fertile soils (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003).

Cowpea plays a critical role in the livelihood of millions of people in the developing countries. The most important part of the cowpea plant used for human consumption is its seeds (Nielsen *et al*., 1997; Ahenkora *et al*., 1998). Indeed, cowpea seeds constitute a major source of protein that nutritionally compensate low-protein tuber crop and cereals

(Hall *et al*., 2003). The total seed protein content ranges from 23 % to 32 % of its weight (Hall *et al*., 2003). The most important amino acids present in cowpea are lysine and tryptophan. Cowpea seeds are rich in minerals and vitamins (Hall *et al*., 2003). The other components are carbohydrates, lipid and crude fiber (Owolabi *et al.*, 2012). Tarawali *et al*. (1997; 2002) stated that in many parts of Africa and Asia, the fresh and dried leaves are also used as a side dish or stew and of high nutritional value. The leaves and the stem are also used to feed animals in West African countries especially during the dry season (Singh and Tarawali, 1997; Tarawali *et al.*, 1997, 2002).

2.1.5 Constraints to production

The constraints to cowpea production have been generally grouped into abiotic and biotic factors (Tamo *et al*., 2003). The most prominent abiotic constraints are drought, heat and low soil fertility while the biotic constraints include the vegetative stage insects (aphids), the flowering insects (flowers Thrips and *Maruca vitrata*), the storage insects (bruchids), bacterial, viral diseases and the parasitic weeds *Striga gesnerioides* and *Alectra vogelii*. (Singh and Tarawali, 1997; Tignegre, 2010). In West Africa, insect pests are the major constraint to cowpea production (Rahie, 1985; Jackai and Daoust, 1986; Karungi *et al;* 2000).

2.2 *Striga gesnerioides*

Parasitic plants are a major problem to today's agriculture because several crop species are their potential hosts (Westwood *et al*., 2010). *Striga gesnerioides* is one of the most important parasitic weeds in cowpea production (Botanga and Timko, 2005; Tignegre, 2010). *Striga* spp probably originated from an area between the Semien Mountains of Ethiopia and the Nubian Hills of Sudan (Atera and Itoh, 2011).

Striga spp are obligate parasitic weeds that attach to the root vascular system of the host plant. They produce abundant and tiny seeds which remain viable in the soil for many years (Musselman and Ayensu, 1984). *S. gesnerioides* is a soil parasite which feeds on cowpea plant, by developing a haustoria through which it sucks nutrients and water (Nweze *et al.*, 2015). *Striga* can cause complete yield loss if susceptible cowpea genotypes are involved **(**Emechebe *et al.*, 1991**).**

Approximately, 30 *Striga* species have been described as parasitising grass species. *Striga gesnerioides* is the only *Striga* species that is virulent to dicots (Atera and Itoh, 2011). The species *S. hermonthica, S. aspera, S. gesnerioides and S asiatica* are the most agronomically significant parasitic weeds. (Hood *et al.*, 1998; Botanga and Timko, 2005). Lane and Bailey (1992) revealed that these parasites are the species of major economic importance in the world and *Striga* genera occur throughout the semi-arid tropics (Hibberd *et al.*, 1996). According to Singh (2002), *Striga gesnerioides* is particularly destructive for cowpea in Sudan-Sahelian areas on sandy and water-stressed soils with 75% of damage occuring during the pre-emergence stage.

2.2.1 Taxonomy and biology

Domain: Eukaryota

Kingdom: Viridiplantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae Order: Scrophulariales

Family: Scrophulariaceae.

Because of the aerial photosynthetic activity occurring after *Striga* emergence, *Striga* spp can be categorized as hemiparasites (Matusova *et al.*, 2005). But, some authors have also considered *Striga* species as holoparasites, based on the low rate or the absence of photosynthesis after emergence (Wolfe and DePamphilis, 1998).

Their entire development before emerging above soil depends on the uptake of water and nutrients and growth hormones from the host. This is why *Striga* species are considered as witch weeds. *Striga gesnerioides* is more dependent on its host than the other species, S. *hermonthica* and *S. asiatica* due to its higher transpiration requirement (Thalouarn *et al.*, 1991).

2.2.2 Geographical distribution

The main distribution areas of *S. gesnerioides* include West and Southern Africa, India, and United State of America (USA). In West Africa, *S. gesnerioides* was reported to occur in Benin, Burkina Faso, Mali, Niger, Nigeria (Cardwell and Lane, 1995) and Ghana (Khan *et al*., 2002; Timko *et al*., 2007).

2.2.3 Species and hosts

According to Kuiper *et al*. (1998), there are approximately 3,000 plant species of parasitic weeds grouped into 17 families. There are several genera in this group among which *Striga* can parasitize cereals and legume crops and then cause damage (Botanga and Timko, 2005). There are several species of *Striga* among which, *S. hermonthica* and *S. aspera* are parasitic on cereals and *S. gesnerioides* parasitizes dicotyledonous crops particularly cowpea (Berner and Williams, 1998).

Tobacco (*Nicotiana tabacum* L.), sweet potato (*Ipomea batatas* (L.) Lam.), *Tephrosia sp.*, *Indigofera tinctoria* L. and *Indigofera spicata* Forsk are other host plants for *S.*

gesnerioides (Musselman and Ayensu, 1984). The *Striga* race attacking *Indigofera* is not harmful to cowpea (Botanga and Timko, 2005).

2.2.4 Damage to crops

Striga gesnerioides damage affects several parts of the cowpea plant (Alonge *et al.*, 2004). The physiological activities of cowpea plant can be disturbed by *S. gesnerioides*. The damages such as leaf photosynthesis reduction, partial flowering, reduced leaf area, poor podding and seed development have also been reported by Alonge *et al.* (2004). These damages are generally intensified by the parasite transpiration in drought conditions (Alonge *et al.*, 2004).

Striga infestation can also reduce the nitrogen and protein content in cowpea plants and its grains respectively because of the concentration of inhibitors which reduce the canopy and the plant growth (Alonge *et al.*, 2004).

The incidence and severity of *S. gesnerioides* depend on the cropping system, the soil type and the genotype involved (Cardwell and Lane, 1995). According to Cardwell and Lane (1995), S. *gesnerioides* severity is affected by climate conditions and this severity is higher on sandy soils than clay soils. *Striga* confinement to Northern Guinea Savannah zones, Sahelian and sandy soils show that *Striga* is a low-fertile area parasite. Therefore, suitable strategies should be designed for an effective control of *S. gesnerioides*.

2.2.5 Life cycle

Striga life cycle (Fig.1) comprises a succession of growth stages that are related to the developmental stages of the host plant (Lane and Bailey, 1992; Matusova *et al.*, 2005). There are biochemical signals that coordinate *Striga* life cycle to the host (Matusova *et*

al., 2005). After *Striga* seeds are shed, they need a post-harvest maturation period of six to seven months to complete the physiological maturity process (Thalouarn and Fer, 1993). *Striga* seeds will remain dormant if temperature is under 25 °C or above 35 °C (Kuiper *et al.*, 1996). The seeds need a pre-conditioning period of 10 to 21 days before they can germinate (Okonkwo, 1991; Lane and Bailey, 1992). These conditions are normally satisfied when the rainy season starts in the semi-arid areas. In a period of two to five days, *Striga* seeds germinate if a stimulus from exudates is produced by cowpea roots within a distance of 2 mm (Lane *et al.*, 1991; Dube and Olivier, 2001) the most important chemical being alectrol (Muller *et al*., 1992).

S. gesnerioides is also stimulated by ethylene but relatively insensitive to the strigol analogues GR 7 and GR 24 (Igbinnosa and Okonkwo, 1992; Maass, 1999). The radicle of *Striga* grows and penetrates the host root, where it forms a tubercle called *haustorium*, which is visible on the surface of the host (Lane *et al.*, 1991). The nutrients contained in the *Striga* seed albumen are very restricted due to its small size which cannot allow the *Striga* radicle to survive more than a week if the connection with its host is not achieved (Berner and Williams, 1998).

The haustorium is an organ designed to drain nutrient, and water from the host to feed the *Striga* plant during its initial and underground development period (Lane and Bailey, 1992). At this stage the injury as a result of *Striga a*ttack is high and *Striga* behaves as an obligate parasite (Lane and Bailey, 1992). Tignegre (1988) stated that the emergence of *Striga* is observed between four to six weeks on susceptible cowpea genotypes. Later on, *Striga* develops stems and leaves and synthesizes chlorophyll (Hibberd *et al.*, 1996). *Striga gesnerioides* is autogamous and this reduces the eventual risk of pollen flow which

subsequently causes the population of *S. gesnerioides* to be uniform (Botanga and Timko, 2005). They have also shown that the existence of *S. gesnerioides* races is associated with a host-driven selection. The Seeds are tiny (0.20 mm to 0.35 mm long), and a weigh 4 to 7 micrograms (μg) each (Dubé and Olivier, 2001). Flowering to seed maturity is achieved in five to seven weeks after cowpea planting, where thousands of seeds (50,000 to 500,000) per plant will be released (Lane and Bailey, 1992). This facilitates *Striga* seed dissemination in the soil, through water, wind, animals and farming tools. *Striga* plants flower around four to seven days after *Striga* emergence (Dubé and Olivier, 2001). About eighty one percent (81%) of *S. gesnerioides* seeds are distributed in the first 15 to 30 cm layer of the soil (Touré *et al.*, 1997). This large amount of seeds produced, associated with the highly degraded soils in semi-arid zones and the poor access of small-scale farmers to herbicides make *S. gesnerioides* eradication very difficult.

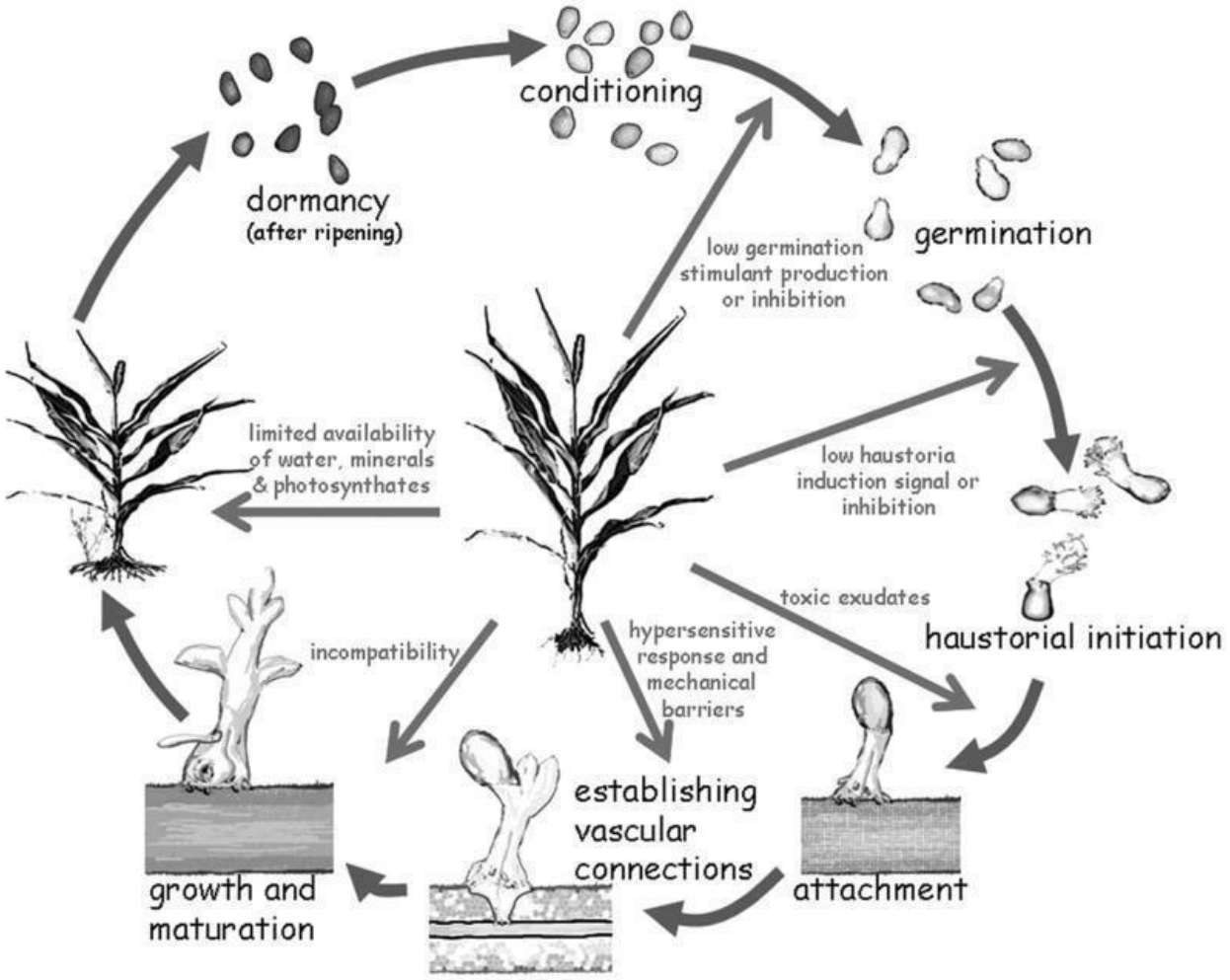

Fig 2.1 Life cycle of *Striga* spp *(https://dl.scciencesocieties.org/images/pupblication/).*

2.2.6 Control measures

In general, *Striga* control is difficult to achieve due to its close association with the host plant (Lane *et al.*, 1997). Several control methods can however, be employed to effectively minimize *Striga* damages.

Germination stimulants (Strigol, s trigyl acetate and alectrol) of *Striga* seeds can be effective in controlling *Striga* by making suicidal germination (Cook *et al.*, 1966; Berner *et al.*, 1997; Berner and Williams, 1998). These approaches are however, expensive to smallholder farmers of Sub-Saharan Africa. Some bacteria like *Pseudomonas seringae*,

when combined in the soil cause more abortions of *S. gesnerioides* seeds than the *Striga*seed germination stimulants (Berner *et al.*, 1997).

Trap-crops can also be used to reduce *Striga* seeds in the soil. *Bagauda farafara*, a variety of *Sorghum bicolor* was found to be the best germination stimulant of *S. gesnerioides* (Berner and Williams, 1998). Other plants like, pigeon pea, *Cajanus cajan*, can also stimulate *S. gesnerioides* germination with a lesser effect. These crops could be used in a farming system management (rotations or mixed crops) for an effective management of *S. gesnerioides* control.

Field solarisation which involves the use of plastic films to heat the soil was revealed to be effective only for destroying *Striga* seeds in the first 2 cm of the soil layer (Parker, 1991), but not effective in reducing the *Striga* seed bank for the entire volume of soil explored by cowpea roots.

Early planting is used as a *Striga* control method as the initial development all stages the cowpea escape *Striga* injury (Muleba *et al.*, 1996; Alonge *et al.*, 2004). However, this might not be completely appropriate, since early planting could expose early maturing cultivars to pod damage due to season-end rainfall.

Jacobson (1994) indicated that with high income-generating crops, herbicides applied at pre-emergence period, combined with soil fumigants, were effective in controlling *Striga.* But, this method is not affordable to small-scale farmers. Thus, the use of resistant genotypes remains the most appropriate *S. gesnerioides* control method on cowpea (Alonge *et al.*, 2004).

2.2.7 Resistance mechanisms

All the stages of *Striga* life cycle including germination, haustorial induction, attachment to the host root and the penetration of the host vascular cells are important for the successful development of *Striga* (Botanga and Timko, 2005). The use of "*in- vitro*" techniques that allow the visualization of the different developmental stages is one of the best ways of studying *Striga* resistance mechanisms.

2.2.7.1 Resistance at germination stage

In some crops like sorghum, certain varieties (eg. N13) induce very small number of *Striga* shoots (Ramaiah, 1987; Lane and Bailey, 1992), which is considered as a form of resistance. Some authors (Ramaiah *et al.*, 1990; Ejeta *et al.*, 1991) revealed that the lowstimulant capacity is governed by a single recessive gene.

Unfortunately in cowpea, no genotype has been found with this type of resistance mechanism (Lane *et al.*, 1991). The chemical signals inducing *Striga* seed germination in maize, sorghum and cowpea are called strigolactones, specifically strigol, sorgholactone and alectrol respectively (Ramaiah *et al.*, 1990; Ejeta *et al.*, 1991; Matusova *et al.*, 2005).

2.2.7.2 Resistance at fixation level

S. gesnerioides tubercle growth can be stopped for weeks if any connection with the host vascular system is established (Botanga and Timko 2005). A study conducted with some cowpea genotype 58-57 revealed a first level of resistance, resulting in an incompatibility, as a product of necrosis before the fixation of the root cortex by the parasite (Lane *et al.*,

1991). According to Hood *et al.* (1998), this resistance mechanism is exhibited by host reaction at the root cortex level. These effects were called hypersensitive reactions, which show vertical resistance and consequently, single genes might be involved.

2.2.7.3 Resistance at and after the penetration of the host vascular cells

In some resistant cowpea genotype (B301), *Striga* seed is able to germinate, forms tubercles, but does not develop shoots (Lane *et al.*, 1991). The Benin *Striga* race SR4 develops tubercles, haustoria and even stems, but their further development is stopped (Lane *et al.*, 1994). This type of resistance mechanisms is comparable to antibiosis due to incompatibility between cowpea and *Striga* (Hood *et al.*, 1998). According to Hood *et al.* (1998), such a resistance mechanism is durable because it is due to the absence of chemical signals or nutrients produced by the host, as requirement for further development of *Striga* plant.

Olivier *et al.* (1991) mentioned that some host tissues are able to modify their structure as a response to the infection as also another form of resistance. Lane and Bailey (1992) stated that the resistance to *S. gesnerioides* in cowpea is likely to remain stable since the resistance mechanisms in most cases involve post-infection reactions and *Striga* is a monocyclic and a soil parasite.

2.2.8 Estimation of yield loss due to *Striga gesnerioides*

2.2.8.1 Definition

Crop yield loss is defined as the difference between the yield gotten from a *Striga* infested plots (also called actual yield) and the yield from the un-infested *Striga* plots (checks) (Evans, 2012).

2.2.8.2 *Striga gesnerioides* yield loss estimation methodologies

Three approaches have been commonly used in the past to estimate yield loss estimates from *Striga*.

(1) Yield loss estimate is obtained by comparing the crop yield in pots or field environments, with or without addition of *Striga* seeds (Andrews, 1946, 1947; Younis and Agabawi, 1965). The principal constraint of this method is that the loss estimation is at that specific level of *Striga* which is realized during the trial.

(2) By generating controlled and infested treatments in the plot, where *Striga* plant that appear above the soil in the check plots are uprooted mechanically (Doggett, 1965; Bebawi and Farah, 1981) or by the use of 2, 4-D (Last, 1960). In this method, *Striga* plants are uprooted or destroyed after its emergence from the soil. Because most of *Striga* injuries appear before its emergence (Ramaiah *et al.*, 1983), this method allows some damage by *Striga* to be added in the "no *Striga*" plot leading to wrong conclusions.

(3) Yield loss can also be estimated by surveys of infested plots and visually estimate the loss centered on *Striga* infestation level, moisture content, soil fertility level and degree of damage to the plant (Vasudeva, 1983).

All these tactics frequently used in literature are subjective and of restricted use in crop loss prediction.

A new approach which is gradually accepted is the regression method because it is a powerful means of attaining statistically-valid and consistent estimation of crop loss (Stynes and Veitch, 1983; Teng, 1985). The main drawback of this method is the validity of the prediction equation represented by R^2 value even though it is possible to improve it by checking soil aspects like fertility (nitrogen),

and humidity in every plot which can then be included as extra independent components in the multiple regression equation (Vasudeva, 1988).

2.3 The flower bud Thrips, *Megalurothrips sjostedti*

Cowpea is subject to heavy insect pest infestation all over the world. The crop is severely attacked at every stage of its growth by insects which generally cause low yield and sometimes total yield losses (Asiwe *et* al., 2005, Oyewale, 2013).

In Africa, especially in Ghana the most important pests of cowpea involves leafhoppers, (*Empoasca spp),* aphids, (*Aphis craccivora* Koch), pod borers, (*Maruca vitrata* (Fab)), pod-sucking bugs (*Clavigralla tomentosicollis* Stål, *Nezera viridula L)* Linnaeus, (*Leptoglossus spp),* bruchids (*Callobruchus spp*.) and flower bud Thrips, (*Megalurothrips sjostedti* (Trybom)), (Singh and Jackai, 1985; Jackai and Adalla, 1997; Obeng-Ofori, 2007).

The flower bud Thrips, *M. sjostedti,* is one of the most important pests of cowpea during the flowering stage. It can cause severe grain yield reductions. According to Ngakou *et al*. (2008), in West Africa, yield losses due to flower bud Thrips were estimated to range from 20-70% depending on infestation level. In some African countries like Tanzania, Ghana, Cameroon and Nigeria yield losses up to 100% were reported (Ezueh, 1981; Price *et al*., 1983).

2.3.1 Biology and its transmission

The developmental stages of *Megalurothrips sjostedti* include an egg stage, two larval stages and the non-feeding stage of pre-pupa and pupa (Afric, 2010). The eggs which are very small measure 0.25 mm long and 0.1 mm wide. Their color is white when they are

newly laid and become pale-yellow at maturity. (www.infonet-biovison.org, 2010). Thrips development from egg to adult stage takes about 19 days at 29 °C and relative humidity (RH) of 58% (Salifu, 1992) and their adult can live up to 23 days or less. The males which are generally smaller than the females are developed from unfertilized eggs. The first and second instar larvae are very small (0.5 to 1.2 mm), elongated and slender. They might also differ in color from pale-yellow, orange or red depending on the species (Africa, 2010). They have piercing-sucking mouthparts and are a small form of the adult but do not have wings (Africa, 2010).

Pre-pupa and pupa instars are intermediate forms between the nymph and the adults. They have small wing buds with no functional wings. During these stages, Thrips do not cause any significant damage because they are inactive and do not feed on the host. Pupa stage may occur on a plant or in the soil beneath, depending on species (Africa, 2010). Adult Thrips, which are shiny black, are found feeding in flower buds and flowers (Singh and van Emden, 1978). They are slender and have long wings (Africa, 2010).

Cowpea infestation by flower bud Thrips starts just before flowering, with the highest activity happening between noon and 1pm at a temperature ranging from 23-24°C (Taylor, 1969). Thrips flight is influenced by temperature and light intensity according to Taylor (1969).

About 5,000 species of Thrips have been described (insects in the order of *Thysanoptera*) (Mound, 1997; Mortiz *et al*., 2001) and most of them feed on fungi and live in leaf litter or on dead wood. Those feeding on higher plants belong mostly to the family Thripidae and include the most important pest species (Moritz *et al*., 2001). Certain flower Thrips reproduce in flowers and feed on the cells of the flower tissue, on pollen grains and on

young developing fruits (Mortiz *et al*., 2001). Several flower-living species are partially predatory on minor insects while others, mainly, feed on leaves.

The main hosts of flower bud Thrips (*Megalurothrips sjostedti*) according to Tamo*'et al*. (1993b), are legumes which include *Vigna unguiculata* (cowpea), *Cajanus cajan* (pigeon pea), *Phaseolus vulgaris* (common beans). They also feed on some other plants considered as minor hosts such as *Arachis hypogaea* (groundnut), while other species attack vegetables.

Flower bud Thrips are present throughout Sub-Saharan Africa, from the humid zones of the West to the semi-arid zones of Kenya and Sudan (Tamo*'et al*., 1993b). But, in Nigeria, they prevail in dry Savannah areas where cowpea is produced (Tamo*'et al*., 1993b).

Abudulai *et al.* (2006) mentioned that flower bud Thrips infestation is found both in the Southern and Northern regions of Ghana where it reduces significantly cowpea production.

2.3.2 Economic Importance

Cowpea is attacked by several insect pests in the field but, actually the flower bud Thrips is believed to be the most important biological constraints to cowpea production (Jackai and Daoust, 1986; Jackai *et al*., 1992). Some species of Thrips have been identified to cause the highest injury in West Africa and other parts of the world. These species include the foliar-feeding *Frankliniella sp* mostly found on blossom or cotton bud (Bottenberg *et al*., 1997). *Frankliniella sp* are also vectors of plant diseases like tospoviruses in vegetables production (Gillot, 2005). Another species *Thrips palmi* causes important yield losses in vegetables in Asia and South America (Jackai and Adalla, 1997).

The flower bud Thrips, *Megalurothrips sjostedti* is the most economically significant insect pest of cowpea in West Africa that causes yield losses between 20 and 70% depending on the level of infestation (Ngakou *et al.*, 2008). According to Cisse and Hall (2010), in Senegal the major losses to cowpea production due to flower bud Thrips were one of the reasons that discouraged farmers from growing cowpea in Eastern and Southern regions.

2.3.3 Infestation control

Thrips control has become important in cowpea production because of their devastating damages reported throughout the world. In order to increase cowpea production, several approaches have been developed to encounter the incidence of flower bud Thrips.

The West African black pepper (*Piper guineense*) (WABP) extract assessed by Oparaeke (2006) for efficacy against flower bud Thrips on cowpea flowers during two years showed that 20% and 10% extracts of WABP at four and six weekly applications, respectively, caused important reduction of flower bud Thrips in flowers. He also noted that, the extract was not inferior to the synthetic insecticide treatment. Similar results were also observed by the reports of Jackai and Oyediran, (1991); Tanzubil, (1991); Ekesi, (2000) and Ogunlana *et al*., (2002).

Applications of extracts from naturally grown plants as insecticides for pest control on the field in Nigeria is well documented and include extracts of *Lonchocarpus species*, *Nicotiana tabacum* L., (Matsumura, 1975), *Chrysanthemum cinerariaefolium* L. (Stoll, 1986), *Syzigium aromaticum* (L) Merr and Perr (Oparaeke *et al*., 2002), *Azadirachta indica* (Olaifa and Adenuga, 1988; Jackai and Oyediran 1991; Tanzubil, 1991; Jackai *et*

al., 1992), *Monodora myristica* and *Allium sativum* L. *and* (Gaertn) Dunal , *Argemone Mexicana* L, *Melia azaderach.* (Pandey *et al.,* 1981). According to these authors, the effectiveness of the extracts for Thrips control has shown a great potential of a biopesticide due to the chemical constituents of WABP.

Oparaeke *et al*. (2000) stated however, that the protection of flowers from Thrips damage requires recurrent and adequate application of botanical extracts as they are slow-acting mortality agents.

Another method that has been reported to reduce flower bud Thrips infestation is intercropping probably due to the effect of shading of the taller plants to the shorter which reduce the abundance and the activity of the thrips (Parella and Lewis, 1997). They also added that, intercropping onion and garlic with tomato reduced Thrips infestations by practically 80%. Comparable results were also reported in several parts of the world. Nampala *et al.* (2002) observed that Thrips population was significantly reduced in cowpea and sorghum intercrops than the sole crop in Eastern Uganda. In Kenya too, the African bean flower Thrips population (*M. sjostedti* and *Hydatothrips adolfifriderici)* on cowpea buds were considerably reduced by intercropping cowpea with sorghum and maize (Parella and Lewis, 1997). However, a main limitation of intercropping is the yield reduction. Atokple (1992) reported cowpea yield, on average is higher when cultivated as sole crop compared to cowpea grown in intercropping with maize, sorghum or millet.

Biological control (use of natural enemies) is also one of the recommended methods to maintain Thrips populations low (Ezuah, 1981).

In pest management, the concept of biological control was generally used to refer to the interactions between pests and their natural enemies (Ezuah, 1981; Jackai and Daoust, 1986; Singh *et al*., 1990). Predators feed frequently on Thrips by reducing severely its population in infested fields (Varela *et al*., 2003). The most important predators comprise anthocorid bugs or minute pirate bugs (*Ovius* spp), lace wigs, predatory mites (*Amblyseius* spp), hoverflies spiders and ground beetles.

Ramachandran *et al.* (2001) mentioned that some natural enemies like minute pirate bugs (true bugs of the order *Hemiptera*, family *Anthocoridae*) and entomogenous nematodes that are particular parasites of Thrips (Loomans *et al*., 1997) were found to cause flower bud Thrips reduction in vegetables.

The egg parasitoid *Megaphragma* spp. (Hym, Trichogrammatidae) (Tamò *et al*., 1993b), and the larval parasitoid *Ceranisus menes* Walker (Hym, Eulophidae) (Tamò *et al*., 1993b; Zenz, 1999) are also some examples of the natural enemies of Thrips.

One of the most important drawbacks with the biological control is the high susceptibility of the natural enemies to insecticides as reported by Abudulai *et al*. (2001).

According to Abudulai *et al*. (2006), one of the well-known pest control methods in cowpea is the use of chemicals. This method has been the most extensively known form of flower bud Thrips control in cowpea that gives reasonable yield (Omo-Ikerodah *et al*., 2009). Afun *et al*. (1991) reported that cowpea grain yield can be increased ten times with the use of insecticides. The use of various strategies that include recurrent applications of expensive insecticides which decreased almost 80% of flower bud Thrips population in cowpea production has been suggested (Morse and Hoddle, 2006). The use of synthetic

insecticides remains the most common control approach of flower bud Thrips in cowpea production in Northern Ghana (Tanzubil *et al.,* 2008). It is also one of the most efficient methods practiced mainly by groups in Northern Ghana (Tanzubil *et al*. 2008). However, insecticides and their application equipments are often not affordable to resource-poor cowpea growers (Morse and Hoddle, 2006; Tanzubil *et al*., 2008). They also induce insecticide resistance in Thrips populations rendering the chemical treatments unsuccessful (Morse and Hoddle, 2006). Moreover, several environmental and health hazards are associated with the frequent use of insecticides (Abudulai *et al*., 2006).

The public awareness of environmental degradation and some economic considerations have made the extensive use of insecticide inappropriate. Thus, in order to reduce their utilization, intensive efforts have led to the development of insect resistant cowpea varieties (Omo-Ikerodah *et al.*, 2009).

Though, good levels of resistance have not been found in cowpea landraces and improved varieties, some genotypes with little levels of resistance to flower bud Thrips exist. According to Omo-Ikerodah *et al*. (2009) and Singh (1977), cowpea genotype TVu 1509 was recognized as having some level of resistance to Thrips. Sanzi, a landrace from Ghana has also been identified with a moderately high level of resistance to flower bud Thrips (Abudulai *et al*., 2006).

It was also reported that cowpea lines such as IT90 K-277-2, KVx 404 8-1, Moussa Local, Sewe, TVx 3236 and IT9I K-180 showed resistance to the flower bud Thrips in West Africa (IITA, 1994).

2.3.4 Host responses

Atokple (1992) stated that the defense mechanisms of host plants against their insect pests may be due to either avoidance which is defined as a mechanism reducing the opportunity of contact between the host and the pest or resistance which takes place when the host tissue is in contact with the pest.

Avoidance mechanism involves early maturity or tolerance to conditions which affect adversely pest development such as high temperature or humidity. These conditions are genetically determined and may be very useful in breeding for disease and pest resistance (Atokple, 1992).

Many studies have been conducted about Host Plant Resistance to Thrips (Mollema *et al*., 1995 and De Jagar *et al*., 1995). Even though cowpea resistance to *Megalurothrips sjostedti* has received little attention, limited informations on this mechanism are available.

According to Ekesi (2000), Host-Plant Resistance is often the result of a combination of resistance mechanisms. Salifu *et al*. (1980) in their studies of resistance mechanisms in cowpea genotype TVx 3236 to flower bud Thrips reported that the resistance was a result of antibiotic mechanisms. These results were also confirmed by Soria and Mollema, (1995) observations on *Megalurothrips sjostedti* and *Frankliniella occidentals* (Pergande) reared on resistant lines of cowpea and cucumber, respectively. They also suggested that the exposure of female Thrips to anti-feedants, toxins or deterrents on resistant genotypes can reduce food consumption and then affect egg production. Furthermore, poor oviposition as a result of inappropriate nutrition was also well established for Thrips (Kirk, 1985).

Tolerance is the attribute of the host plant to develop and reproduce normally regardless of Thrips infestation or more than what is needed to cause damage to a susceptible host. It is effective when the host plant supports as many pests as susceptible varieties without showing a significant reduction in grain yield or plant productivity (Ejeta *et al*., 1991).

2.3.5 Common symptoms of infestation

The symptoms produced by *Megalurothrips sjostedti* starts at the terminal leaf bud stage of the cowpea plant and extend to flower buds and then leads to leaves and stems necrosis (Ezuah, 1981). Omo-Ikerodah *et al*. (2009) gave more information on the common symptoms of flower bud Thrips infestation. It encompasses a mixture of non-elongation of peduncles, flower buds browning and flower bud abscission. Abudulai *et al.*, (2006) reported that the fact that Thrips feed on racemes, terminal leaf buds, or flower buds cause browning, distortion and abscission of floral parts.

The major symptoms when the plants are severely infested include inflorescence distortion and discoloration, abortion, reduced the production of pollen and flower loss, leaf defoliation leading to death of the entire plant and extreme yield reduction (Childers and Achor, 1995).

The Quantitative Traits Loci (QTL) analysis for resistance to *Thrips tabacci* and *Frankliniella* species done by Muchero *et al*. (2010) led to the conclusion that feeding by the Thrips on susceptible genotypes produced the characteristic scaring along the mid-rib of affected leaves causing distorted and curled leaflets.

2.3.6 Alternate host plants of *Megalurothrips sjostedti*

During the long dry season, insect pests feeding on cowpea need to find alternative host to stay alive or to diapause. (Fatokun, 1993b). According to Tamo *et al.* (1993b) and Arodokun *et al.* (2000) *Megalurothrips sjostedti* do not go through diapause during the dry season. There are capable of feeding and reproducing on a large number of alterative hosts plants in the absence of cowpea. In West Africa, the first report of *M. sjostedti* on alternative host plant was given by Taylor (1974). Later studies by Tamo *et al.*, (1993b) and Zenz, (1999) gave more detail on alternative host plant for diverse ecological area extending from the coast of Benin and Ghana, up to the Sudan savanna in Burkina Faso. Most of the alterative host plants belong to the family of Fabaceae (Table 2.1) (Tamo *et al.*, 1993a).

Table 2.1 Flowering season, habitat, and location of most important host plants for *Megalurothrips Sjostedti* in West and Central Africa (adapted from Tamo et al (1993b) and Zenz (1999)) Host plant Family Habitat

Flowering during the main dry season		
Berlinia grandiflora	Caesalpiniaceae	Wetland, River (Savanna)
Centrosema pubescens	Fabaceae	Ubiquitus
Milletia thonningii	Fabaceae	Firmland (Savanna)
Pueraria phaseoloides	Fabaceae	Ubiquitus
Flowering during the main rainy season		
Afromosia laxiflora	Fabaceae	Firmland (Savanna)
Centrosema pubescens	Fabaceae	Wetland (Savanna)
Dolichos africanus	Fabaceae	Firmland (Savanna)
Flowering during the intermediate period		
Sesbania candida	Fabaceae	Firmland (Savanna)
Tephrosia candida	Fabaceae	Firmland (Savanna)
Tephrosia platycarpa	Fabaceae	Firmland (Savanna)

2.3.7 Host-plant resistance

The definitions of Host-Plant Resistance are many and diverse. According to Snelling (1941) resistance is defined as "including those mechanism which enable a plant to avoid, tolerate or recover from attacks under conditions that will cause great injury to other plant of the same species". Kumar (1984) defined it as the intrinsic aptitude of a crop plant to limit, delay or overcome pest infestation and then improve the yield and/or the quality of the harvestable crop produce.

Host-Plant Resistance can be classified into three main categories which are nonpreference, antibiosis and tolerance (Painter, 1951). Non-preference (As well known as antixenosis) is the ability of the plant to either provide stimuli which are unattractive to the pest (color, odor, texture such as silky hairs, repellents or antifeedants) or fail to offer stimuli that are attractive to the pest affecting the behavior of the pest (Kogan and Omar, 1978).

Antibiosis is the kind of resistance in which the host plant causes injury, death, reduced longevity or reduced reproduction of the pest. Often both resistant and susceptible genotypes will have the same basic reaction to a pest however the resistant will respond more quickly or more dramatically than the susceptible genotype, decreasing the amount of damage the pest causes. Plant that express antibiosis affect the pest biology (Kogan and Omar, 1978).

Painter (1951), defined tolerance as "a basis of resistance in which the plant shows an ability to grow and reproduce itself or repair injury to a marked degree in spite of supporting a population as large as a susceptible host".

According to Reese (1994), tolerance is more advantageous in a pest management program than both antibiosis and antixenosis since it is compatible with additional control approaches and several biotype considerations.

2.3.8 Advantages and drawbacks of Host-Plant Resistance

Using insect-resistant genotypes is economically, ecologically, and environmentally helpful. Economic profits occur since crop harvests are protected from loss to pest and money is saved by not spraying chemicals that would have been applied to susceptible genotypes. Most of the time, seed of insect-resistant genotypes are not expensive, or little more compared to susceptible lines. The increased number of species in the agro ecosystem because of the reduced use of chemicals is one of the ecological and environmental benefits of using of Host-plant resistance.

The disadvantages of resistant cultivars are the long time that it takes to breed resistant cultivars; the specificity of the resistant variety for a particular pest, whereas pesticides are frequently active for many pests; resistance needs to be introduced for each new genotype; the capacity of the pest to adapt to the resistance might limit the permanency of the resistant genotypes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of study location

The experiments were conducted from July 2015 to April 2016 in field and pot conditions at the Manga Station of Council for Scientific and Industrial Research-Savannah Agricultural Research Institute (CSIR-SARI). Manga is geographically located within latitude 11.02° and longitude 0.27°, with an altitude of 224 meters above sea level. The area is situated in the Sudan Savanna agro-ecological zone of Ghana. The mean annual rainfall of the area during the period of the experiment was approximately 44.33 mm. The average annual temperature was about 29.44^{o}C, the highest being observed from February to April 2016. The relative humidity (RH) of the location fluctuated significantly, dropping in the dry season and rising during the rainy season with an average humidity of 55.4 %.

The study was conducted in two stages. The first step was carried out in the field and the second stage in pots experiment.

3.2. Planting materials

Two hundred and fifty one (251) Recombinants Inbred Lines RILs at F_8 generation (F8) (Appendix 1) derived from a cross between two cowpea lines, 'Sanzi', resistant to *Megalurothrips sjostedti* (Omo-Ikerodah *et al*., 2009) and 'IT97K-499-35' resistant to *Striga gesnerioides* (Omoigui, 2007), by single seed descent (SSD) method were used in the study.

3.3 Experimental procedure

3.3.1 Field experiment

The field studies was carried out under rain fed conditions (between July and September) and under irrigation during the dry season.

Two hundred and fifty one (251) Recombinant Inbred Lines and the parents Sanzi and IT97K-499-35 were planted in a selected field known to be a hot spot for *Striga* and flower Thrips (Fth). Due to the lack of seeds, each RIL was planted in a single row of 2 meters in one replication. Systemic Insecticide K-optimal (lambda-cyhalothrin (15g/l) and Acetamiprid 20g/l)) was applied during the critical stage of the plant, when there was a need and also to increase the chance of getting enough seeds. During this preliminary screening, data collected included plants at 50% flowering, presence or absence of *Striga* plants, number of *Striga* plants attached, total number of *Striga* per plot and *Striga* height.

Thrips population densities were assessed by randomly picking ten (10) flowers from each plot early in the morning between 6 am and 8 am. The flowers were all placed in a well labelled container containing 40% ethanol and kept in the laboratory. Twenty four hours after sampling, the flowers were teased out and put under a stereomicroscope to facilitate the Thrips counting.

After harvesting, the same materials (251 RIL's) were planted in two plots. One plot was fully protected with a contact insecticide whiles the other plot was protected until it reached the flower bud initiation to full flowering stage where insecticide application was stopped. Each plot had three (3) replications and planting was done in a single row of 2 meter long. To ensure a high population of Thrips, a susceptible variety Vita 7 was planted ten (10) days before the establishment of the experiment around the field. Sampling of ten

(10) flowers was done at flower bud initiation and at the full flowering for Thrips count using the same procedure described earlier. A presence or absence of *Striga* was recorded by visual observation on the different plots from thirty five (35) days after planting (DAP).

3.3.2 Pot experiment

A pot experiment was carried out to confirm whether there was no *Striga* attachment to the roots of those found without *Striga* emergence during the field experiment. (Appendix 2). The cowpea lines were arranged in a randomized complete block designs with three replications. The pots were artificially infested with five grams (5g) of *Striga* seeds. Three holes were made in each pot and two seeds sown into each hole making six seeds per pot. Two weeks after planting, the plants were thinned to maintain a total of three plants per pot. From thirty five (35) days after planting (DAP), the pots were monitored on daily basis to check for *Striga* emergence. At maturity, the early pods were harvested on single plant basis to get some seeds from each plant. This was followed with washing off the soil from the roots of the plants to confirm that there were no *Striga* attachment to the roots of those that did not record *Striga* emergence.

3.4 Evaluation of promising *Striga* resistant lines through yield loss assessment

Twelve (12) RIL's were selected based on their good agronomic traits on the field (white seed coat and big size and early maturity). The 12 consisted of five (5) *Striga* resistant lines, five (5) *Striga* susceptible lines and the two parents (IT97K-499-35 and Sanzi) as checks.

Table 3.1 Characteristics of Germplasm used to determine yield losses by *S. gesnerioides* infestation.

Genotypes	Days to	Growth	seed	seed	maturity	habit
	color	texture	parents			
IT97k-499-35	69	Erect	White	Smooth	Sanzi 67	Spreading
Brown	Rough					
R. progenies						
16 A	60	Erect	White	Rough		
19 B	65	Erect	White	Rough		
35	68	Erect	White	Rough		
155 A2	61	Erect	White	Rough		
191	68	Erect	White	rough	**S. progenies**	
12 B	61	Erect	White	Rough		
22 A	55	Erect	White	Rough		
25	62	Erect	White	Rough		
112 A	62	Erect	White	Rough		
211 A	57	Erect	White	Rough		

R: resistant; **S**: susceptible

The experiment was designed as a split plot with *Striga* infested and no *Striga* infested as main plots and the 12 lines served as sub plots which were randomly applied in each main plot in four replications. The soil used to fill the pots was subjected to steam sterilisation. A metallic barrel was used for the sterilization of the soil. A wire mesh was fitted at 1/3 of the length of the barrel from the bottom. This served as a separator between the soil and the water. The setup was placed on fire. Water was poured in the barrel to fill up to the level where the wire mesh is fitted, jute sack was then laid over the wire mesh before filling the remaining two thirds with soil. The soil was covered with jute sack. The steam generated from the boiling water was allowed to pass through the soil for about an hour and half to heat up the soil up to 100°C. The fire was put off upon attaining the 100°C to allow the soil to cool down. The soil was then scooped and spread on a plastic sheet to allow it to further cool down under shade before filling the plastic pots.

Half of the pots (forty eight) were infested with five grams (5g) with one year old seeds of *S. gesnerioides.* The infestation was done by removing a third of the soil content in every pot and mixing thoroughly with the *Striga* seeds and then re-poured into the main pot. The other half (forty eight pots) were not infested and used as a control. All the pots were watered and allowed to drain for twenty four (24) hours before planting. Three holes were made in each pot and two seed were sown into each hole. Two weeks after planting, the plants were thinned to maintain three (3) plants per pot. The pots were irrigated as when it is needed and kept weed-free through hand pulling. Monitored spray was done. At thirty five (35) days after planting based on visual observation *Striga* emergence was recorded daily. The other agronomic data collected included first day of flowering, 50% flowering, plant height, number of peduncles per plant and at 50% maturity. At harvest, plants were harvested individually into separate envelopes. The roots of the plants were gently washed in a basin with water to examine for attachment of *Striga* plants. The presence or absence of *Striga* was recorded for each plant. Plants with *Striga* attachment or with *Striga* emergence were categorized as susceptible and those free from *Striga* infestation, without any attachment were considered as resistant lines (Singh and Emebeche, 1990).

The post-harvest data collected included number of pods per plant, dry pod weight, number of seeds per pod, hundred seed weight and also fresh and dried matter weight. The dried biomass was obtained after drying all the plants in an oven for twenty four (24) hours.

Yield loss assessment due to *Striga* infestation was estimated using the formula:

$$YL = \frac{yield\ in\ uninfested\ pot - yield\ in\ infested\ pots}{yield\ in\ uninfestded\ pot} \times 100$$

YL: yield losses

3.5 Statistical analysis

All field data collected were subjected to analysis of variance (ANOVA) using the Genstat analytical software (version 12.1.0.3338). Varietal means were compared using Least Significant Difference at 5% level of probability (LSD 5%).

CHAPTER FOUR

RESULTS

4.1 Cowpea RILs reaction to natural *Striga gesnerioides* in the field screening

The result of the field experiment on Recombinant Inbred Lines (RILs) of advanced cowpea progenies derived from a cross of IT97K-499-35 (Resistant parent) and Sanzi (susceptible parent) are presented in Table 4 1 and Plate 4.1. Sixty six (66) RILs out of

the 251 (26.29%) used for this trial were found resistant. The susceptible lines of cowpea had germinated *Striga* plantlets emerged from the soil. The symptoms expressed by these susceptible genotypes included stunted growth, defoliation and reduced size of young leaves, leaf necrosis, chlorosis, and senescence.

Table 4.1. Reaction of cowpea RILs derived from a cross of IT97K-499-35× Sanzi to *S. gesnerioides* infestation in field trial. Manga station, 2016.

RILs	field trial	RILs	field trial	RILs	field trial
	R		R		R
1B	R	73	R	184	R
12C	R	80	R	188	R
16A	R	89B	R	191	R
		72B		179B	
19B	R	96A	R	195	R

1A

22A	R	96B	R	197A	R	22B
	R		R	197B	R	
	R		R	200	R	
	R		R	201A	R	
	R		R	201B	R	
	R		R	210	R	
	R		R	211A	R	
	R	155A1	R	212	R	
40A1	R	155A2	R	213	R	
40A2	R	155B*	R	214	R	
40B	R	157	R	249	R	
40C1	R	162	R	251B	R	
46	R	165A	R	256A	R	
47	R	178A	R	257	R	
72A	R	178B1	R	259*	R	

R: Resistant, **S**: Susceptible

Table 4.1. Continued

RILs	field trial	RILs	field trial	RILs	field trial
260	R		S	109A	S
265	R		S	109B	S
270			S	110	S
275			S	112A	S
279			S	112B	S
280			S	113	S
IT 97k-499-35	R	63B	S	114A	S

97

23
25
28
30A
33B
35
100
104A
151
152
153

55
56 R 59A
R 59B
R 62
R 63A

3	S	64	S	114B	S	7B
	S	65A	S	119	S	
	S	65B	S	121	S	7C
	S	68	S	124	S	
	S	70A	S	125	S	8A
	S	70B	S	126	S	
12A	S	74	S	128	S	9
12B	S	79	S	129	S	
14	S	81	S	130A	S	11
15	S	84	S	131B	S	
21	S	85A	S	134B	S	
29	S	86B	S	135	S	
33A	S	90	S	136	S	
37	S	92C	S	141A	S	
40C2	S	93	S	141B	S	
42A	S	94	S	143	S	
42B	S	95A	S	144A	S	
43A	S	95B	S	144B	S	
43B	S	98	S	145	S	
44	S	104B	S	148	S	
45	S	105A	S	149	S	
48	S	105B	S	150A2	S	
49	S	106	S	150B	S	
51A	S	107A	S	154 A	S	
51B	S	107B	S	158A	S	
54	S	108	S	158B	S	

R: Resistant, **S**: Susceptible

Table 4.1 Continued

RILs	field trial	RILs	field trial	RILs	field trial
	S		S		S
	S				S
164	S	208	S	247A	S
165B*	S	209	S	247B	S
166A1	S	215	S	248A	S
166B	S	216A	S	248B	S
160B		202		245	
161		205	S	246B	

167	S	216B	S	251A	S
168B1	S	220	S	254A	S
168B2	S	221	S	254B	
169	S	223	S	255	
170A	S	224	S	256B	
170B	S	225	S	258A	
171A	S	226	S	258B	
171B	S	227	S	261	
174A	S	229A	S	262	
174B	S	229B	S	263	
175	S	230	S	266	
176	S	232A	S	268	
177	S	232B	S	269B	
178B2	S	234A	S	273A	
179A	S	234B	S	273B	
179C	S	234C	S	276	
180	S	235	S	277	
181	S	237A	S	278	
182	S	237B	S	282A	
183	S	238	S	282B	
186	S	239C	S	Sanzi	
187	S	240	S	Apagbaala	S
190	S	242A	S		
192A	S	242B	S		
192B	S	242C	S		
199	S	244	S		

R: Resistant, **S**: Susceptible

168A	S	217	S	252	S	S	S
							S

S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S

Plate 4.1. *S. gesnerioides* field screening (*Striga* free plot (left); *Striga* infested plot (right))

4.2 Status of RILs resistance evaluated under natural Thrips infestation in the field

Generally the Thrips infestation was very low and therefore Thrips population sampled were very small. They ranged from zero to eleven (0 -11) per plot making it difficul t to rank the genotypes into the categories (resistant and susceptible). Seventy (70) RILs which did not show any Thrips in all the three (3) replications are presented in Table 4.2 and those which did not record Thrips (75 RILs) in two (2) replications ou t of the three are presented in Table 4.3.

The damage index (scores) were therefore not calculated due to the total absence of flower Thrips in these plots.

Table 4.2 Cowpea RILs recording zero (0) Thrips in all the three replications

Genotypes recording 0 Thrips in all the 3 replications										
14	128	223	158A	258A	15	143	227	160B	282B	
28			145				235		166A1	33B
56			149				249		166B	42B

64	152	255	168B2	63A
80	161	262	174A	65A
81	169 268 179A 70A 82 182 280 216B 86B 84 183 104A 234A			
	89A 97 Sanzi 105A 237B 89B			
100	184	114B	242C	8A
106 188 12B 246B 92C 119 213 141B 248B 96A 125 221 144A 256B 96B				

Table 4.3. Cowpea RILs recording zero (0) Thrips in two replications

Genotypes recording 0 Thrips in 2 replications				
9 151 245 134B 229B 11 153 252 144B 232A				
35	167	257	150A1	234B
44 173 265	155B*	234C 55	176 266	
165A	237A 58	180 270	170B	239C
73	186	275	171A	242A
90	191	276	171B	247A
98	195	277	178A	248A
110	208	278	178C	259A
126	209	279	179B	51B
129	210	107A	192A	59B
135	212	107B	192B	6B1
148	214	112A	19A	85B
150	236	12A	1A	IT97K-499-35

4.3 Reaction of cowpea RILs to artificial *Striga gesnerioides* in pot experiments In order to confirm the resistant status of the RILs found to be resistant during the field trial, the sixty six (66) RILs which were found to be resistant (no *Striga* emergence) during the field experiment were re-evaluated through pot experiment. After the pot experiment, 27 RILs were found to be resistant (no *Striga* emergence or *Striga* attachment) whiles 39 were susceptible (induced *Striga* emergence or *Striga* attachment at the roots level). The number of days to flowering and maturity varied from 35 to 73 and 60 to 86 respectively. The new status of these RILs is shown in Table 4.4**.**

Table 4.4 Reaction of cowpea RILs derived from a cross of IT97K-499-35× Sanzi to *S. gesnerioides* infestation in field trial and pot experiment. Manga station, 2016.

		Field trial	pot trial		
SN	Genotypes	status	status	50% flow. (days)	50% mat. (days)
1	23	R	R	38	63
2	35	R	R	43	67
3	46	R	R	53	66
4	151	R	R	37	65

5	162	R	R	62	80
		R	R		78
		R	R		73
		R	R		81
		R	R		70
		R	R	37	66
		R	R	58	70
		R	R	56	72
	155A1	R	R	53	65
	155A2	R	R	36	60
	16A	R	R	35	60
	178A	R	R	71	86
	19B	R	R	44	64
	1A	R	R	69	72
	201A	R	R	73	86
	22B	R	R	50	63
	251B	R	R	58	66
	40A1	R	R	58	72
	40A2	R	R	41	66
	40B	R	R	43	62
	40C1	R	R	63	76
	89B	R	R	65	81
	96B	R	R	61	85
	IT97K-499-35	R	R	46	68
	25	R	S	49	66
	28	R	S	65	85
	47	R	S	60	79
	73	R	S	66	75
	80	R	S	53	78
	97	R	S	58	79

R: resistant, **S**: susceptible, **flow**: flowering, **mat**: maturity

Table 4.4. Continued

SN	Genotypes	Field trial status	pot trial status	50% flow. (days)	50% mat. (days)
36	152	R	S	66	74
37	153	R	S	57	73
38	157	R	S	55	72
39	188	R	S	51	65
6	184	56			
7	191	38.5			

8 249 60
9 257 56
10 279 11 280
12 12C
13 14 15 16 17 18 19 20 21 22 23 24
25 26 27 28 29 30 31 32 33
34

35	100	R	S	51	63
40	195	R	S	57	71

41	200	R	S	69	84
42	210	R	S	58	70
43	212	R	S	67	80
44	213	R	S	54	69
45	214	R	S	50	62
46	260	R	S	55	76
47	265	R	S	64	70
48	270	R	S	60	78
49	275	R	S	52	62
50	104A	R	S	63	86
51	155B*	R	S	58	69
52	165A	R	S	44	62
53	178B1	R	S	53	68
54	179B	R	S	71	88
55	197A	R	S	63	78
56	197B	R	S	42	60
57	1B	R	S	62	80
58	201B	R	S	41	68
59	211A	R	S	54	68
60	22A	R	S	42	60
61	256A	R	S	58	74
62	259*	R	S	62	67
63	30A	R	S	55	68
64	33B	R	S	49	68
65	72A	R	S	51	67
66	72B	R	S	53	70
67	96A	R	S	51	73
68	Apagbaala	S	S	49	71
69	sanzi	S	S	39	66

R: resistant, **S**: susceptible, **flow**: flowering, **mat**: maturity

4.4 Evaluation of *Striga* promising lines

Based on agronomic traits, twelve promising *Striga* lines were evaluated in pot experiments.

4.4.1 Days to flowering and maturity

Days to flowering and maturity varied from 41 to 55 and 63 to 73 days from sowing. Under *Striga* infestation, the earliest genotypes in terms of days to flowering were 191 and 25 which flowered 44 and 45 days respectively (Table 4.5). Days to flowering differed significantly between the genotypes in both infested (DF=11; P< 0.001) and not infested (DF=11; P< 0.001). Under no *Striga* infestation, the genotypes 25 and 112A flowered earlier than the rest of the genotypes (41 and 43 days). 155A2 took more days (9 and 7 days) to flower (50 days). The remaining genotypes were considered as medium maturity cultivars based on the days to flowering (43-49 days). The resistant progenies except 155A2 also flowered earlier than the resistant parent IT97k-499-35 (49 days).

Under *Striga* infestation, all the resistant lines flowered and matured almost at the same time as in no *Striga* infested pots whiles the susceptible lines delayed in flowering and maturity (Table 4.5).

The resistant genotype 19B for instance flowered at 48 DAP and matured at 65- 66 days DAP in the non-infested and *Striga* infested pots respectively.

The susceptible genotype 12B flowered at 48-55 DAP and matured at 65-73 days DAP in the non-infested and *Striga* infested pots respectively.

Significant differences were also observed among the genotypes under no infestation (DF = 11; P = 0.008) and infestation (DF = 11; P < 0.001) conditions in terms of days to maturity (Table 4.5).

Table 4.5. Mean days to flowering and maturity of cowpea RILs on no infestation and *S. gesnerioides* infested plots. Manga station, 2016

Genotypes	Days to Flowering		Days to Maturity	
	No infestation	Infestation	No infestation	Infestation
Parents				
IT97k-499-35	49	48	68	69
Sanzi	46	49	66	73
R. progenies				
16 A	47	48	68	70
19 B	48	48	65	66
35	47	46	65	66
155 A2	50	51	67	67
191	44	44	62	63
S. progenies				
12 B	48	55	65	73
22 A	43	47	69	73
25	41	45	62	67
112 A	43	48	63	68
211 A	46	51	67	73
Mean	45.81	48.38	65.56	68.75
LSD (5%)	3.641	2.089	3.75	2.833
CV (%)	5.5	3	4	2.9

Values represent means of four replications.

4.4.2 Seed yield and dry biomass per hectare

The analysis of variance revealed significant differences between the genotypes under *Striga* infestation ($P < 0.001$) and no *Striga* infestation ($P < 0.001$).
Among the progenies, the resistant progeny 16A, under no infestation produced the greatest yield (754.2 kg ha^{-1}) followed by 25 (473.3 kg ha^{-1}) and 19 B (470.1 kg ha^{-1}).

(Table 4.6) .The rest of the cultivars recorded dry grain yield ranging from 320.1 to 554.2 kg ha^{-1}. The genotype, 155A2, a resistant cultivar recorded the smallest grain yield (320.1 kg ha^{-1}).

The cultivar 16A which recorded the greatest yield under no infestation (754.2 kg ha^{-1}) also recorded the highest yield under *Striga* infestation. (750 kg ha^{-1}). Ironically, the susceptible cultivar 25, one of the highest grain producers under no *Striga* infestation (436.1 kg ha^{-1}) also had one of the lowest grain yield under the infestation (338.6 kg ha^{-1}). In general the reduction in grain yield was higher in the susceptible progenies than the resistant ones.

Dry biomass yield showed significant differences among the *Striga* infested ($P < 0.001$) and uninfested ($P < 0.001$) conditions.

The mean values of dry fodder yield were 1507 kg ha^{-1} under no *Striga* condition and 1126 kg ha^{-1} in the infested conditions. The genotypes with the highest dry biomass under uninfested conditions were 155A2 and 12 B respectively with 2234 kg ha^{-1} and 1901 kg ha^{-1}. The smallest fodder yield was recorded for the cultivar 25 with yield of 876 kg ha^{-1}. The dry biomass yields for the other genotypes ranged from 1076 to 1812 kg ha^{-1}. Under the *Striga* infestation condition, the genotype 155A2 still recorded the highest fodder likewise in the no infestation. The genotype 12B saw its dry fodder yield drastically dropped from 1901 kg ha^{-1} in the non-infested condition to 1002 kg ha^{-1} under the infested one. The genotype 16A also recorded good production of fodder in both infested (1813 kg ha^{-1}) and no infested condition (1898 kg ha^{-1}).

Table 4.6. Mean grain weight and dry biomass of cowpea RILs under no infestation and *S. gesnerioides* infested plots.

Genotypes	Grain yield (kg/ha)		Dry biomass (kg/ha)	
	Uninfested	Infested	Uninfested	Infested
parents				
IT97k-49935	554.2	545.8	1812	1779
Sanzi	488.8	302.8	1251	557
R. progenies				
16 A	754.2	750	1898	1813
19 B	470.1	455.6	1627	1548
35	397.4	389.7	1099	1036
155 A2	320.1	313.2	2234	2209
191	390.3	385.4	1424	1314
S. progenies				
12 B	445.8	183.3	1901	1002
22 A	436.1	270.8	1567	810
25	473.3	338.6	876	379
112 A	389.3	259	1076	412
211 A	481.1	340.3	1317	656
Mean	466.7	377.9	1507	1126
LSD (5%)	95.79	116.1	336	170.1
CV (%)	14.3	21.4	15.5	10.5

Values represent means of four replications

4.4.3 Plant height and number of pods per plant

The number of pod per plant were significantly different in both infested (DF = 11; P < 0.001) and non-infested environment (DF=11; P<0.001).

The analysis of variance indicated a significant difference for the plant height in both infested (DF = 11; P < 0.001) and non-infested (DF = 11; P < 0.001) environments.

The plants of the resistant progenies were taller than the infested susceptible ones (Table

4.7). The resistant parent IT97k-499-35 was the tallest plant (35.61 cm) followed by 16A and 191 with plant heights of 31.63 cm and 31.53 cm respectively. The susceptible cultivars 12B and 112A recorded the shortest plants height with 15.74 cm and 17.79 cm

respectively, in the uninfested pots.*Striga* infestation induced reduced plant height among the susceptible genotypes as compared to the resistant progenies.

Among the progenies, the highest mean number of pods per plant was recorded in the susceptible genotype, 22A (10) under no *Striga* infestation, but under *Striga* infestation the number of pods drastically reduced to 7. However the resistant progeny 19B which recorded an average number of pods when into infested condition (8.42) showed almost the same number of pods in the infested environment (8.37).

Table 4.7. Mean plant height, number of pods per plant of cowpea RILs under no infestation and *S. gesnerioides* infested plots.

Genotypes	Plant height (cm)		Mean Number of pods /plant	
	Uninfested	Infested	Uninfested	Infested
parents				
IT97k-49935	35.61	36.04	11.19	11
Sanzi	21.83	16.63	12	9
R. progenies				
16 A	31.63	31.16	7.5	7.02
19 B	29.32	29.11	8.42	8.37
35	26.1	25.87	7.9	8
155 A2	24.76	24.34	7.55	7.4
191	31.53	30.35	8.32	8.25
S. progenies				
12 B	15.74	12.83	7.55	4.05
22 A	28.38	23.03	10.28	7.37
25	24.85	20.6	9	6.42
112 A	17.79	13.8	8.47	5.92
211 A	28.16	22.9	9.38	6.1
Mean	26.31	23.89	8.96	7.41
LSD (5%)	1.74	2.11	1.756	2.15
CV (%)	4.6	6.1	13.6	20.1

Values represent means of four replications

4.5 Grain yield and dry biomass loss due to *Striga gesnerioides*

The grain and fodder loss caused by *Striga gesnerioides* infestation (Table 4.8) were estimated using the mean dry grain and biomass yield per hectare.

In general the dry grain and biomass yield loss were higher in the susceptible lines compared to the resistant progenies.

For the resistant progenies (16A and 19B) the dry grain yield losses ranged from 4.5 kg ha^{-1} (0.55%) to 14.5 kg ha^{-1} (3.08%) respectively. In the susceptible ones (genotypes),

grain yield losses oscillated from 134.7 kg ha^{-1} (28.45%) to 262.5 kg ha^{-1} (58.88%) for the genotypes.

The higher grain yield loss (58.88%) was recorded for the susceptible progeny 12B followed by the susceptible line 22A which registered 37.9% grain yield loss. The grain yield losses for the other susceptible progenies (25,112A and 211A) varied from 28.45% to 33.47%. Grain yield losses for the resistant progenies were found to be between 0.55% for the cultivar 16 A to 3.08 % for the genotype 19B. The resistant progeny 16A also showed a lower, yield loss (0.55%) than the resistant parent IT97K-499-35 (1.51%). Dry biomass losses for the susceptible progenies ranged from 889 kg ha^{-1} (47.29%) to 664 kg ha^{-1} (61.71%) for the cultivars 12B and 112A respectively. The biomass yield losses for the other susceptible lines varied from 48.3% to 56.71 %.

Similarly for the dry grain yield, the resistant progenies did not show any significant biomass losses.

With regard to the biomass losses, the cultivar 155A2 performed better in both *Striga* infested (2209 kg ha^{-1}) and non-infested (2234 kg ha^{-1}) then to the resistant parent IT97K-499-35, and also recorded the least biomass loss (1.1%) (Table 4.8).

Table 4.8. Percentage dry grain and biomass loss per hectare under to *S. gesnerioides* infestation

	Grain yield (kg/ha)		**Yield losses (%)**	**Dry biomass (kg/ha)**		**Biomass losses (%)**
Genotypes	No *Striga*	*Striga*		No *Striga*	*Striga*	
parents						
IT97k-499-35	554.2	545.8	**1.51**	1812	1779	**1.82**
Sanzi	488.8	302.8	**38.05**	1251	557	**55.47**

R. progenies						
16 A	754.2	750	**0.55**	1898	1813	**4.47**
19 B	470.1	455.6	**3.08**	1627	1548	**4.85**
35	397.4	389.7	**1.93**	1099	1036	**5.73**
155 A2	320.1	313.2	**2.15**	2234	2209	**1.11**
191	390.3	385.4	**1.25**	1424	1314	**7.72**
S. progenies						
12 B	445.8	183.3	**58.88**	1901	1002	**47.29**
22 A	436.1	270.8	**37.9**	1567	810	**48.3**
25	473.3	338.6	**28.45**	876	379	**56.73**
112 A	389.3	259	**33.47**	1076	412	**61.71**
211 A	481.1	340.3	**29.26**	1317	656	**50.18**
Mean	466.7	377.9		1507	1126	
LSD (5%)	95.79	116.1		336	170.1	
CV (%)	14.3	21.4		15.5	10.5	

Values represent the means of four replications

4.6 Promising *Striga* resistant lines

After the field and the pot trials, some few lines were identified as *Striga* resistant lines**.** (Table 4.9). The genotype 16A is one of the most promising lines in terms of dry grain yield. Its performance in grain yield (754.2 kg ha^{-1}) and dry biomass (1898 kg ha^{-1}) is higher than the resistant parent IT 497k-499-35 with (554.2 kg ha^{-1}) and (1812 kg ha^{-1}). The genotype 155A2 had an average production of dry grain but performed better in terms of biomass. It can be used as a dual purpose variety.

Other lines were also identified as resistant to *Striga* but unfortunately did not have the agronomic traits preferred by farmers. These lines can be used in others breeding programs. The table 4.9 also gives a few lines which are resistant to *Striga* and show less flower Thrips population.

Table 4.9. *Striga* resistant and Thrips promising lines obtained after field and pot trials

Characteristics	**Genotypes**				
***Striga* promising lines**	16 A	19 B	35	155 A2	191
***Striga* resistant and less Flower Thrips population**	1A 184 280	35 191	96 B 249	151 257	178 A 279
breeding purpose (could be used as breeding material)	1A 40 A2 96 B 184 279	12 C 40 B 151 201 A 280	22B 40 C 1 155 A1 249	23 46 162 251 B	40A1 89 B 178 257

CHAPTER FIVE

DISCUSSION

One of the best methods to control *S. gesnerioides* could be the development of resistant cowpea lines. This appears as a sustainable strategy for resource poor farmers since extra inputs are not required. Toure *et al.* (2008) found some cowpea lines that were resistant

to *S. gesnerioides* attack. The first identification of resistance to *S. gesnerioides* came from field experiments in Burkina Faso where the varieties Suvita-2 (previously known as Gorom Local) and 58-57 recorded zero or very low emergence of *Striga gesnerioides* (Aggarwal *et al.*, 1984).

5.1 Field screening for resistance to *Striga gesnerioides*

Field screenings for *Striga* infestation have been efficient in selecting cowpea lines with resistance to *S. gesnerioides* and estimating yield loss by *Striga gesnerioides*, (Muleba *et al*., 1997). The aim of the field screening was to identify some resistant Recombinant Inbred Lines (RILs) obtained by a cross between a *Striga* resistant line IT97K-499-35 and Thrips resistant Sanzi (Omo-Ikerodah, 2009).

The present field study recorded high emergence of *Striga gesnerioides* per plot (243 shoots) and this is similar to the data recorded for other studies (Carsky *et al.*, 2003; Kamara *et al.*, 2008) .The high *Striga* emergence was an indication that the site was really a hot spot for *S. gesnerioides* and the field was uniformly infested with the high concentration of *Striga* seeds in the soil.

Moreover, the identification of susceptible and resistant RILs also conformed to the selection procedure by Singh and Emebeche (1990).

The field screening showed 26.2% of the genotypes without *Striga* shoots or emergence (66 out of the 251 RILs). However, a rigorous screening of the 66 genotypes in infested pots revealed that only 10.75% were truly resistant to *Striga*. A susceptible genotype could be heavily infested underground without any *Striga* emergence as a results of several factors.

Striga sp. seeds need warm stratification for a certain time at a right temperature (approximativeley 30°C) before the seeds start responding to germination stimulants (Matusova *et al.,* 2004).

According to Kim *et al.* (2002), one of the major disadvantages of screening under natural infestation remains the variability in *Striga* seeds dissemination and cultivars escaping infestation. Baptiste *et al*. (2013), stated that the high interference with locations such as soil and climatic factors observed in the field is making the field screening less accurate.

Screening of large segregating population should therefore be started in adequately infested pots which offer the opportunity to identify individuals with *Striga* emergence as well as washing the roots of those without *Striga* to check for attachment or not. This method is more cost effective and saves time and energy since a complete phenotyping could be achieved in one set of study.

5.2 Pot screenings

Field screening under artificial infestation is not always practical due to the fact that it can cause *Striga* seeds spreading to novel regions and it is moreover not consistent because breeders do not have any control of the parasite density and distribution (Haussmann *et al.,* 2000). Pot screening has been operative as an alternative technique to confirm uniform infestation of *Striga* seeds.

After the pot experiment the number of resistant lines were reduced from sixty six (66 RILs) recorded after the field screening to twenty seven (27 RILs) after the pots experiment. This is essentially due to the high level of infestation (five grams of *Striga* seed per pot), the uniformity and a better control of the environment. A previous study

done by Baptiste *et al.* (2013), confirmed the reliability of the pot screening compared to field screening.

The increased number of susceptible Recombinant Inbred Lines found among the 66 could also be explained by the fact that this number included the genotypes which showed no emerged seedlings of *Striga* but have *Striga* attached to their roots. According to Ba (1983), some cowpea genotypes stimulate the *Striga* to germinate and the plantlets were allowed to penetrate the cowpea root tissues, but failed to grow more. A similar mechanism was observed by Lane (1989), from laboratory work with B301 variety which is one of the parents of IT97K-499-35. The study conducted by Lane (1989), revealed the presence of stimulation and germination of the *Striga* seeds, attachment and haustorial formation but failed to grow any further.

After both field and pot screening for *Striga* resistance, and taking into consideration farmers chosen traits, the genotypes 16A, 19B, 35, 155A2 and 191 were identified as promising *Striga* resistant lines.

The mechanisms of resistance of these cultivars are not known but they can be related to resistance mechanism expressed by B301, one of the parents of IT97K-499-35. Indeed Lane *et al.* (1993), reported different mechanisms of resistance to *Striga* gesnerioides in two cultivars B301 and 58-57. Firstly the host tissue nearby invading *Striga* radicles became necrotic in association with premature death of the *Striga* plant and lack of tubercle development. This mechanism was expressed in both lines. The other mechanism was only involved in the cultivar B301; the *Striga* radicles infected the cowpea roots and tubercles and were established but remained very small with incomplete stem development.

5.3 *Striga* promising lines

The results of the current study have shown that *Striga* infestation delayed the flowering and maturity of genotypes essentially for the susceptible ones. The susceptible genotypes have also shown a huge reduction in grain yield and dry biomass in the *Striga* infested environment compared to the resistant ones where the difference between the infested and uninfested were not significant.

The study also confirmed that *Striga* infestation induce stunted growth hence the significant reduction of plant height at 50% flowering recorded for the susceptible genotypes. It also had an effect on the production of number of pods per plant. These data corroborated with previous studies (Press, 1995; Alonge, 1999; Gworgor *et al*., 1991), which produced similar results.

The stunted growth of genotypes, 12B, 22A, 25, 112A and 211A, can be attributed to *Striga* infestation which resulted in low grain yield of these genotypes. The reduced vegetative growth of the susceptible varieties resulted in reduced leaf area, photosynthetic capacity and therefore affected flowering, podding and seed production due to inadequate water (Alonge, 1999).

According to Press (1995), the lower biomass accumulation by the susceptible genotypes could be the result of competition among the host and the weed for solutes, as well as carbon, water, and minor rate of photosynthesis in the leaves of *Striga* infested plant. The reduced photosynthesis might have resulted in lower number of pods per plant and translocation of photosynthate to the sink.

Graves *et al.* (1992) showed that the low chlorophyll content which characterizes susceptible genotypes may account for the reduced development of the susceptible cowpea genotypes causing a decrease in both grain and biomass yield. The low biomass yield could also be attributed to the reduced shoot growth of the susceptible genotypes. The same phenomenon has also been reported for both cereals infected with *Striga hermonthica* and for cowpea infected with *S. gesnerioides* (Graves *et al.*, 1992).

The resistant cultivars showed a relative good growth compared to the susceptible lines in the infested pots. The relative good growth and the reduced export of assimilate to the weed would have ensured sufficient biomass accumulation and seed development as suggested by Gworgwor *et al.* (1991) on *S. gesnerioides*.

The superior growth of some genotypes like 16A, 19B, 35, 155A2 and 191 indicated the positive relationship between crop vigour and crop performance even in *Striga* infested pots.

5.4 Low performance of the resistant varieties

The grain yield of all the lines ranged from 300 to 800 kg ha^{-1} which was lower than the grain yield potential of 1500 to 2500 kg ha^{-1} registered for *Striga* and *Alectra* resistant cowpea lines by Singh (2002) in a better condition. According to literature, cowpea is a warm weather species which produces better yield in the dry Savannah zones where the temperature varies from 20°C to 35°C. The very high temperature (39.5 °C for March and 40.2°C recorded in April) and the heat during the experiment could possibly be responsible for the general low grain and folder yields recorded. According to Bagnall and King (1987), cowpea grain yield is very sensitive to conditions of the environment regardless of its hardiness. Grain yield may have influenced by the high temperatures at

flowering stage which resulted in decreased number of pods, and therefore affected the yield. It is also confirmed by Prasad *et al*, (2002), who reported that exposure to temperatures above 28 C also reduced photosynthesis, seed number and seed yield in kidney bean (*Phaseolus vulgaris L.).*

The current data showed that some of the cultivars (155A2, 191 and 35) that were highly resistant (no *Striga* recorded) were also amongst the lowest yielders. Olusoji (2012) obtained comparable results with *Alectra vogeli*. He pointed out that the performance of such genotypes could be more affected by their inherent low yield potential rather than by the parasite. Omoigui (2007), also revealed that resistance to *Striga* does not necessary convert to higher yield by a specific genotype as shown by B301 and IT 97k-205-8 which recorded no emerged *Striga* and low yield (less than 1000 kg ha^{-1}).

According to Alonge *et al.* (2004), the reduction in the seed yield of highly resistant cultivars B301, IT90k-76 and IT90k-59, may have been partially due to the reduction in their root nodulation and root growth by the parasite even though there was no attachment on these genotypes in most cases. A comparable statement with *Alectra vogeli* was also reported by Alonge *et al.,* (2001a). They indicated that it is likely the seeds of these weeds contain toxins which leaked into the soil and masked their root development. This might cause insufficient nitrogen and nutrient absorption for vegetative development and therefore reduced grain yield (Alonge *et al.,* 2001a).

5.5 Grain and biomass loss due to *Striga gesnerioides*

This current study has shown that all the resistant cowpea cultivars (16A; 19B; 35; 155

A2 and 191) exhibited lower grain yield and dry biomass loss compared to the susceptible ones (12B; 22A; 25; 112A and 211A) indicating that these cultivars could play an essential role in controlling *Striga* in the endemic areas.

The susceptible genotypes recorded an average yield loss of 37.66 % for dry grain yield which is quite consistent with the yield loss of 31±4% with a range of 26-65% observed by Aggarwal and Ouedraogo (1989). According to these authors, the loss can be attributed exclusively to the genotype effect as a consequence of *Striga* direct parasitism of susceptible cowpea lines (Muleba *et al.*, 1996).

Striga gesnerioides diverts the host nutrient into themselves via the haustorium which establishes contact with the host tissues (xylem and phloem) (Okonkwo and Nwoke 1978; Okwonkwo, 1966). Consequently, this competition among host and parasite for water, and essential metabolites could be the explanation for the yield loss according to Stewart and Press (1990). Setty and Nanjapp (1985) and Kuijt (1969), revealed that the osmotic pressure of the parasite is higher in both leaf and root than its host making the *Striga* more competitive. The use of high yielding *Striga* resistant varieties coupled with good agronomic practices can therefore help to reduce the yield losses in soil infested with *Striga* in the traditional farming systems.

5.6 *Megalurothrips sjostedti* population

The flower Thrips population recorded under natural field experiment was very low compared to results from similar studies (Alabi *et al*., 2003), where the number can sometimes reach four hundred (400 per flower) for some particulars genotypes. The flower sampling for Thrips counting was done from December ending to the beginning of

January 2016, approximativeley from 6 am to 8 am.

According to Saliou (2015), the flower Thrips population varied over the time of day, with higher population at 10 am, 1 pm, and 4 pm than 7 am. This difference could be attributed to variation in temperature and time of the day which moreover affect flower opening and closing in the host plant (Ekesi *et al*., 1999, Ige *et al.,* 2011). This factor could explain the lower number of *Megalurothrips sjostedti* recorded during this trial.

Light trap monitoring and sampling of cowpea fields throughout the dry and wet season done by Bottenberg *et al.* (1997), in Nigeria have shown that a large number of pests including *Megalurothrips sjostedti* populations are low during dry season compared to the wet season. They stated that flower infestation by *Megalurothrips sjostedti* was relatively low (less than 1Fth/flower) during the dry period which amplified quickly during the raining season. Afun *et al.* (1991) and Alghali (1991), similarly reported a low insect population on cowpea during the second half dry season in Fadama zone in the Bida region (Nigeria). These data corroborate this study where most of the flowers recorded zero flowers Thrips.

The flower sampling and the Thrips counting occurred in December and January which are the coldest months. The minimum temperatures recorded were 20.1°C for December and 19.4°C for January. According to Tamo (1991), a temperature under 15°C and above 35°C severely reduce the survival of all growth phase of the flower Thrips. This might probably explain the lower number of Thrips recorded during this screening.

In the dry season, the harmattan wind is known to be severe in the Northern and Upper East regions of Ghana. The harmattan wind usually starts around December to the end of January but, however, 2016 was exceptional, it lasted up till February ending.

Bottenberg *et al*., (1997) through a similar study carried out in the Northeast of Nigeria, on aphids stated that harmattan winds carried aphids from dry season cowpea production area in the Hadejia wetlands about 200 km North-East of Kano. These same authors also

mentioned that Thrips can also be carried year round by dominant wind over long distance. Even though, the population of *Megalurothrips sjostedti* was very small, a particular attention should be paid to some of the Recombi nant Inbred Lines (RILs) because of the consistent number of flower Thrips found in all the three replication like the Thrips resistant parent Sanzi.

The low number of Thrips recorded did not allow the scoring of the different RILs in terms of damages due to Thrips. Even though it is challenging to classify Thrips resistant cultivars by using the Thrips number as the only selection criterion (Ekvised*et al*., 2006), the fact that those particular lines showed the same number of Thrips (zero Thrips) in all the three replication as the resistant parent Sanzi could signify that it did not happen by chance and those RILs might have a higher chance to be resistant compared to the other ones. It is therefore important to take a closer look at these lines in a further evaluation of these lines in multi-locations.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The study revealed different reactions of cowpea RILs to *Striga gesnerioides* during the field and the pot experiments. Out of the 251 RILs used, 27 RILS were found resistant similar to the resistant check IT97K-499-35, whiles 224 RILs were susceptible. The study has also shown a low number of flower Thrips after the flower sampling, due to the weather conditions. This made the screening for *Megalurothrips Sjostedti* more challenging.

Yield loss assessment showed that the *Striga* resistant genotypes suffered less yield loss compared to the susceptible ones and therefore resistant genotypes can be one of the best means to minimize yield loss. These genotypes that expressed complete resistance are potential lines that will serve as resistant genotypes. The latest discovery of new sources of resistance to *Striga* provides an excellent way to supply farmers with new genotypes to replace their susceptible varieties.

6.2 RECOMMENDATIONS

The level of Thrips infestation was very low during the study period which was also confirmed in other populations that were evaluated under the same conditions for Thrips resistance by the lead Scientist of Legume Innovation Lab at Manga. One of the reasons could be due to the bad weather during the dry season. The harmattan period during the period was unusually prolonged, lasting for about three months and this was immediately followed by very high day and night temperatures with very low relative humidity. It is therefore recommended that the population should be evaluated again under rainy season in multi-locations to be able to make a better judgement about the performance of the lines against heavy Thrips infestation.

The phenotypic data for *Striga* and Thrips will be shared with University of California

Riverside partners for Quantitative Trait Loci (QTL) mapping for identification of SNP markers for flower Thrips and *Striga* resistance for future use in marker-assisted breeding.

The use of molecular markers has become an important tool in cultivar development, the conventional breeding method should therefore be complemented with Marker Assisted Selection (MAS) to increase the efficiency and the effectiveness of *Striga* and Thrips breeding.

Repeating this work also in the rainy season and in pot experiments will also help to know the real potential yield of the promising *Striga* resistant lines that have been selected. The yield potential in Northern Ghana could also be improved by applying fertilizer as it is the practice in other countries such as Burkina Faso, Niger, Mali and Northern Nigeria.

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APPENDICES

Appendix 1: List of Recombinant Inbred Lines (RILs) used in field experiment

Genotypes
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	97	181	244	12B
	98	182	245	12C
	100	183	249	130A
	106	184	252	131B
	108	186	255	134B
	110	187	257	141A
	113	188	260	141B
	119	190	261	144A
	121	191	262	144B
	124	195	263	150A2
	125	199	265	150B
	126	200	266	154 A
	128	202	268	155A1
	129	205	270	155A2
	135	208	275	155B*
	136	209	276	158A
	143	210	277	158B
	145	212	278	160B
	148	213	279	165A
	149	214	280	165B*
	151	215	104A	166A1
	152	217	104B	166B
	153	220	105A	168A
	157	221	105B	168B1
	161	223	107A	168B2
	162	224	107B	16A
	164	225	109A	170A
	167	226	109B	170B
	169	227	112A	171A
	175	230	112B	171B
	176	235	114A	174A
	177	238	114B	174B
	180	240	12A	178A
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Appendix 1 contd (RILs used in field experiment)

Genotypes				
178B1	229B	248B	40A1	70B
178B2	22A	251A	40A2	72A
179A	22B	251B	40B	72B
179B	232A	254A	40C1	7B
179C	232B	254B	40C2	7C
192A	234A	256A	42A	85A
192B	234B	256B	42B	86B
197A	234C	258A	43A	89B
197B	237A	258B	43B	8A
19B	237B	259*	51A	92C
1A	239C	269B	51B	95A
1B	242A	273A	59A	95B
201A	242B	273B	59B	96A
201B	242C	282A	63A	96B
211A	246B	282B	63B	Apagbaala
216A	247A	30A	65A	IT 97k-499-35
216B	247B	33A	65B	Sanzi
229A	248A	33B	70A	

Appendix 2: RILs used in pots experiment

Genotypes				
23	162	270	197A	33B
25	184	275	197B	40A1
28	188	279	19B	40A2
35	191	280	1A	40B
46	195	104A	1B	40C1
47	200	12C	201A	72A
73	210	155A1	201B	72B
80	212	155A2	211A	89B
97	213	155B*	22A	96A
100	214	165A	22B	96B
151	249	16A	251B	Apagbaala
152	257	178A	256A	IT97K-499-35
153	260	178B1	259*	Sanzi
157	265	179B	30A	

Appendix 3: ANOVA TABLES

Appendix 3.1: Analysis of Variance table for days to 50 % flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	57.781	19.26	1.48	
Striga infestation	1	157.594	157.594	12.11	0.04
Residual (a)	3	39.031	13.01	3.06	
Genotypes	11	561.781	51.071	12	<.001
Genotypes ×*Striga* infestation	11	139.031	12.639	2.97	0.003
Residual (b)	66	280.938	4.257		
Total	95	1236.16			

Appendix 3.2: Analysis of Variance table for days to maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	11.615	3.872	0.36	
Striga infestation	1	243.844	243.844	22.54	0.018
Residual (a)	3	32.448	10.816	2.03	
Genotypes	11	573.031	52.094	9.76	<.001
Genotypes ×*Striga* infestion	11	177.531	16.139	3.02	0.003
Residual (b)	66	352.187	5.336		
Total	95	1390.66			

Appendix 3.3: Analysis of Variance table for biomass weight (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	54477	18159	0.34	
Striga infestation	1	3477020	3477020	64.55	0.004
Residual (a)	3	161588	53863	1.57	
Genotypes	11	2.1E+07	1885789	55.03	<.001
Genotypes ×*Striga* infestation	11	2560853	232805	6.79	<.001
Residual (b)	66	2261621	34267		
Total	95	2.9E+07			

Appendix 3.4: Analysis of Variance table for grain yield (kg/ha)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	5863	1954	0.12	
Striga infestation	1	189441	189441	11.29	0.044
Residual (a)	3	50323	16774	3.07	
Genotypes	11	1335651	121423	22.19	<.001
Genotypes × *Striga* infestation	11	182980	16635	3.04	0.002
Residual (b)	66	361166	5472		
Total	95	2125425			

Appendix 3.5: Analysis of Variance table for plant height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	5.863	1.954	2.17	
Striga infestation	1	140.505	140.505	156.15	0.001
Residual (a)	3	2.699	0.9	0.5	
Genotypes	11	3608.57	328.051	181.64	<.001
Genotypes × *Striga* infestation	11	115.076	10.461	5.79	<.001
Residual (b)	66	119.196	1.806		
Total	95	3991.91			

Appendix 3.6: Analysis of Variance table for number of pods/plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	3	18.582	6.194	1.75	
Striga infestation	1	57.893	57.893	16.37	0.027
Residual (a)	3	10.609	3.536	1.9	
Genotypes	11	182.229	16.566	8.91	<.001
Genotypes × *Striga* infestation	11	49.748	4.523	2.43	0.013
Residual (b)	66	122.746	1.86		
Total	95	441.806			

Appendix 4: Weather data during experiment period.

		Jul-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-16	Jan-16	Feb-16	Mar-16	Apr-16
Temp (0C)	max	32.52	30.6	32.6	35.3	37.4	31.9	34.7	37.6	39.5	40.2
	min	23.8	23.5	23.4	23.8	20.4	20.1	19.4	21.2	24.6	27.9
	mean	28.12	27.8	29	29.5	31.1	26	27.2	29.4	32.1	34.2
RH (%)	max	92	95	94	93	67	39	40	36	50	74
	min	72	78	73	63	27	20	17	15	25	41
	mean	82	86	84	78	46	30	28	25	37	58
Rainfall(mm)	max										
	min										
	mean	112.8	284.8	-	45.7	-	-	-	-	-	-

Source: Manga research station, 2016.