

Feeding and oviposition preference of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for selected poaceous plant species

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ABSTRACT

The Fall armyworm (FAW), *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), is an agricultural pest native to the Americas. FAW is polyphagous with 274 larval host plant species being reported. Crop losses are caused by the larvae which are mainly controlled with insecticides. FAW is difficult to control by means of insecticide applications since the larvae feed inside the whorl of maize plants and are able to develop resistance within a relatively short period of time. Cultural control methods such as the push-pull strategy and trap cropping were investigated to determine if these could be used along with insecticides to control FAW. In push-pull systems plant volatiles play an important part in insect-plant interactions. Gravid moths may be drawn to the 'pull' component of the system or 'attractant' which emit more attractive volatiles than the main crop. If the larvae are unable to survive on this 'pull' crop, it may be regarded as a trap crop. The aim of this study was to evaluate the oviposition and feeding preference of *Spodoptera frugiperda* on selected poaceous host plants including maize, sorghum, Napier grass, *Brachiaria* spp and Vetiver grass for inclusion in a push-pull strategy. Volatile compounds were collected via entrainment of intact plants. The collected volatiles were analysed by means of gas chromatography (GC) and three main components occurring in all four plant species were identified viz. decanal, squalene and limonene. Electroantennography (EAG) was used to evaluate and record the olfactory responses of FAW moths. Antennae were exposed to the three compounds identified with GC. It was determined that maize and Napier emit more volatiles during the evening. Since FAW moths respond to an array of volatiles, gravid female moths may not profoundly respond to specific volatile cues. No-choice, two-choice and multiple-choice bioassays were conducted in Petri-dishes in which leaf material were provided to larvae. Host suitability for larval development was evaluated by rearing larvae on leaf tissue of the respective plant species in an insect rearing room. Maize was most preferred by larvae and higher numbers of larvae were able to reach the pupal stage when feeding on maize and their development to the pupal stage was significantly faster than on other plant species. Larval preference for Vetiver, *Brachiaria* and Napier grass was low. In the feeding studies, no larvae survived on Vetiver and *Brachiaria* while survival on Napier grass was also very low. These three grass species could therefore be considered as trap crops if moths prefer these plants as oviposition sites over maize. Moth oviposition preference studies

suggested that gravid female moths are not particular in choosing a plant for oviposition and they often laid eggs on plants that were unsuitable for larval development and on substrates such as pots and the oviposition cages as well. It was concluded that gravid moth's oviposition choice may be overridden by the dispersal behaviour of larvae.

Keywords: *Spodoptera frugiperda*, push-pull strategy, trap crops, feeding preference, oviposition

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Chapter 1: Introduction and literature review

1.1. Introduction

The Fall armyworm (FAW), *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), is an agricultural pest originating in the tropical and subtropical environments of South America (Sparks, 1979). Two sympatric and morphologically identical strains of FAW exist. The strains are identified on the basis of feeding preferences, one population feeding on maize and sorghum and the other feeding on rice and forage grasses (Meagher *et al.*, 2004). Both strains cause major damage to crops.

Crop losses are caused by the larval stages of FAW. Yield losses commonly exceed 30% and it is not uncommon for an entire crop to be lost (Aguirre *et al.*, 2016). FAW may cause significant damage to crops such as maize, *Zea mays* L. (Poaceae), sorghum, *Sorghum bicolor* L. Moench (Poaceae) turf grass, rice, *Oryza sativa* L. (Poaceae) cotton, *Gossypium hirsutum* (Malvaceae) and groundnut (*Arachis hypogaea* L. (Fabaceae) (Perez-Zubiri *et al.*, 2016; Aguirre *et al.*, 2016). FAW is polyphagous and has been reported to have up to 274 different host plants. It does however prefer species of the grass family (Poaceae) as host plants (Silvain and Ti-A-Hing, 1985; Castro and Pitre, 1988; Montezamo *et al.*, 2018). Because of its wide host range, FAW is one of the most harmful pests that threaten annual crops in tropical regions (Mello da Silva *et al.*, 2015). Feeding on a wide range of host plants may be an important survival strategy for FAW, but this and the fact that it occurs over a large area from where it invades different regions, makes it difficult to control (Knipling, 1980; Ullah *et al.*, 2016). Control is further impeded by the different phenologies and growing seasons of the host plants. Host plants that grow in different seasons, yet in proximity to each other facilitate movement of FAW between crops (Mello da Silva *et al.*, 2015). This availability of different host plants may result in FAW populations with new or different food preferences, as seen with the occurrence of two different strains (Meagher *et al.*, 2004; Mello da Silva *et al.*, 2015).

1.2. Biology and Ecology

The FAW adults are nocturnal, emerging at dusk to feed, mate and oviposit (Sparks, 1979). Female moths sit near the top of the plant, extending their ovipositor to secrete a sex pheromone which attracts the males. Females can only mate once a night, but can mate for a number of nights. The female will typically oviposits her eggs on the underside of leaves, but in high densities eggs are oviposited all over the plant as well as other plants, window panes and flags, to name a few. The eggs are laid in clusters and are covered with a coating of scales for protection. The eggs hatch in 2-4 days when temperatures are between 21 – 26 °C (Sparks, 1979). The number of eggs laid varies and up to 2047 eggs per female have been reported (Castro and Pitre, 1988). Larvae that hatch consume the egg shells before continuing to feed on foliage (Sparks, 1979). The FAW lifecycle typically lasts four weeks in optimal warm temperatures (Sparks, 1979). Larval development can pass through as many as seven instars when reared on maize (Andrews, 1998). Due to their cannibalistic behaviour, only a few smaller larvae or one large larva is usually found per plant (Vilarinho *et al.*, 2011). Some larvae disperse from as early as 2-3 days old due to their cannibalistic behavior, but the majority of the larvae disperse round the 3rd instar (Rojas *et al.*, 2018). Once fully grown, the 6th instar moves to the ground and pupates in the soil (Sparks, 1979). At temperatures of 24 – 28 °C the pupal stage can last for 9 - 11 days (Sparks, 1979; Andrews, 1988). After emergence moths typically spend the first night feeding and do not mate (Sparks, 1979).

Fall armyworm, unlike some other lepidopteran pests such as the maize stemborer, do not undergo diapause (Sparks, 1986), and are typically found in tropical and subtropical regions where they infest crops year-round (Sparks, 1979; Knipling, 1980). The populations are sustained in these areas and spread to other areas during spring (Knipling, 1980). The size of the area occupied by FAW can be 10-20 times larger than the size of the original, overwintering area (Knipling, 1980). Moths are thought to migrate over long distances by being carried by the wind (Sparks, 1979; Knipling, 1980).

1.3. Host plant selection by insects

To qualify as a host plant, females need to be able to find and accept the plant for oviposition and the larvae must accept the plant as a host and must be able to fully develop on the plant when it is used as a food source (Saeed *et al.*, 2010; Henniges-Janssen *et al.*, 2014).

In response to tactile, olfactory and visual cues, insects will generally exhibit a preference for particular plant species, cultivars or crop stages (Shelton and Nault, 2004). Plant species and individual plants differ in their quality and suitability as hosts to insects and have evolved a diverse array of mechanisms and adaptations to limit the damage caused by herbivorous insects (Rausher, 1983; Cheruiyot *et al.*, 2017). This variation in plant quality has given rise to the discriminatory behavioral responses of insects (Rausher, 1983; Sauvion *et al.*, 2017). Host plant quality affects female life history traits and also larval growth and larval defense against natural enemies. The quality and quantity of plant tissues consumed during larval stages affect reproduction in females (Sauvion *et al.*, 2017).

Host selection can be described as a series of decisions made by the insect that ultimately lead to the acceptance or rejection of the plant as a host (Rausher, 1983). Host plant selection can be divided into the 'host plant finding' and 'host plant acceptance' phases (Finch and Collier, 2004; 2012). By planting non-host plants in the vicinity of the host plants, the additional diversity created disrupts the insects from selecting otherwise acceptable host plants. These disruptions are generally caused through a number of different mechanisms such as: 1) physical obstruction, 2) visual camouflage, 3) masking of host plant odors, 4) repellent chemicals or 5) the presence of non-host plants that alter the physiology of the host plant (Finch and Collier, 2004, 2012; Sauvion *et al.*, 2017).

Insects recognise and respond to chemical cues given off by plants (Calatayud *et al.*, 2008b; Cheruiyot *et al.*, 2017). This response is usually a multifaceted process involving visual and semiochemical stimuli over long distances and tactile and gustatory cues over short distances (Cheruiyot *et al.*, 2017; Sauvion *et al.*, 2017). After landing, information on plant suitability and quality is obtained through sensory cues. Female acceptance of the plant as a host is based on contact cues where both physical (e.g. pubescence and surface texture) and chemical (volatile and/or surface

chemicals) cues play a role (Calatayud *et al.*, 2008b). In order to make a successful host plant selection, certain behavioural and physiological adaptations of the insect must be met by the host plant (Henniges-Janssen *et al.*, 2014; Sauvion *et al.*, 2017).

There are a number of theories as to how a host plant is chosen by an insect. Perhaps the most common theory is the optimal oviposition theory, also known as the 'mother knows best' principle. In this theory it is assumed that juvenile life stages have little or no opportunity to change their developmental location. It is therefore up to the mother to locate a suitable host plant for the development of her offspring (Thompson 1988; Mayhew, 2001; Cheruiyot *et al.*, 2017). However, females may also choose hosts based on factors that affect their own survival rather than that of their offspring (Mayhew, 2001; Cheruiyot *et al.*, 2017). As plant species differ in their chemistry and physical traits, choice/preference tests can be conducted to determine the ability of the juvenile to feed, grow, survive and develop on different host plants (Cheruiyot *et al.*, 2017; Sauvion *et al.*, 2017).

Plants exhibit variation in their suitability as hosts for insects and evolved an array of mechanisms to limit the degree of damage caused by insect herbivores. These include direct defences such as toxin production, repellents and structural defences such as thorns and trichomes (Calatayud *et al.*, 2008a; Sauvion *et al.*, 2017; Cheruiyot *et al.*, 2018). The association that plants have with other surrounding vegetation is able to influence herbivorous insects, in some cases deterring the insect from the host plant. Defences against herbivore attack can be shared among plants growing within the vicinity of each other (Sauvion *et al.*, 2017).

Plants are capable of emitting a wide variety of volatiles. These volatiles are emitted from different plant organs such as the leaves, flowers, stem and roots. Functions of plant volatiles include activating defences in neighbouring plants, pollinator attraction or attraction of predators and parasitoids by herbivorous insects defoliating the leaves (Paré and Tumlinson, 1999; Sauvion *et al.*, 2017).

Insects, on the other hand recognise and respond to signals emitted by the plants to determine whether the plant is a species on which it can feed and reproduce (Cheruiyot *et al.*, 2017; Sauvion *et al.*, 2017). Insects use visual and semiochemical stimuli to find a host plant (Finch and Collier, 2012; Cheruiyot *et al.*, 2017; Sauvion *et al.*, 2017). Plants exude volatiles (semiochemicals) that act in the attraction or

repulsion of insect species (Showler, 2001; Midega *et al.*, 2005; Sauvion *et al.*, 2017), or harbour natural enemies of herbivorous insect species (Calatayud *et al.*, 2008a; Cheruiyot *et al.*, 2017; Sauvion *et al.*, 2017). These volatiles can be detected over long distances (McCall *et al.*, 1993; Sauvion *et al.*, 2017), but only when an insect comes within a few meters of the plant that volatile and visual cues will stimulate it to land on the plant (Finch and Collier, 2012). Differences in oviposition orientation on different host plants may be initiated by volatiles or chemical cues (Midega *et al.*, 2005; Saeed *et al.*, 2010; Sauvion *et al.*, 2017). Insects typically receive these volatiles by means of olfactory receptors located on the antennae (Sauvion *et al.*, 2017).

Maize, Napier grass, (*Pennisetum purpureum* Schumach. 1827) (Poaceae) and Sudan grass (*Sorghum vulgare* var. *sudanense*) (Poaceae) produce compounds that are attractive to female stemborer moths, and they are therefore more likely to choose these plants as oviposition sites (Midega *et al.*, 2005). Trichomes, nectaries, plant waxiness as well as various deterrents and attractants also play a role in the attraction or repulsion of female moths, inducing the preference for one plant over another (Showler, 2001; Sauvion *et al.*, 2017). Plants also release different volatile signatures during their different development stages. Therefore females may also be drawn to a particular life stage of the plant (Sauvion *et al.*, 2017).

1.4. Poaceous plants

Although FAW has a very large host range, poaceous plants are preferred over other plants (Table 1.1) (Silvain and Ti-A-Hing, 1985; Castro and Pitre, 1988). While lepidopteran pest species such as FAW, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) and *Eldana saccharina* (Walker) (Lepidoptera: Noctuidae) oviposit heavily on some grasses, only a few grass species are favourable for the completion of their life cycles (Songa *et al.*, 2002). The selection of a favourable host plant for larval development is crucial to the larvae's survival (Sauvion *et al.*, 2017; Calatayud *et al.*, 2008a).

Table 1.1: Important poaceous host plants of *Spodoptera frugiperda* relevant to Africa (Buntin 1986; Pashley, 1988; Andrews, 1998)

Species	Common name
<i>Avena sativa</i> L.	Oats
<i>Axonopus affinis</i> Chase	Carpet grass
<i>Brachiaria platyphylla</i> (Nash)	Signal grass
<i>Chloris gayana</i> Kunth	Rhodes grass
<i>Cynodon dactylon</i> (Pers.)	Bermudagrass
<i>Dactyloctenium aegyptium</i> (Beauv.)	Crowfoot grass
<i>Hordeum vulgare</i> L.	Barley
<i>Oryza sativa</i> L.	Rice
<i>Panicum maximum</i> Jacq.	Guinea grass
<i>Panicum miliaceum</i> L.	Broomcorn millet
<i>Panicum texanum</i> Buckl.	Texas millet
<i>Panicum purpureum</i> Schum.	Napier grass
<i>Phleum pratense</i> L.	Timothy grass
<i>Poa pratensis</i> L.	Kentucky bluegrass
<i>Saccharum officinarum</i> L.	Sugarcane
<i>Secale cereale</i> L.	Rye
<i>Sorghum sudanense</i> Hitch.	Johnson grass
<i>Sorghum vulgare</i> (Pers.)	Sorghum
<i>Triticum aestivum</i> L.	Wheat
<i>Zea mays</i> L.	Maize

1.4.1. Maize

Maize is one of the most important crops attacked by FAW. Plants are attacked during any of the growth stages, but younger plants are preferred for oviposition. It has been reported that maize is preferred by FAW larvae for feeding and by the moths for oviposition when a choice is given (Castro and Pitre, 1988). Larvae feed in the whorl of the maize plant where the young leaves are present as well as on reproductive parts, the ears and tassels (Vilarinho *et al.*, 2011). Maize growth stages vary in susceptibility to attacks by FAW. During the mid-vegetative growth stages, larvae can be found within the whorl of the plant where larval feeding causes tattering of developing leaves. Studies show that young maize plants are more susceptible to damage by FAW compared to older plants (Buntin, 1986).

Infestation of seedlings and plants during the early whorl stage can cause complete defoliation of the plants. If no damage occurs to the apical meristem, the plant can

tolerate complete defoliation (Buntin, 1986). However, if the apical meristem of the plant is damaged, it results in the modification of plant architecture through loss of primary and secondary tiller formation where no ears will develop, and can lead to seedling death. An infestation during late whorl stages may cause injury to the developing tassels (Buntin, 1986). Once the tassel emerges the larvae move to the developing ear. Larval feeding on silks reduces the pollination potential leading to a reduction in kernel numbers on the ear. Larvae may also feed on the ears itself causing direct damage to the product, or may feed at the base of the ear resulting in ear droppage (Buntin, 1986).

1.4.2. Sorghum

Sorghum is another important grain crop attacked by FAW. Plants in the vegetative stages are usually damaged, but FAW will also feed on soft grains in the panicle. Defoliation patterns are similar to those reported in maize. Young larvae are typically found on the expanded leaves while older larvae can be found inside the whorl. Similar to maize, different growth stages of sorghum vary in their susceptibility to FAW infestation. Defoliation of sorghum plants during the mid- to late-whorl stages resulted in significant losses in yield (Buntin, 1986).

1.5. Management strategies

1.5.1. Chemical control

Currently the use of chemical pesticides is the main method of FAW control (Ríos-Díez and Saldamando-Benjumea, 2011). Chemical pesticide use is often unpractical for small-scale farmers that are resource poor (Khan and Pickett, 2004). FAW is difficult to control by means of chemicals since the larvae tend to hide and feed within the whorl of the maize plant (Sparks, 1979; Vilarinho *et al.*, 2011). In addition, due to its short life cycle, FAW has the ability to quickly evolve resistance to pesticides (Yu *et al.*, 2003). The adverse effects of pesticides on human health and the environment are well known (Khan and Pickett, 2004).

1.5.2. Cultural control in agroecosystems

The biodiversity within an agroecosystem provides the farmer with services beyond the production of food and income (Altieri, 1999; Sauvion *et al.*, 2017). Other services provided by the biodiversity of agroecosystems include the recycling of nutrients,

microclimate control, hydrological process regulation, undesirable organism abundance regulation and noxious chemical detoxification (Altieri, 1999). Many of these services are lost due to the simplification of the environment in agroecosystems (Altieri, 1999; Crowder and Jabbour, 2014). This in turn can lead to significant economic and environmental costs. In agriculture, there is an increasing need to supply crops with external inputs such as fertilizers and insecticides due to the loss of components that regulate soil nutrients and pest control (Altieri, 1999). It is therefore important to enhance the functional biodiversity within the agroecosystem as a key strategy of maintaining stability in the ecosystem (Altieri, 1999; Finch and Collier, 2012; Crowder and Jabbour, 2014).

Traditional farming systems are now recognized by scientists as models of efficiency in crop management strategies that are more affordable for subsistence farmers (Altieri, 1999). The study of these strategies provides important guidelines for water-use efficiency, soil conservation, soil fertility management and pest control. The biodiversity found in agroecosystems greatly varies with the age, diversity, structure and management of these systems (Altieri, 1999). To maintain a desired biodiversity in agroecosystems, it is important to determine management practices that will ultimately encourage biodiversity components that are needed for ecosystem services to flourish (Altieri, 1999; Sauvion *et al.*, 2017) (Figure 1.1).

In a study conducted by Botha *et al.* (2017) in South Africa, higher numbers of invertebrates were found in maize fields as well as in the adjacent vegetation when there was an increase in the abundance of grasses (Poaceae). This suggests grasses are important for supporting arthropod diversity.

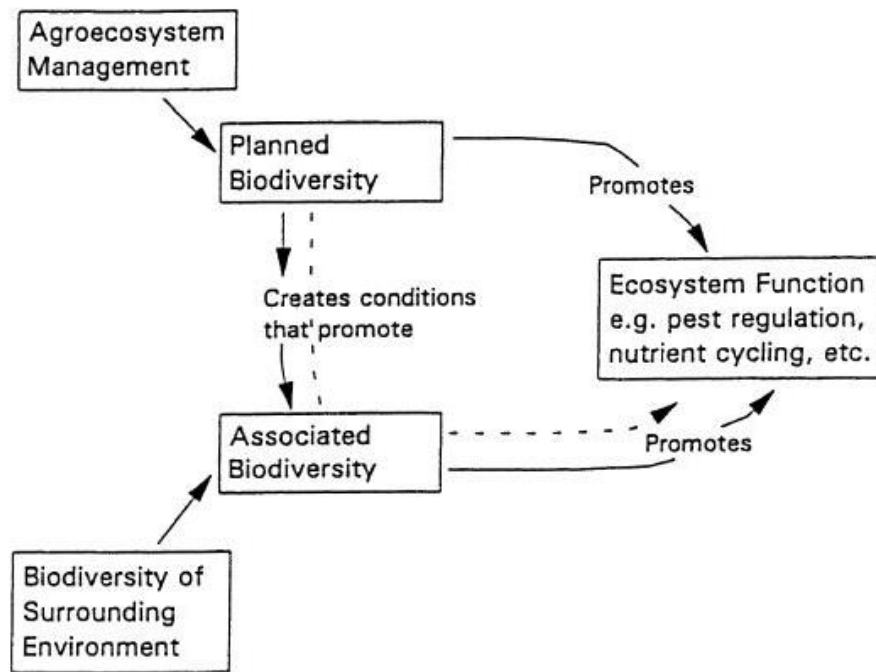


Figure 1.1: The relationship between planned biodiversity (determined by the farmer) and associated biodiversity (biodiversity that results from the nature of the surrounding environment) when attempting to promote optimum ecosystem functions (Altieri, 1999).

1.5.3. Cultural control in pest management

The assumption that diversity of an ecosystem fosters its stability has been largely debated by agricultural ecologists (Altieri, 1999). By planting vast areas of crop monocultures within agroecosystems, cause instability of the natural ecosystem and as a result insect pest problems are significantly increased (Altieri, 1999; Sauvion *et al.*, 2017). Agroecosystems that focus on providing for the needs of the human population become subject to heavy pest damage as a result of decreased biodiversity (Altieri, 1999; Sauvion *et al.*, 2017). Most studies conclude that when certain plant species are planted in conjunction with the primary host species of a specialized herbivore, the specialized herbivore abundance is lower than when compared to mono-cropping systems (Altieri, 1999; Finch and Collier, 2000, 2012). Many studies suggest that the larger the diversity within the agroecosystem and the longer the period that the diversity remains undisturbed, the greater the promotion of insect stability (Altieri, 1999; Botha *et al.*, 2017). Two non-mutually exclusive hypotheses exist to explain the reason for greater insect stability in more diverse environments (Finch and Collier, 2000, 2012). These hypotheses are: 1) The natural enemy

hypothesis which states that pest populations are decreased in more diverse ecosystems as the diverse ecosystem has a larger availability of resources for natural enemies and 2) the resource concentration hypothesis which states that due to increased diversity within a habitat, problem insect species have difficulty finding their host plants due to the chemical and/or physical confusion caused by the surrounding plants (Sauvion *et al.*, 2017).

Management strategies that exploit the interactions between insects and plants have been under investigation for many years (Knipling, 1980). The development of efficient control strategies requires knowledge of the biological relationships of insect species with different host plants (Greenberg *et al.*, 2001) and host plants may play a role in the spread, population increase and outbreaks of these insect species (Saeed *et al.*, 2010). When resources are limited, insects tend to make accurate judgements when it comes to host plant location, but as diversity increases this host plant finding becomes more and more inaccurate (Bernays, 2001). Experiments determining the favorability of different host plants may therefore be useful in designing practical management systems such as cultural control strategies using habitat management or push-pull systems and trap crops to manage and control FAW (Knipling, 1980; Saeed *et al.*, 2010; Midega *et al.*, 2018).

1.5.4. Push-pull systems

The term push-pull was first conceived by Pyke *et al.* (1987) who suggested this approach as a strategy for insect pest management. Push-pull strategies attempt to modify the environment and use a combination of behavior-modifying stimuli to lower pest populations while simultaneously creating a favorable environment for the populations of their natural enemies (Khan and Pickett, 2004; Cook *et al.*, 2007). For a push-pull system to be successful, an understanding of the pest's biology, behavioral and chemical ecology, and interactions with its host, natural enemies and conspecifics must be clearly understood (Cook *et al.*, 2007; Calatayud *et al.*, 2008a). The components of the push-pull system will change to suit the specific pest involved (Cook *et al.*, 2007).

Push-pull strategies developed to control insect pests aim to decrease their abundance on a protected resource i.e. the crop. Behavior-modifying stimuli used for push-pull strategies typically rely on visual and chemical cues or signals (Cook *et al.*,

2007; Finch and Collier, 2012). The aim of the push-pull strategy is to make the protected crop difficult to locate and unattractive or unsuitable for the pest (Cook *et al.*, 2007). Insect pests can be deterred (pushed) by stimuli from plant species that mask the host plant or by being repellent as indicated in Figure 1.2 (Finch and Collier, 2012; Calatayud *et al.*, 2008b). Both repellent and highly aromatic plants, such as marigolds (*Tagetes erecta* L.) act by disrupting the 'normal' sequence of insect behavior (Finch and Collier, 2012).

At the same time, the insect pest species can be attracted (pull) using highly attractive stimuli such as trap crops, where they become concentrated providing easy methods of elimination (Cook *et al.*, 2007; Calatayud *et al.*, 2008b; Finch and Collier, 2012;). Attractive odors released by the plant stimulate insect behavior by (1) causing the insect to turn into the wind (anemotaxis) and then flying upwind to the source of the odor and (2) causing the insect to land on a visually acceptable surface, such as a plant (Finch and Collier, 2012). This strategy makes use of non-toxic mechanisms of pest control and can therefore be used together with other population-reducing methods targeted at pests (Cook *et al.*, 2007).

Push-pull strategies use a combination of various pest management tactics to gain control of the insect pest. Behavioral manipulation primarily makes use of visual or chemical signals. Semiochemicals tend to have the most potential for the use in pest management. Habitat diversification strategies, such as trap cropping and intercropping, are behavioral manipulative methods that have become popular in pest management strategies (Hokkanen, 1991; Shelton and Badenez-Perez, 2006; Cook *et al.*, 2007).

Stimuli for push components include the following: 1) visual cues: these include the manipulation of the host colour and shape or size in the attempt to inhibit host orientation and acceptance by the insect pest. It is unpractical to try altering these aspects in a host plant, but by understanding how pests use visual stimuli, these aspects can at least be disrupted or minimized; 2) non-host volatiles: volatiles that are derived from non-hosts can be used to mask host odors and evoke avoidance and repellent behaviors in the pest species; 3) Host-derived semiochemicals: these play an important role in host recognition and are often very specific. Inappropriate ratios of host volatiles cause host-directed orientation by the insect to be terminated. Herbivore

induced plant volatiles are produced by plants in response to herbivore attack and can either deter other potential herbivores by indicating competition or attract the natural enemies of the herbivore and 4) Oviposition deterrents: These are synthetic compounds or are produced by plants and ultimately prevent a host from being oviposited on. They encourage females to avoid laying their eggs on a host plant by communicating that the host had been previously oviposited upon, the female will try avoiding intraspecific competition and therefore avoiding the plant (Figure 1.2) (Cook *et al.*, 2007).

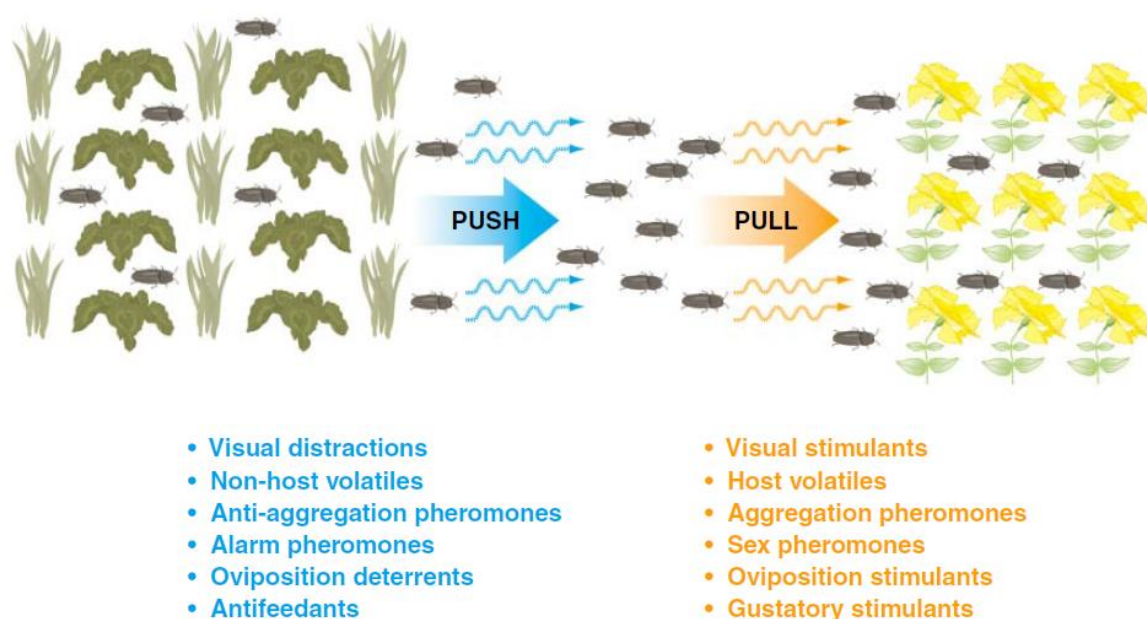


Figure 1.2: A diagrammatic representation of the generalised mode of action of the push-pull strategy (Cook *et al.*, 2007).

1.5.5. Trap cropping

The main principle of trap cropping rests on the knowledge that almost all insects tend to prefer certain plant species over other plant species, cultivars or crop stages (Hokkanen, 1991; Shelton and Nault, 2004; Van den Berg, 2006b). These preferences can be manipulated for the use of insect control in the form of trap cropping. Trap cropping is usually composed of one or more plant species that are grown to attract pest insects away from the main crop in the attempt to protect the main crop (Shelton and Nault, 2004). Trap cropping falls into the category of habitat manipulation of an agroecosystem for the purpose of pest management. These manipulations can fall into the categories of within crop, within farm or landscape level (Shelton and

Badenez-Perez, 2006). In agricultural pest management, trap cropping has the potential to reduce crop damage while simultaneously reducing the use of conventional pesticides (Keeping *et al.*, 2006; Holden *et al.*, 2012). Trap cropping was a common method of pest management prior to the introduction of synthetic pesticides (Shelton and Badenez-Perez, 2006; Cheruiyot *et al.*, 2017). The cost of pesticides combined with the number of treatments required can result in trap cropping simply being a much more cost effective way of coping with insect pests (Hokkanen, 1991). Another motivation for using trap crops is that insect pests are able to quickly evolve resistance to commonly used pesticides. By using trap crops, the main crop seldomly needs to be treated with insecticides (Hokkanen, 1991).

Trap crops are plants that are grown in close proximity to the main crop to attract insect pests away from the main crop (Hokkanen, 1991; Shelton and Nault, 2004). Trap crops may be planted according to the spatial pattern of the main crop across the landscape. Factors such as vegetation distribution, shape, size, configuration and type also play a role in trap crop parameters (Shelton and Badenez-Perez, 2006). In perimeter trap cropping, trap crops are planted around the border of the main crop. This allows the outer borders to become infested with insect pests where they can be controlled using pesticides (Shelton and Badenez-Perez, 2006). Sequential trap cropping involves trap crops that are planted earlier or later than the main crop in order to enhance the effectiveness of the trap crop against the target pest (Shelton and Badenez-Perez, 2006). Multiple trap cropping makes use of different trap crops planted together in order to control different species of insect pests or, alternatively, to effectively control one insect pest species. This method combines the growth stages of the different plant species that are attractive to the pest at different times to successfully control it (Shelton and Badenez-Perez, 2006). Push-pull trap cropping or “stimulo-deterrent diversion” uses a combination of a trap crop (the pull component) and a repellent intercrop to divert the pest away from the main crop (Shelton and Badenez-Perez, 2006).

Trap crops can be plants of a preferred growth stage, cultivar or species that are more attractive to the pest species than the main crop (Hokkanen, 1991; Cook *et al.*, 2007). Trap crops may be manipulated to attract the insect pest at critical stages in the phenology of the pest or crop (Shelton and Nault, 2004). In plant-based systems, vegetation diversification such as intercropping and trap cropping, can be used as a

natural means of exploiting plant stimuli (Keeping *et al.*, 2006; Cook *et al.*, 2007). In order for the trap crop to be successful, it should be more favorable to the insect pest than the protected crop for most of the growing season (Hokkanen, 1991; Zhou *et al.*, 2010). This can be achieved by using plants that are a more preferred species, or more preferred cultivar, than the main crop. Both the main crop and the trap crop should be grown at the same time (Hokkanen, 1991). The chosen crop must act as a sink for the target pest, while meeting the following two requirements: 1) the pest must be attracted to the trap crop and 2) the trap crop must retain the insects after they arrive (Holden *et al.*, 2012). Protection of the main crop is achieved either by preventing insect pests from reaching the main crop or by concentrating the insect pests on the trap crop where they can be controlled (Hokkanen, 1991). Plants that are highly attractive to pests but are unsuitable for their larvae to survive upon can be used as dead-end trap crops (Shelton and Badenez-Perez, 2006; Cook *et al.*, 2007). Dead-end trap crops serve as a sink for pests, preventing their dispersal from the trap crop to the main crop as the season progresses (Shelton and Badenez-Perez, 2006).

Intercropping reduces pest density within crops by disrupting host location. In addition, an enhanced natural enemy abundance due to vegetation diversification may lead to increased herbivore mortality due to predation and parasitism (Hokkanen, 1991; Shelton and Nault, 2004; Cook *et al.*, 2007). Some plants are able to emit secondary metabolites that directly mask the cues herbivorous insects use to locate host plants. On the other hand, the cues emitted by the host plant in response to herbivory may successfully attract natural enemies of the insect pest. Both of these phenomena enable the utilization of these plants as successful trap crops for insect pest species (Cheruiyot *et al.*, 2017). Most host plants can function as a trap-crop provided it is grown within the vicinity of the main crop. Insects that fly over host trap-crops will be stimulated to land and oviposit. These alternative host plants used as trap crops work as interception barriers, ensuring that the number of eggs oviposited on the main crop is reduced (Finch and Collier, 2012). But insects have also adapted to make use of the most abundant host plant even if it not the most preferred. As a result, even when trap-crop plants are more favorable and grown within the vicinity of the crop, because the crop is more abundant it will still be targeted by the insect pest (Finch and Collier, 2012). Trap-crops should ideally be planted around the main crop so that they can act as a barrier. When a crop field is too large, even when a trap-crop is planted around

the field, it cannot adequately prevent insect pest species from reaching the main crop. The trap-crop 'barrier' would have to be very wide to produce measurable effects, and this is not always practical (Finch and Collier, 2012). In Kenya, trap cropping against lepidopteran stemborers with Napier grass is successful because the maize fields (the main crop) are small. Therefore, Napier grass can easily be planted around the maize field and as an added benefit, Napier grass grows taller than maize, acting as a barrier (Finch and Collier, 2012).

Few trap crops alone provide adequate pest management (Keeping *et al.*, 2006; Holden *et al.*, 2012) and work more effectively when used together with pesticides in an IPM system (Shelton and Badenez-Perez, 2006; Holden *et al.*, 2012). The potential of using trap crops alone, however, has been demonstrated in many glasshouses and particularly when it comes to poaceous crops (Keeping *et al.*, 2006). In Africa, it has been reported that lepidopteran stemborers such as *B. fusca* and *C. partellus*, show preference for gramineous plants (Van den Berg, 2006ab; Khan *et al.*, 2007; Midega *et al.*, 2010). Napier grass, Vetiver grass, Sudan grass (*Sorghum vulgare* var. *sudanense*) and Brachiaria grass have been recommended as trap plants around maize fields for stemborers in east and southern Africa (Shelton and Badenez-Perez, 2006; Van den Berg, 2006ab; Khan *et al.*, 2007; Midega *et al.*, 2010; Cheruiyot *et al.*, 2018).

1.6. Stemborers and cultural control in eastern and southern Africa

Host plant recognition and selection is primarily conducted by gravid female Lepidoptera, and as newly hatched larvae are often limited in their dispersal capabilities, oviposition is important because it determines the survival of their progeny. It is therefore important for gravid female moths to determine which plants are acceptable for oviposition and feeding. The process of determining which plants are suitable host plants is governed by interactions based on sensory cells and physical and chemical characteristics of the plant (Sauvion *et al.*, 2017).

Lepidopteran stemborers feed on the inside of monocotyledonous plants belonging to Poaceae, Cyperaceae and Typhaceae. In east and southern Africa, lepidopteran stemborers typically attack cereal crops (Le Ru *et al.*, 2006, 2014). In sub-Saharan Africa poaceous crops are grown in small plots surrounded by wild grasses which are

alternative hosts for the lepidopteran pests (Khan *et al.*, 2007; Moolman *et al.*, 2014; Le Ru *et al.*, 2006, 2014). It has previously been recommended that these grasses should be removed and destroyed in order to reduce stemborer infestation on the main crop, but recent studies have shown that these grasses have potential to act as trap plants. Some grasses and legumes have been shown to repel gravid moths, allowing researchers to develop a stimulo-deterrent or push-pull approach to stemborer control (Khan *et al.*, 2007). Trap crops and push-pull systems can be used as an alternative or in addition to pesticide treatments (Cook *et al.*, 2007).

In the push-pull system, stemborer species are repelled from crops by repellent non-host intercrops, namely Molasses grass, Silverleaf desmodium or Greenleaf desmodium (*Desmodium intortum*), and are concentrated on attractive trap plants such as Napier grass, Vetiver grass or Sudan grass (Muyeko *et al.*, 2003; Khan and Pickett, 2004; Van den Berg, 2006a, b; Cook *et al.*, 2007; Khan *et al.*, 2007; Cheruiyot *et al.*, 2017). These wild grasses act as trap plants for attracted gravid female moths but prevent the development of their larvae, therefore providing a natural control (Muyeko *et al.*, 2003).

It has been reported by Midega *et al.* (2005), that a push-pull system developed for lepidopteran stemborers, namely *C. partellus* and *B. fusca*, also resulted in decreasing the numbers of FAW on maize. Greenleaf desmodium was used as a deterrent and planted in-between maize, while *Brachiaria* cv Mulato II was planted as a trap-crop around the maize field. The trap plants have been shown to be unsuitable for the development of FAW larvae resulting in high mortality rates. There is still a need to explain the mechanism behind this method of FAW control to allow for the optimization of this strategy (Midega *et al.*, 2007).

Specific effects of varieties of plant species available in southern Africa to be used as push-pull crops are unknown. It is therefore necessary to conduct choice test experiments to assess the efficiency of local available plant species to be used in push-pull systems or trap crop systems for the control of FAW.

1.6.1. *Brachiaria* grasses

Species of the genus *Brachiaria* (Poaceae) are extensively grown as pasture crops in tropical Latin America and Africa. Although there are over 100 species in the genus, only a few have been commercially exploited. Signal grass (*Brachiaria brizantha*)

(Hochst. Ex A. Rich) has been adopted in combination with desmodium as part of a push-pull strategy used to control cereal stemborers. Studies show that cereal stemborer moths such as *C. partellus* preferred *B. brizantha* for oviposition when compared to maize (Cheruiyot *et al.*, 2017). The cultivar Mulato II supports minimal feeding and therefore larval survival of *C. partellus* (Cheruiyot *et al.*, 2017).

In a study conducted by Cheruiyot *et al.* (2017), female *C. partellus* moths preferred *B. brizantha* cv. Marandu, *B. brizantha* cv. Piata and *B. brizantha* cv. Xaraes for oviposition when compared to maize. It has been suggested by Cheruiyot *et al.* (2017), that the preference for these *Brachiaria* species may in part be due to the difference in trichome density compared to the trichome density of maize. Trichomes provide the plant with structural resistance or chemical resistance, in the form of glandular trichomes, repelling attacking insects or restricting their movements (Cheruiyot *et al.*, 2017). In studies conducted with *B. fusca*, rough and pubescent surfaces made it difficult for the female moths to sweep and insert their ovipositors therefore prohibiting oviposition (Calatayud *et al.*, 2006).

The study conducted by Cheruiyot *et al.* (2017) indicated that *C. partellus* larvae however, tended to prefer leaves of maize rather than *Brachiaria* spp. The non-preference for *Brachiaria* spp. by the larvae may be due to chemical and/or physical characteristics and/or poor nutrient quality of the plant, causing the larvae to disperse away from the plant. Even though *Brachiaria* spp. have lower densities of trichomes than other host plants, they still did not support feeding of *C. partellus* larvae, suggesting that there are other factors affecting larval feeding preference. Larval mortality on *Brachiaria* spp. has been documented to be very high, and as a result *Brachiaria* can act as a sink for *C. partellus*. Therefore, *Brachiaria* can be considered a “dead-end” trap crop (Cheruiyot *et al.*, 2017).

In Africa, *B. brizantha* cv. Mulato II is a highly drought-resistant fodder crop used in conjunction with *Desmodium* spp. in push-pull agroecosystem management systems (Cheruiyot *et al.*, 2018). It has been documented that FAW will occasionally feed on *Brachiaria* species (*Brachiaria platyphylla* (Nash)) growing in close proximity to maize (Pashley, 1988).

1.6.2. Napier grass

Napier grass (*P. purpureum*) is an alternative host for stemborers such as *B. fusca*, *C. partellus*, *S. calamistis* and *E. saccharina* (Songa *et al.*, 2002; Moolman *et al.*, 2014). Napier grass is a host plant that is as attractive to lepidopteran stemborers and is used by resource-poor farmers in Africa. A wide variety of grasses exist and it is easy to obtain and use in stemborer management systems (Midega *et al.*, 2005; Van den Berg, 2006b). In previous studies on maize crops surrounded by Napier grass, these maize crops had lower incidences of stemborer oviposition compared to maize monocrops (Midega *et al.*, 2005; Van den Berg, 2006a). Napier grass releases large amounts of electrophysiologically active green leaf volatiles at the time when most oviposition occurs (scotophase), therefore is more attractive to ovipositioning moths (Cook *et al.*, 2007). In addition, Napier grass acted as a barrier to colonizing female moths as the grass is taller than maize (Midega *et al.*, 2005; Finch and Collier, 2012). When phytophagous insects encounter high vegetation, such as Napier grass, they tend to either turn back or fly over the barrier. Therefore, Napier grass can also be a physical obstacle between stemborer moths and maize fields (Finch and Collier, 2012). It has been observed that stemborer larval survival on Napier grass is poor, this has been attributed to the sticky sap produced by Napier in response to predation (Khan *et al.*, 2000; Songa *et al.*, 2002; Van den Berg, 2006a; Cook *et al.*, 2007). This sticky sap restricts larval development, causing only a few larvae to survive (Cook *et al.*, 2007). It has also been observed by Van den Berg (2006a) that the migration of *C. partellus* larvae to the whorl was severely hampered by the trichomes present on Napier grass. Napier grass, however is not always suited to farming conditions in low rainfall areas (Van den Berg, 2006a).

1.6.3. Vetiver grass

Vetiver grass (*Chrysopogon zizanioides* (L.) Roberty = *Vetiveria zizanioides* (L.) Nash (Poales: Poaceae) is a species that has been more commonly used as a soil erosion management tool as well as for sustaining agricultural productivity (Van den Berg, 2006b; Lu *et al.*, 2017). As a result, Vetiver is easily accessed and available in many African countries, making it a strong candidate as a possible trap crop or 'pull' plant in lepidopteran stemborer management systems (Van den Berg, 2006b). It has been reported in China that Vetiver is often damaged by *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) (Van den Berg, 2006b; Lu *et al.*, 2017). It was also reported

that stemborer larval mortality on the Vetiver was high despite being an attractive plant for adult female moths to oviposit on; this indicates that Vetiver has the potential to be a trap crop (Van den Berg, 2006b; Zheng *et al.*, 2009).

1.6.4. Maize

Maize can be used as a pull crop for FAW when other crops are grown for their economic importance (Rhino *et al.*, 2014). Maize can be intercropped with beans in order to reduce FAW damage to beans (Mensah and Sequeira, 2004). Alternative cover crops that are poorer host plants for FAW may reduce migrating populations (Meagher *et al.*, 2004). Choice of host plant can also play a role in the susceptibility of FAW to different insecticides (Wood *et al.*, 1981).

1.7. Aim of the study

The aim of this study was to evaluate the oviposition and feeding preference of *Spodoptera frugiperda* on selected poaceous host plants including maize, sorghum, Napier grass, *Brachiaria* spp and Vetiver grass for inclusion in a Push-Pull strategy.

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Chapter 2: Gas chromatography of selected poaceous plants and electroantennogram responses of *Spodoptera frugiperda* moths

Abstract

Plant volatiles play an important role in plant-insect interactions. Plants emit many volatiles with varying compositions and ratios of emissions dependent on the phenology of the plant and time of day. Female moths use various sensory cues to locate, select and accept suitable host plants. Volatile compounds were collected via entrainment of intact plants (maize, sorghum, Napier, Brachiaria and Vetiver). The collected volatiles were analysed by means of gas chromatography (GC) and three main components occurring in all four plant species were identified viz. decanal, squalene and limonene. Electroantennography (EAG) was used to evaluate and record the olfactory responses of Fall armyworm moths. Antennae were exposed to the three compounds identified with GC. Maize and Napier emit more volatiles during the evening. Since FAW moths respond to an array of volatiles, gravid female moths may not profoundly respond to specific volatile cues. It was concluded that gravid moth's oviposition choice may be overridden by the dispersal behaviour of larvae. From this study, gravid FAW moths do not seem to rely on volatile detection and tactile cues may play a larger role in host preference. This study indicated the need for further studies of FAW moth and larval sensitivity to plant volatiles for host plant location and selection.

Key words: Fall armyworm, host location, kairomones, oviposition

2.1. Introduction

Millions of people rely on the production of maize, *Zea mays* L. (Poales: Poaceae) and sorghum, *Sorghum bicolor* Moench. (Poales: Poaceae), as food crops in sub-Saharan Africa (Chamberlain *et al.*, 2006; Midega *et al.*, 2018). It is therefore important to understand the mechanisms used by herbivorous insects to recognize and find suitable hosts. It has ecological, evolutionary and economic significance as herbivorous insects play vital roles in ecosystem functioning by mediating transfer of energy and nutrients (Schmitz, 2008), while also competing with humans for food (Piementel, 2004). Fall armyworm (FAW), *Spodoptera frugiperda* Smith (Noctuidae), is an economically important pest on maize and other poaceous crops in Africa (Midega *et al.*, 2018). In the first year after the pest was reported in Africa, the cost of losses caused by FAW to maize, sorghum, rice and sugarcane in Africa were estimated at approximately \$13 383 (Abrahams *et al.*, 2017). FAW is polyphagous and has been reported to have up to 274 different host plants, but prefers species of the grass family (Poaceae) as host plants (Silvain & Ti-A-Hing, 1985; Castro & Pitre, 1988; Montezano *et al.*, 2018).

A cultural control mechanism, the push- pull system, which is based on the use of plant volatiles to control the lepidopteran stemborers, *Busseola fusca* Fuller (Noctuidae) and *Chilo partellus* (Swinhoe) (Crambidae), had been developed for Africa. The system involves the use of a trap crop (pull component) surrounding the cash crop field and a repellent intercrop (push component) in-between the rows of the cash crop. A number of wild grasses such as Vetiver grass, *Vetiveria zizanioides* (L.) Nash (Poales: Poaceae), *Brachiaria* spp. and Napier grass, (*Pennisetum purpureum* Schumach. 1827) (Poaceae) have been used as “pull” crops, attracting gravid stemborer moths, in the system with *Desmodium* spp (*D. uncinatum* or *D. intortum*) acting as the push component (Chamberlain *et al.*, 2006; Khan *et al.*, 2006; Khan *et al.*, 2007; Midega *et al.*, 2018). This method of stemborer control relies on attractant semiochemicals released by ‘pull’ plants and repellent semiochemicals released by the ‘push’ plants (Khan *et al.*, 2000).

Accurate host plant location is a crucial ability for an insect to ensure that its nutritional requirements are met and that a suitable oviposition site is found. Many insects have the ability to locate their host plants amongst an array of other plants. Plant volatiles

play an important role in an insect's ability to locate a suitable host (Bruce *et al.*, 2005; Calatayud *et al.*, 2014). The majority of phytophagous insects use a mixture of ubiquitous volatile compounds, rather than unique, host-specific compounds in the host finding process (Bruce *et al.*, 2005; Raguso 2008).

Many phytophagous insects rely on their antennae to identify volatile cues to find food, mates and suitable oviposition sites (Lu *et al.*, 2017). Female moths use various sensory cues to locate, select and accept suitable host plants. Before landing, olfaction plays an important role when it comes to locating and recognising a suitable host plant. Insects show different sensitivities to host semiochemicals throughout the day. Oviposition by gravid female moths generally occurs during scotophase or at dusk (Sparks, 1979; Chamberlain *et al.*, 2006). This could be an adaption to take advantage of the release of volatile cues during this time (Chamberlain *et al.*, 2006). Specific volatile compounds which are found accross different potential host plants of FAW could indicate why female moths choose to oviposit their eggs on maize, sorghum, and potentially on other plants such as Napier grass, Vetiver grass or Brachiaria grass. Findings will provide a reference for further research aimed at understanding the chemical ecology of FAW.

2.2. Materials and methods

Insects

Spodoptera frugiperda larvae were collected on maize from various areas in South Africa during 2018 and used to establish a rearing colony. Rearing was done in a rearing chamber at 26.5 ± 1 °C, RH $65 \pm 5\%$ and 14L:10D photoperiod. Larvae were reared individually in plastic containers (52 mm high and 30 mm in diameter) with a mesh infused lid and provided with fresh maize leaf tissue every two days. Once the moths emerged from the pupae, they were sexed and placed into 2 L plastic bottles containing moist crusher stones, cotton wool with a sugar water solution and a piece of maize consisting of the whorl and leaves as an oviposition substrate following the methods described by Kruger *et al.* (2012). Eggs were collected daily and placed in small plastic containers (52 mm high and 30 mm in diameter) which were kept in a glass desiccator (150 mm diameter) in which RH was maintained at $70 \pm 5\%$ using a

potassium hydroxide solution according to the method of Solomon (1951). The desiccators were also kept in the same rearing chamber as described above until neonates hatched from these eggs.

Plants

Maize (Cultivar: DKC 80-10), sorghum (cultivar: NS 5511) and *Brachiaria brizantha* (Cultivar: Marandu) seeds were planted, while Napier grass and Vetiver grass were propagated from cuttings. All plant species were planted in 2 L pots and watered daily.

2.2.1. Headspace volatiles from intact plants

Headspace volatile samples were collected via entrainment of intact plants. Headspace volatiles accumulated for two hours in aluminium bags placed over the leaves, and were then drawn over 197 mg Tenax TA[®] adsorbent (Marks International Ltd., Pontyclun, UK) in stainless steel tubes. Volatiles from maize, sorghum, Brachiaria, Napier and Vetiver were collected between 21:00 and 23:00 pm. In addition, volatiles were collected from maize and Napier between 12:00 and 14:00 pm. Three different plants were used for each of the different plant species, including maize and Napier during the day, giving a total of 21 entrainments.

Analysis of the volatiles: Headspace volatiles were desorbed with a thermal desorption unit (Unity, Markes International Ltd., Pontyclun, UK) as described by Augustyn *et al.* (2010). The volatilised sample was transferred to an Agilent gas chromatograph (model 6890 N, Chemetrix, SA) coupled to a model 5975B mass selective detector (Augustyn *et al.*, 2010). Compounds were identified with the aid of a NIST version 5 library and the identities were verified by analysis of authentic reference standards (Sigma- Aldrich, UK). Terpenes were separated on a Phenomenex Zebron ZB-5 column (cross-linked 5% phenyl polymethylsiloxane) 30 m x 0.25 mm i.d. x 0.25 µm film thickness, using the instrumental conditions described by Augustyn *et al.* (2010).

2.2.2. Electroantennogram analyses

Three of the identified headspace volatiles were evaluated with respect to any antennal activity of female *S. frugiperda* moths using a GC-FID-EAG system. Odor standards were prepared by diluting pure compounds in hexane (Chromasol, Sigma-Aldrich, Steinheim, Germany). For each compound to be tested, in the EAG setup, 10

μl of the compounds were separately diluted with 1.5 ml hexane. The following chemicals were used: (Decanal, Squalene and Limonene) (all from Sigma-Aldrich, Steinheim, Germany). The purity of the commercially available chemicals ranged from 92 to 99%.

GC analyses were carried out on a Varian 3800 (Varian Inc., Walnut Creek, CA, USA) and a CP 8400 auto sampler. The respective peaks were recorded and integrated with Star Chromatography Workstation ver. 6.3 software. Analysis were performed using a Zebron ZB-5 fused silica capillary column (5% phenyl - 95% dimethylpolysiloxane, 30 m x 0.53 mm i.d. x 1.50 μm film thickness) (Phenomenex, Torrance, CA, USA) and simultaneously detected with a flame ionising detector set at 300 °C and an electroantennographic detector (EAG) (Syntech, Hilversum, The Netherlands). Helium was used as carrier gas and the column flow was set at 5.0 mL.min⁻¹. The oven temperature was programmed to start at 60 °C for 5 minutes and increased to 280 °C at a rate of 20 °C.min⁻¹. It was held at this temperature for 10 minutes. Two microliter of each standard was injected in a splitless mode with the injector temperature set at 300 °C.

A Y-shaped GlasSeal capillary tubing connectors (Supelco, Bellefonte, Pennsylvania) were used to divide the effluent of the column between the two detectors. The individual gas flow streams were transported using two pieces of deactivated fused silica capillary (0.3 m x 0.53 mm ID) leading to the FID and the EAG. The EAG system was based on an EAG interface with an IDAC-2 signal acquisition interface and a CS-55 stimulus controller (Syntech, Hilversum, The Netherlands). The outflow of the column was mixed with cold humidified air (600 ml/ min).

2.3. Results

2.3.1. Headspace volatiles from intact plants

All plant species evaluated emitted volatiles during the evening. Plant volatile emissions were generally very similar in type and composition (Figure 2.1). Maize and Napier released many more compounds during the day than during the evening (Table 2.1). The general trend indicated that larger volumes of volatiles are released during the evening despite there being fewer compounds (Figures 2.2 and 2.3).

6-Methyl-5-hepten-2-one was only released by maize during the day. Limonene and 1-hexadecanol (CAS) were released by maize and sorghum during the evening. Alpha-zingiberene was released by maize during the day and evening. Butanoic acid, butyl ester (CAS) was released by maize, sorghum and Brachiaria in the evening. N-octanal was released by sorghum in the evening and maize during the day as well as the evening. 3-Hexen-1-ol, acetate, (Z)-(CAS) was released by Brachiaria during the evening. Nonanal and decanal were released by maize and Napier during the day and evening, and by sorghum, Brachiaria and Vetiver during the evening (Table 2.1).

Table 2.1: Compounds of headspace volatiles of different plants species. All components were emitted by plants nocturnally unless stated otherwise.

Plant volatile components	Maize	Sorghum	Brachiaria	Napier	Vetiver	Maize Day	Napier Day
6-Methyl-5-hepten-2-one						✓	
Octadecanoic acid (CAS)						✓	
Limonene	✓	✓					
1-Hexadecanol (CAS)	✓	✓					
10-Demethylsqualene		✓		✓			
Alpha-zingiberene	✓					✓	
Butanoic acid, butyl ester (CAS)	✓	✓	✓				
N-octanal	✓	✓				✓	
3-Hexen-1-ol, acetate, (Z)-(CAS)			✓	✓			✓
Geranyl acetone		✓				✓	✓
1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS)		✓				✓	✓
Hexadecanoic acid (CAS)		✓	✓		✓	✓	✓
Squalene	✓		✓		✓	✓	✓
Nonanal (CAS)	✓	✓	✓	✓	✓	✓	✓
Decanal	✓	✓	✓	✓	✓	✓	✓

“✓” indicates presence of volatiles

Abundance

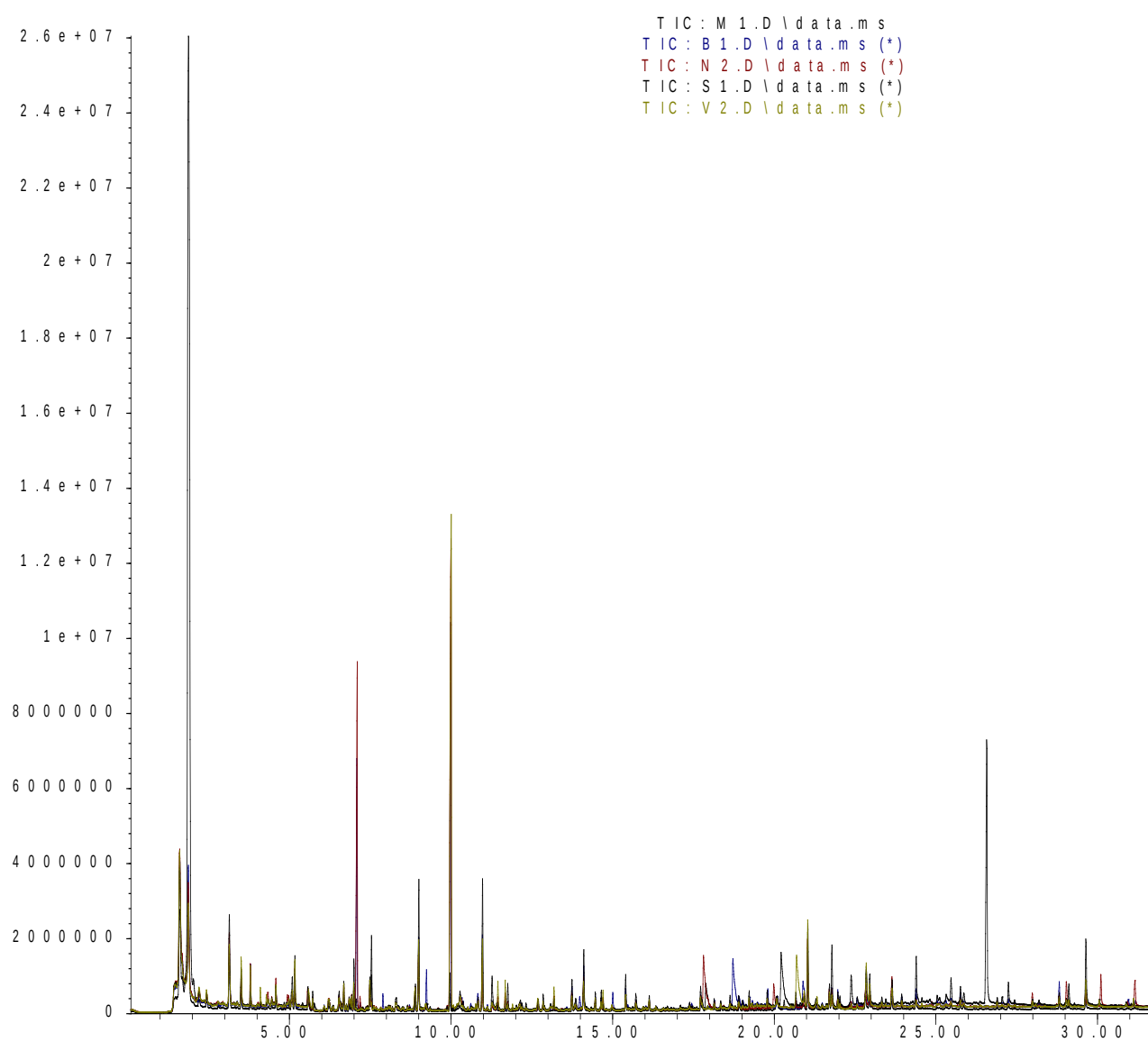


Figure 2.1: Gas chromatography of nocturnal chemical headspace profiles of maize, sorghum, Brachiaria, Napier and Vetiver. Black = maize; blue = Brachiaria; red = Napier; black = sorghum; yellow = Vetiver.

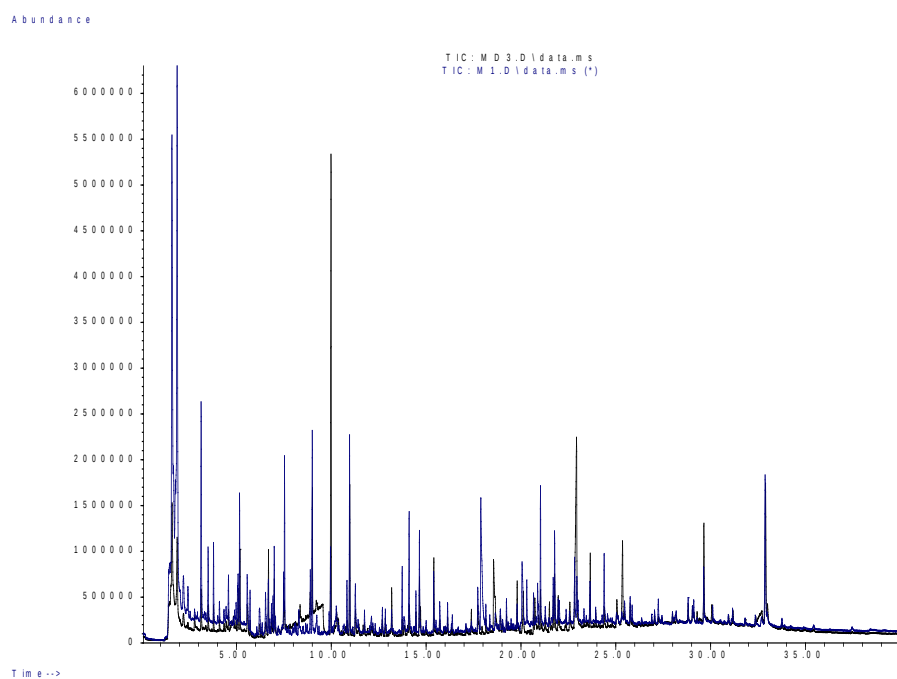


Figure 2.2: Gas chromatography of headspace volatiles emitted by maize nocturnally compared to headspace volatiles emitted diurnally. Black = maize diurnal; blue = maize nocturnal.

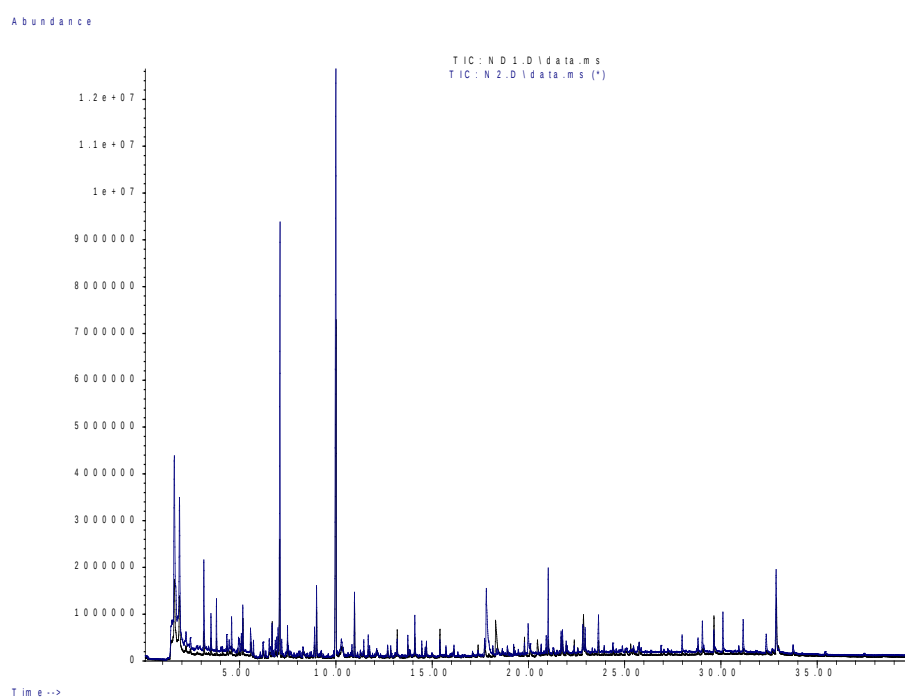


Figure 2.3: Gas chromatography of headspace volatiles emitted by Napier nocturnally compared to headspace volatiles emitted diurnally. Black = Napier diurnal; blue = Napier nocturnal.

2.3.2. Electroantennogram analysis

Three out of the four moths showed reactions at 700.0 s and 900.0 s (Figure 2.4).

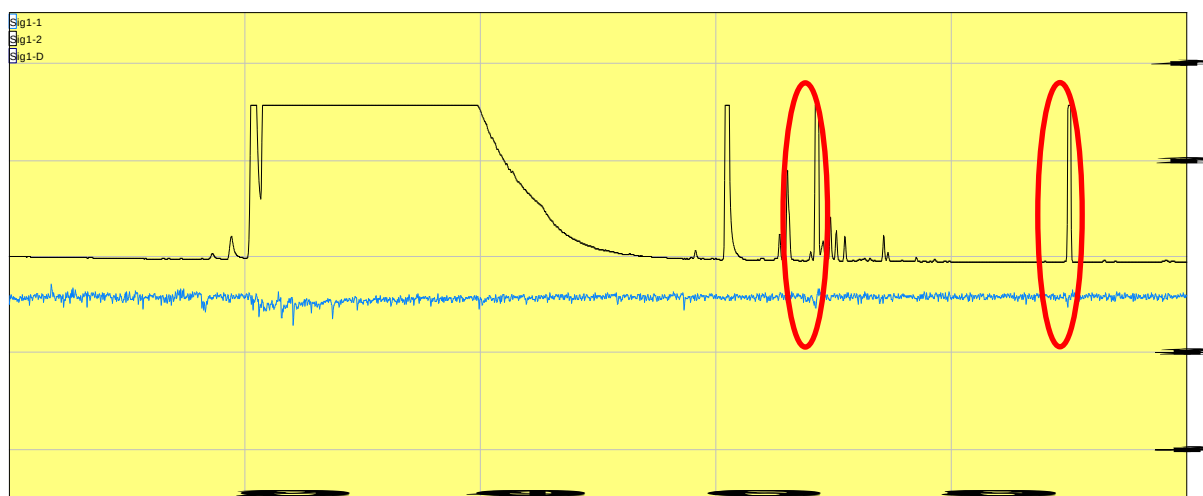


Figure 2.4: Electroantennography of *Spodoptera frugiperda* reactions to Decanal (700 s), Limonene (900 s), and Squalene (900 s).

2.4. Discussion

Plants are able to synthesize a large diversity of volatile organic compounds (VOCs). VOCs facilitate the plants interaction with its environment. VOCs can attract pollinators, act as seed dispensers and protect the plant from herbivores, parasites and pathogens (Dudareva *et al.*, 2013). Based on their biosynthetic origin, VOCs can be divided into several groups, including terpenoids, phenylpropanoids and fatty acid derivatives (Dudareva *et al.*, 2013).

N-octanol and decanal are aldehydes. Aldehydes in plants contribute to the aroma and flavour of numerous edible plants. Some forms of aldehyde aid the plant as defence mechanisms when it undergoes microbial attack (Trombetta *et al.*, 2002).

Alpha-zingiberene is a terpenoid (more specifically a sesquiterpene) (Koo and Gang, 2012). Isoprenoids, or terpenoids, are the largest family of natural compounds consisting of more than 40 000 different molecules. The terpenoid biosynthetic pathway generated both primary and secondary metabolites that play an essential role in plant growth and survival (Aharoni *et al.*, 2005). Geranyl acetone belongs to a group of terpenoid flavour volatile compounds that are generally present at low levels in plants. This group of terpenoids contributes to the flavour of plants. A reduction in

geranyl acetone leads to a reduction in flavour (Simkin *et al.*, 2004). FAW antennae reactions in this study showed a reaction to limonene, which is classified as a terpenoid, and concurs with the study by Malo *et al.* (2004). Although not tested in this study, hexan-1-ol (classified as a green leaf volatile) also elicited a response in the study by Malo *et al.* (2004).

Volatile fatty acid derivatives are a group of VOCs that include 1-hexanal, cis-3-hexanol, nonanal and methyl jasmonate, which arise from C₃ fatty acids, linoleic or linolenic (Dudareva *et al.*, 2013). Green leaf volatiles are produced by linolenic and linoleic acids through the lipoxygenase pathway. When the plant is damaged, these components are liberated from the cell membranes. They are then converted via hydroperoxide lyase enzymes to hexanal and (E)-2-hexenal. These then undergo further reactions to give other C₆ aldehydes, alcohols and esters (Chamberlain *et al.*, 2006). This same pathway occurs when conditions change from light to dark. Although the change from light to dark does not physically damage the plant, it has been suggested that the change from a state where there is a saturation of photosynthetic flux density to a state of darkness disturbs the cell membranes, inducing wounding responses (Chamberlain *et al.*, 2006). Nonanal was emitted by all the plants in this study despite the time of day. 1-hexanol was emitted by maize during the day, while 3-hexen-1-ol was emitted by Napier and Vetiver during the evening only and by Napier during the day. In the case of Napier and maize, these compounds could also have been induced by the change from light to dark as suggested by Chamberlain *et al.* (2006). In a study by Birkett *et al.* (2006), *B. fusca* showed a greater sensitivity to green leaf volatile compounds emitted by indigenous grasses compared to *C. partellus*. *Buseola fusca* is indigenous to Africa while *C. partellus* is exotic, suggesting that *B. fusca* had time to co-evolve with the grasses allowing them to be more sensitive to their volatiles. The question could then be asked if this could also be true for the exotic FAW in Africa?

In this study, more green leaf volatiles were found than other types of semiochemicals. Birkett *et al.* (2006) indicated that green leaf volatiles play a key role in host location as they tend to be present in higher concentrations in the plant. In addition plants produced larger volumes of hexanal, (E)-2-hexenal, (Z)-3-hexen-1-ol, and (Z)-3-hexen-1-yl acetate during scotophase, which suggested that they could be important for gravid stemborer moths for host location (Birkett *et al.*, 2006; Chamberlain *et al.*,

2006). In a study conducted by Khan *et al.* (2000), six key compounds (octanal, nonanal, linalool, naphthalene, 4-allylanisole and eugenol) were identified to mediate host location and oviposition in gravid stemborer females. In a follow-up study conducted by Birkett *et al.* (2006), all the poaceous 'pull' hosts used in their study (including Napier grass) were found to contain these compounds, suggesting a reason for their success as 'pull' components in a push-pull system.

Maize and Napier tended to emit a larger volume of volatiles during the evening. This was in accordance to the findings of a study by Schmelz *et al.* (2001). Since oviposition by female moths generally occurs during scotophase or at dusk (Sparks, 1979; Chamberlain *et al.*, 2006), this could be an adaption to make use of the larger amount of volatiles released at that time (Chamberlain *et al.*, 2006). Gas chromatography–electroantennographic detection (GC-EAD) techniques have shown that the chemoreceptors on the antennae of insect species can only detect a portion of the released volatiles and the compounds that dominate the volatile blend are often not the most important compounds in terms of behaviour (Cha *et al.*, 2008, Piñero and Dorn, 2007; Webster *et al.*, 2008). A better understanding of the oviposition behaviour of FAW is needed to determine if and how plant kairomones influence the host finding and orientation behaviour of gravid FAW moths therefore warrants further research.

FAW larvae are highly mobile and polyphagous (Chang *et al.*, 1985; Rojas *et al.*, 2018), and will often disperse away from the plant that the gravid female has chosen as an oviposition site (Rojas *et al.*, 2018). As a result, a gravid moth's oviposition choice may be overridden by the dispersal behaviour of larvae. The volatile detection to locate a suitable substrate on which to oviposit may therefore not be very important for FAW moths. Oviposition studies by Rojas *et al.* (2003) and Orsucci *et al.* (2018) found FAW moths are not impartial to laying on non-host plants rather than host plants, and generally do not show a marked preference for host plants compared to other plants. The response of FAW to a wide array of volatile compounds as was demonstrated by Malo *et al.* (2004), may explain the non-preference of ovipositing female moths towards host plants which are preferred by larvae for feeding (see chapter 3).

Host selection by female moths is deemed crucial for the survival of the larvae. Generally, larvae have restricted mobility and are not able to actively search for a

preferred host (Signoretti *et al.*, 2012). However, FAW larvae are highly mobile (Chang *et al.*, 1985). Laboratory studies have demonstrated that larvae prefer certain plants over others (Hardy *et al.*, 1985; Pashley, 1988; Murúa *et al.*, 2015). FAW larvae frequently respond to plant volatiles, moving to plants with older damage symptoms compared to plants with more recent damage in Y-tube olfactometer bioassays (Rojas *et al.*, 2003). This suggests that the larvae could be comparatively more influenced by plant volatiles than gravid moths.

A study by Williams *et al.* (1986), found that female FAW prefer not to oviposit on surfaces (towels) treated with aqueous extracts of maize leaves when compared to towels containing only the solvent. This inhibitory effect of oviposition does, however, suggest the presence of deterrent compounds in host plants (Rojas *et al.*, 2003). Deterrents emitted by maize and sorghum are typically released as a reaction to feeding by FAW larvae. It has been suggested these deterrents stimulate gravid moths to migrate away from the area before depositing their eggs (Williams *et al.*, 1986).

Conclusion

Gravid FAW moths do not rely solely on volatile detection. Tactile cues may play a larger role in host plant preference. FAW larvae may be more sensitive to plant volatiles as they tend to disperse away from the oviposition site. Further studies should focus on identifying and isolating volatile compounds emitted by the grasses tested in this study to determine if any of these have repellent effects on female moths.

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Chapter 3: *Spodoptera frugiperda* larval feeding preference and development on selected poaceous plant species

Abstract

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), or Fall Armyworm (FAW) is a highly destructive pest of maize with the larvae being the damaging stage. They are characterized by their high mobility and polyphagy, making them difficult to control and manage. The effects of plants on growth and development of insects is directly linked to their performance. The quality of the host plant determines the success of the insect's reproductive strategies as well as their fecundity. The characteristics of an ideal trap crop species are that it should be preferred by moths for oviposition and that it should be a poor larval host. The aim of this study was to determine the preference of FAW larvae for specific plant species that have potential as trap crops for this pest and to determine the suitability of five different poaceous plant species for larval development. The plant species were: maize, sorghum, Vetiver, Brachiaria and Napier grass. No-choice, two-choice and multiple-choice bioassays were conducted in Petri-dishes in which leaf material were provided to larvae. Host suitability for larval development was evaluated by rearing larvae on leaf tissue of the respective plant species in an insect rearing room. Maize was most preferred by larvae and higher numbers of larvae were able to reach the pupal stage when feeding on maize and their development to the pupal stage was significantly faster than on other plant species. Larval preference for Vetiver, Brachiaria and Napier grass was low. In the feeding studies, no larvae survived on Vetiver and Brachiaria while survival on Napier grass was also very low. These three grass species could therefore be considered as trap crops if moths prefer these plants as oviposition sites over maize. Moth oviposition preference studies suggested that gravid female moths are not particular when it comes to choosing a plant for oviposition and they often laid eggs on plants that were unsuitable for larval development and on substrates such as pots and the oviposition cages as well. This could be due to a mixed population of the Corn and Rice strains of FAW and the high mobility and polyphagous nature of the larvae.

Key words: Fall Armyworm, feeding preference, oviposition, trap crops

3.1. Introduction

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), or Fall Armyworm (FAW) is a new and destructive pest of maize in Africa. Larvae are characterized by their high mobility and polyphagy, making them difficult to control and manage (Chang *et al.*, 1985; Montezamo *et al.*, 2018). Although the larvae are polyphagous in nature, they have a preference for poaceous plant species (Barros *et al.*, 2010) such as maize, sorghum, wheat and rice (Gouin *et al.*, 2017). Studies of the preference of female moths to oviposit on certain plants and suitability of plants for larval development provide information whether certain hosts could be exploited as trap plants for pests (Hokkanen, 1991; Shelton and Nault, 2004). Plant species that are preferred by ovipositing moths but of which host suitability is poor could be used as trap plants or in habitat management systems such as the push-pull system developed for stemborers (Hokkanen, 1991; Shelton and Nault, 2004; Khan *et al.*, 2007) and the FAW (Midega *et al.*, 2018). Studies on the preference and development rate of larvae on different poaceous plants is important to determine if any of these plants (e.g. *Brachiaria*, Napier or Vetiver) could potentially act as trap crops, or even dead-end trap crops, for FAW.

The effects of the plant on the growth and development of the insect is directly linked to their performance. The quality of the host plant will determine the success of the insect's reproductive strategies as well as their fecundity (Shahout *et al.*, 2011; Sauvion *et al.*, 2017). Biological studies on suitability preference and consumption of different host plants are therefore important to determine for example the effects of different nutritional components on FAW (Silva *et al.*, 2017).

The characteristics of an ideal trap crop species are that it should be preferred by moths for oviposition and that it should be a poor larval host (Shelton and Nault, 2004; Khan *et al.*, 2007). In the Lepidoptera, host plant selection is made by the female moth, on behalf of larvae (Berdegué *et al.*, 1998; Rojas *et al.* 2018). It is believed that there is a positive correlation between adult host preference and larval performance, but realistically, such a relationship does not always exist. Variation in this relationship could be due to the fact that host choice and larval performance often differs under different ecological conditions and selection pressures. In addition to this, many lepidopteran species have highly mobile larvae that are able to conduct host plant selection themselves (Berdegué *et al.*, 1998).

The aim of this study was to determine the preference of *S. frugiperda* larvae for specific plant species that may have potential as trap crops. The specific objectives were to: 1) determine preference of neonate larvae for different poaceous plant species using no-choice, two-choice and multi-choice tests, 2) determine suitability of five different poaceous plant species for larval development and 3) determine if moths prefer certain poaceous plant species for oviposition.

3.2. Materials and Methods

Insects

Spodoptera frugiperda larvae were collected from various areas in South Africa during 2018 and used to establish a stock colony. Rearing was done in a rearing chamber at 26.5 ± 1 °C, RH $65 \pm 5\%$ and 14L:10D photoperiod. Larvae were reared individually in plastic containers (52 mm high and 30 mm in diameter) with a mesh infused lid and provided with fresh maize (*Zea mays*) (Poaceae) leaf tissue every two days. Once the moths emerged from the pupae, they were sexed and placed into 2 L plastic bottles containing moist crusher stones, cotton wool saturated with a sugar water solution and a piece of maize stem (15 cm long) consisting of the whorl and leaves following the method described by Kruger *et al.* (2012). Eggs were collected daily and placed in small plastic containers (52 mm high and 30 mm in diameter) which were kept in a glass desiccator (150 mm diameter) where the RH was maintained at $70 \pm 5\%$ using a potassium hydroxide solution according to the method of Solomon (1951). The desiccators were also kept in the same rearing chamber as described above until neonates hatched from these eggs.

3.2.1. Larval development

Maize (cultivar: DKC 80-10), sorghum, *Sorghum bicolor* (Poaceae) (cultivar: NS 5511) and *Brachiaria brizantha* (Cultivar: Marandu) seeds were planted, while Napier grass, *Pennisetum purpureum* Schumach. 1827. (Poaceae) and Vetiver grass *Chrysopogon zizanioides* (L.) Roberty (Poaceae) were propagated from cuttings. All plant species were planted in 2 L pots and watered daily.

Petri dishes (12 cm diameter) were fitted with filter paper. The filter paper was sprayed with distilled water to prevent desiccation of the leaf material of the respective test plants inside the Petri dishes. Neonate larvae were used in all the experiments. One

larva was placed per Petri dish and fresh leaf tissue provided as food every second day. Leaf tissue of the different host plant species was provided ad libitum. Pupae were weighed two days after pupation. The number of days for larvae to complete their cycle was recorded. Forty larvae (replicates), each in its own Petri dish were evaluated on each of the respective plant species.

All statistical analyses were done on SPSS Statistics v 22. Data on percentage survival as well as mean number of days survived by larvae on the respective poaceous plants species were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test), but these assumptions were not met. The respective data sets were therefore analysed by means of Kruskal–Wallis test and pair-wise comparison of the means was done using Mann–Whitney test. Data for mean pupal mass from larvae that fed on the respective host plants were normally distributed and homoscedastic and were analysed by means of one-way Anova.

3.2.2. Larval feeding preference for selected poaceous plants

The plant species mentioned above were used in this experiment. Leaf cuttings from these potential host plant species were used in no-choice, two-choice and multi-choice tests.

The tests were conducted in petri dishes (12 cm in diameter) fitted with filter paper. Since the width of the leaves of the different host plant species differed, a standardized size of 2 cm long leaf tissue pieces were used in all tests. These leaf tissue pieces were placed on the filter paper which was kept moist to prevent desiccation of the leaf tissue. Each of the three different choice tests consisted of 20 petri dishes per host plant species (no-choice test) or host plant combination (two-choice and multi-choice tests). The no-choice test was done by placing one leaf tissue piece of a plant species in the center and one *S. frugiperda* neonate larva inside but towards the side of the petri dish. For the two-choice test, two pieces of leaf tissue from different host plants were placed per Petri dish. One neonate larva was placed between the two leaf tissue pieces in the center of each Petri dish. For the multi-choice test, one piece of leaf tissue from three different host plants was placed per Petri dish. One neonate larva was placed in the center of each Petri dish at an equal distance away from the respective leaf pieces. The position of the larva inside each petri dish was recorded after 24 and 48 hours, for example if the larva was on the host plant leaf, and if so for two- and multi-choice test, on which host plant leaf. Wandering

larvae not on a leaf were also recorded. All experiments were conducted in a rearing chamber at 25 ± 1 °C, $65 \pm 5\%$ humidity and 14L:10D photoperiod.

The data were analysed using Statistica v13.3. The proportion of insects that made a choice was calculated, and data were analysed by means of a binomial distribution test. Insects that did not make a choice were not included in the analyses, but were indicated on the graphs. Bonferroni correction was used to adjust for multi means comparisons of the multi-choice test. The order of preference was calculated by pooling the data and calculating the percentage preference of each host plant species.

3.2.3. Oviposition preference

The plant species and FAW stock colony used in oviposition preference tests were the same as that described above. Moth preference for certain host plants were evaluated by means of no-choice and multiple-choice tests.

Oviposition studies were carried out in wooden cages with the sides covered with mesh (1 m x 1 m). One plant of each of three plant species were placed at equal distances apart inside the cage. One pair (male and female) of *S. frugiperda* moths were released inside the cage and allowed to mate and lay eggs for a period of five days. A sugar water solution with cotton wool was also placed within the cages. Plants were watered and cages were checked for the presence of eggs every second day. The eggs were collected and counted. Eggs laid in all the multi-choice tests were pooled per plant species and expressed as a proportion of the total number of eggs laid. These proportions were compared by means of binomial distribution tests. Bonferroni correction was used to adjust for multi means comparisons of the multi-choice test.

3.3. Results

3.3.1. Larval development

The mass of pupae reared from larvae that fed on maize, sorghum and Napier grass was similar and did not differ significantly (data not shown). However, percentage larval survival was 87.5, 30 and 10 percent on maize, sorghum and Napier grass respectively. Significantly more larvae survived on maize than on sorghum and Napier grass as well as on sorghum than on Napier grass (Figure 3.1). *Brachiaria* and *Vetiver*

grass were unsuitable for larval survival and no survival was recorded on any of these two species after 12 days. Larvae survived significantly longer on maize and sorghum compared to Brachiaria, Napier and Vetiver (Figure 3.2). There was no significant difference in the time that larvae survived on Brachiaria, Napier and Vetiver grass (Figure 3.2). Larval development time ranged between 17 and 44 days and was significantly longer on sorghum and Napier grass than on maize (Figure 3.3). The number of larvae that reached pupal stage for the respective plant species was: maize, 35; sorghum, 12 and Napier, 4. No larvae survived on Brachiaria and Vetiver grass.

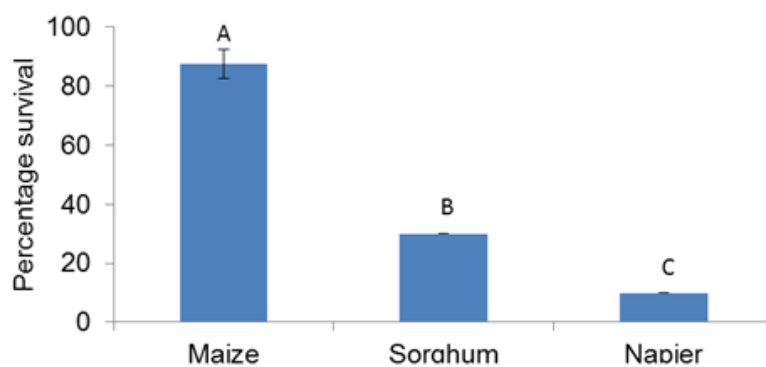


Figure 3.1: Percentage of *Spodoptera frugiperda* larvae that survived to the pupal stage on selected poaceous plants. No larvae survived on Brachiaria and Vetiver grass and where therefore not shown on the graph. Different higher case letters above bars indicate significant differences ($P < 0.05$).

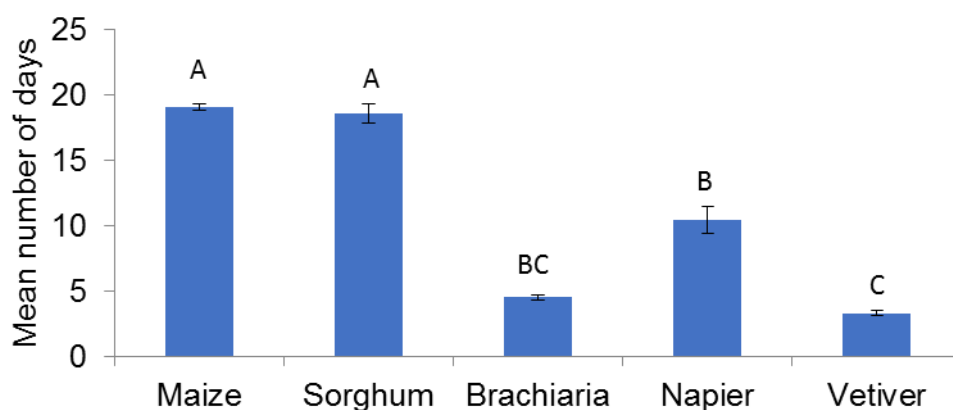


Figure 3.2: Mean number of days (+SE) that *Spodoptera frugiperda* larvae survived on selected poaceous plants. Different higher case letters above bars indicate significant differences ($P < 0.05$).

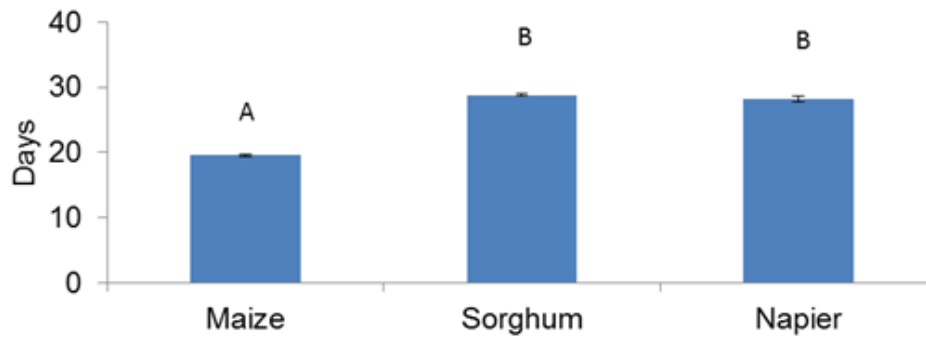


Figure 3.3: Mean larval development time (+SE) of *Spodoptera frugiperda* larvae on selected poaceous plants. Different higher case letters above bars indicate significant differences ($P < 0.05$).

3.3.2. Larval feeding preference for selected poaceous plants

There was no difference in the number of larvae choosing either sorghum or maize leaf tissue after 24 or 28 hrs (Figure 3.4). There were, however, significantly more larvae present on Brachiaria leaf tissue after 48 hrs than there were at 24 hrs. Significantly fewer larvae were present on Napier grass at 48 hrs compared to 24 hrs while no larvae were present on the Vetiver leaf tissue at 48 hrs (Figure 3.4). At 48 hours, significantly more larvae were wandering inside petri dishes (i.e. made a 'no-choice' decision than present on the leaf tissue) (Figure 3.4). In the Brachiaria, Napier and Vetiver grass no-choice tests, significantly more larvae were wandering than on the leaf tissue at both 24 and 48 hours (Figure 3.4).

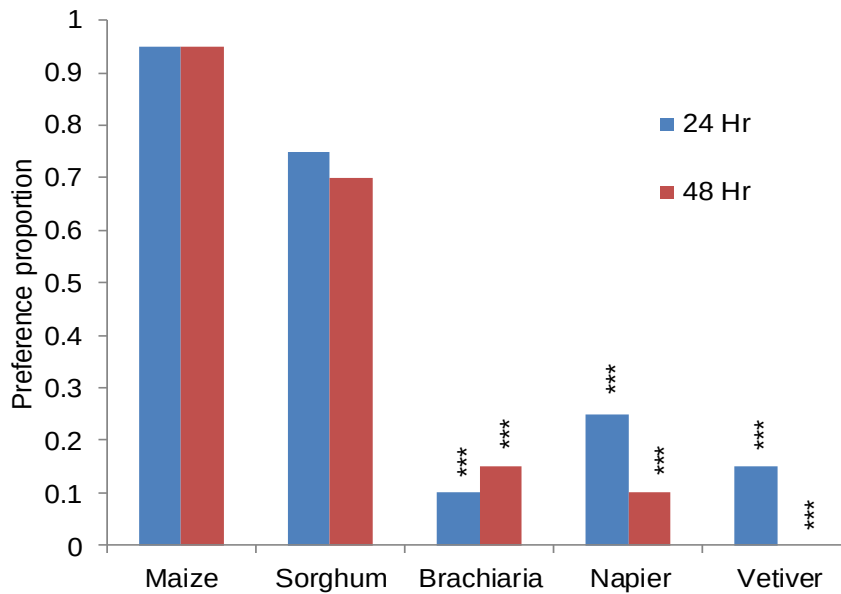


Figure 3.4: The response of *Spodoptera frugiperda* larvae in no-choice tests with poaceous plant species after 24 and 48 hours. Data over time were compared per plant species. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

In the two-choice tests, maize was significantly more preferred than sorghum, Brachiaria, Napier and Vetiver after 24 and 48 hours (Figures 3.5 and 3.6). Sorghum was similarly preferred to the other three grass species while Napier grass was significantly more preferred than Brachiaria (Figures 3.5 and 3.6) as well as Vetiver, after 24 and 48 hours (Figure 3.5). Vetiver grass was the least preferred by larvae (Figure 3.5 and 3.6).

The order of preference for the different plant species in two-choice tests was maize > sorghum > Napier > Brachiaria > Vetiver (Figures 3.5 and 3.6). The numbers of larvae which did not make any choice (NC) (and was therefore considered wandering) was very high in all the two-choice combinations with Brachiaria, Vetiver and Napier grass (Figures 3.5 and 3.6). When sorghum or maize was included in the choice tests with these grasses, the incidences of no-choices were much lower and large numbers of larvae responded positively to these two crops (Figures 3.5 and 3.6).

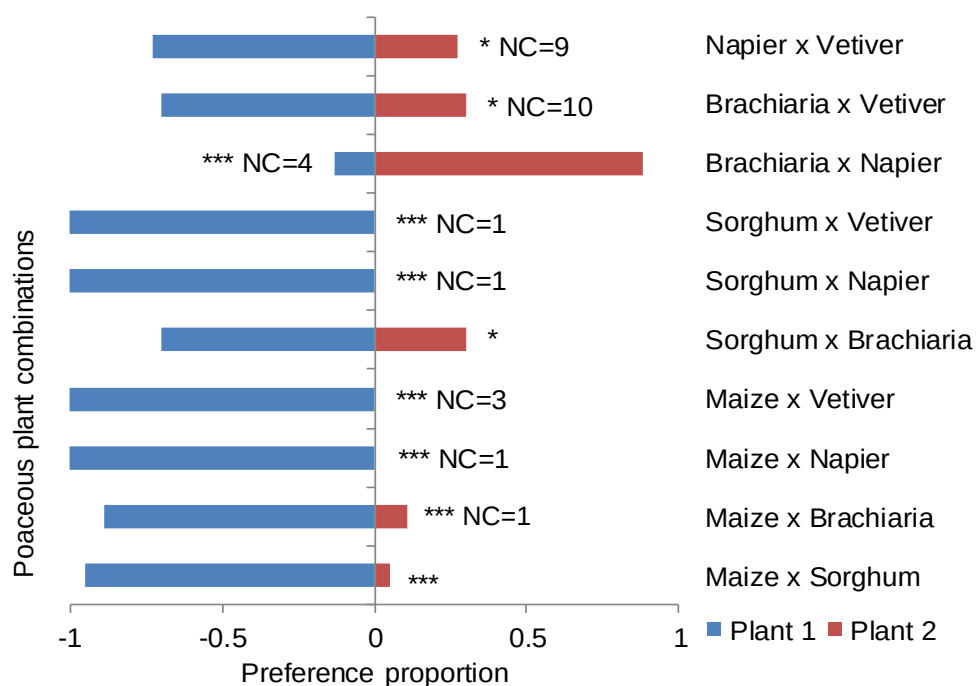


Figure 3.5: The preference of *Spodoptera frugiperda* larvae for different poaceous plant species in two-choice tests after 24 hours. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. 'NC' indicates the number of larvae (out of 20 per combination) that did not make a choice.

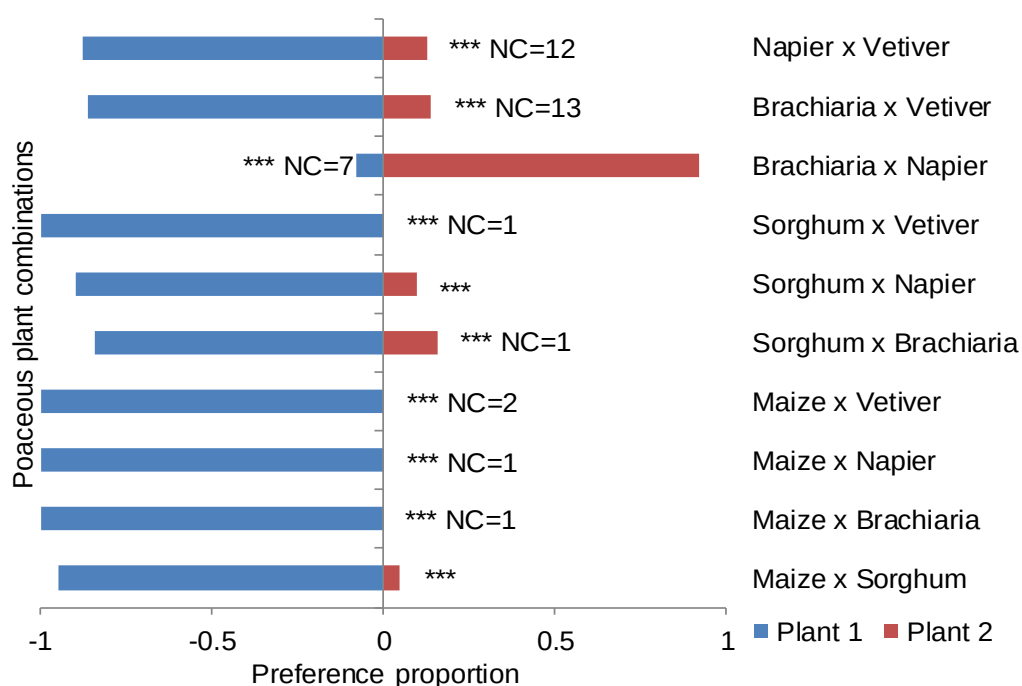


Figure 3.6: The preference of *Spodoptera frugiperda* larvae in two-choice tests with poaceous plant species after 48 hours. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. 'NC' indicates the number of larvae (out of 20 per combination) that did not make a choice.

In all the multi-choice test in which maize were included, almost no larvae were recorded on the other plant species (Figures 3.7 and 3.8). The multi-choice experiments in which sorghum was included also showed that a large number of larvae preferred sorghum leaves to the other species which in many cases were not selected by any larvae. The incidences of no-choices by larvae were markedly higher in multi-choice combinations in which maize was not included as a choice (Figures 3.7 and 3.8).

The order of preference for the different plant species in multi-choice experiments were maize > sorghum > Brachiaria > Napier > Vetiver (Figures 3.7 and 3.8).

In experiments in which maize was not provided as a choice and only sorghum, Brachiaria and Vetiver grass was used, high numbers of larvae did not make any choices (Figures 3.7 and 3.8).

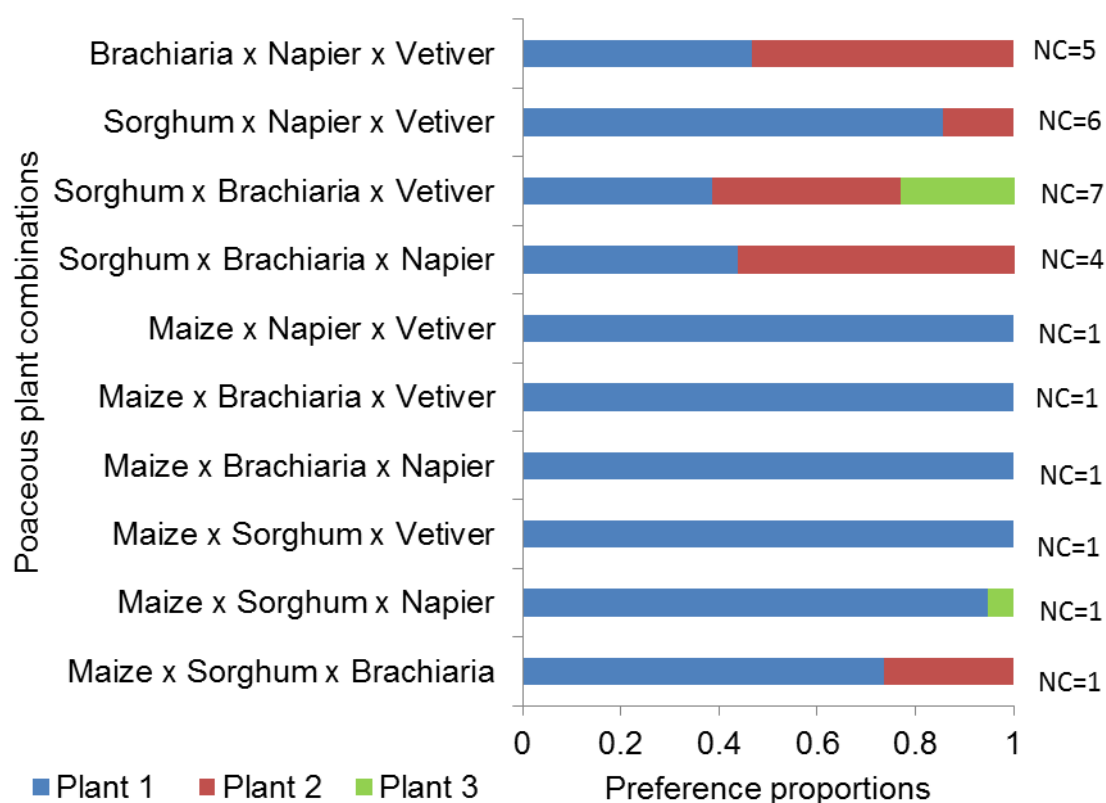


Figure 3.7: The preference of *Spodoptera frugiperda* larvae in multi-choice tests with poaceous plant species after 24 hours. Significant differences in preference are provided in table 3.1. 'NC' indicates the number of larvae (out of 20 per combination) that did not make a choice.

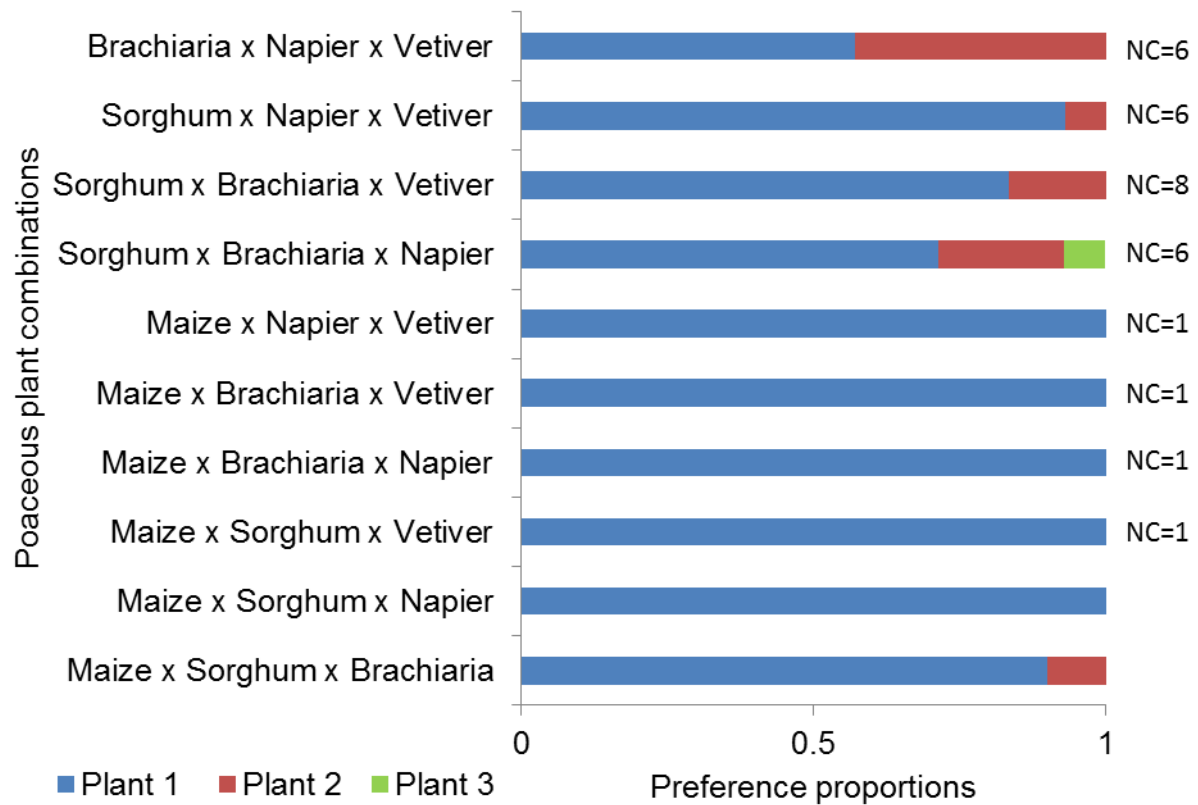


Figure 3.8: The preference of *Spodoptera frugiperda* larvae in multi-choice tests with poaceous plant species after 48 hours. Significant differences in preference are provided in table 3.1. 'NC' indicates the number of larvae (out of 20 per combination) that did not make a choice.

Table 3.1: Results of the binomial distribution test of *Spodoptera frugiperda* larval multi-choice preference tests with poaceous plant species after 24 and 48 hours.

Plant combinations	Specific combinations	24 hours P-value	48 hours P-value
Maize x Sorghum x Brachiaria	Maize x Sorghum	**	***
	Maize x Brachiaria	***	***
	Sorghum x Brachiaria	*	NS
Maize x Sorghum x Napier	Maize x Sorghum	***	***
	Maize x Napier	***	***
	Sorghum x Napier	NS	NS
Maize x Sorghum x Vetiver	Maize x Sorghum	***	***
	Maize x Vetiver	***	***
	Sorghum x Vetiver	NS	NS
Maize x Brachiaria x Napier	Maize x Brachiaria	***	***
	Maize x Napier	***	***
	Brachiaria x Napier	NS	NS
Maize x Brachiaria x Vetiver	Maize x Brachiaria	***	***
	Maize x Vetiver	***	***
	Brachiaria x Vetiver	NS	NS
Maize x Napier x Vetiver	Maize x Napier	***	***
	Maize x Vetiver	***	***
	Napier x Vetiver	NS	NS
Sorghum x Brachiaria x Napier	Sorghum x Brachiaria	NS	**
	Sorghum x Napier	**	***
	Brachiaria x Napier	***	NS
Sorghum x Brachiaria x Vetiver	Sorghum x Brachiaria	NS	***
	Sorghum x Vetiver	NS	***
	Brachiaria x Vetiver	NS	NS
Sorghum x Napier x Vetiver	Sorghum x Napier	***	***
	Sorghum x Vetiver	***	***
	Napier x Vetiver	NS	NS
Brachiaria x Napier x Vetiver	Brachiaria x Napier	NS	NS
	Brachiaria x Vetiver	***	***
	Napier x Vetiver	***	**

Significance indicated by *P<0.05; **P<0.01; ***P<0.001 and NS = no significant difference.

3.3.3. Oviposition preference

There were no significant patterns in oviposition preference in any of the choice test combinations (Data not shown). In many of the replicates no eggs were laid or eggs were laid on the cages. While we attempted to get at least six successful replicates per choice test, the maximum number where preference was shown, was only two per choice test combination. In many of the replicates on oviposition, only one of the females oviposited. Descriptive data are therefore provided (Figure 3.9 – Figure 3.17).

In the no-choice oviposition tests with maize, no eggs were laid on maize or oviposition cages (Figure 3.9). In the no-choice tests with sorghum and Napier, more eggs were laid on the plants than on cages (only two replicates). In no-choice tests with Brachiaria and Vetiver however, many more eggs were laid on the cages than on plants (Figure 3.9).

In the multi-choice test in which maize occurred, generally fewer eggs were laid on the cage or others (Figures 3.10, 3.11 and 3.13) The exception was the combination maize x sorghum x vetiver, where maize had the highest number of eggs (Figure 3.12). In all multi-choice combinations where maize was absent and replaced by sorghum, many eggs were laid on the cages (Figures 3.14 and 3.15). The exception was the combination sorghum x Napier x Vetiver, where sorghum had the highest number of eggs (Figure 3.16). In the multi-choice tests with Brachiaria x Napier x Vetiver, eggs were only laid on Brachiaria and the cage with 60 and 951 eggs respectively (Figure 3.17).

After pooling the data from the various multi-choice tests, no significant difference in the number of eggs laid on the respective plant species was found. No definite preference for any of these plant species was therefore detected.

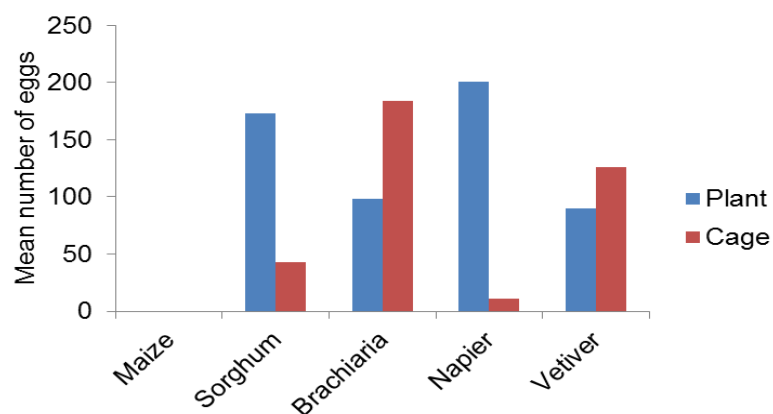


Figure 3.9: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in no-choice tests.

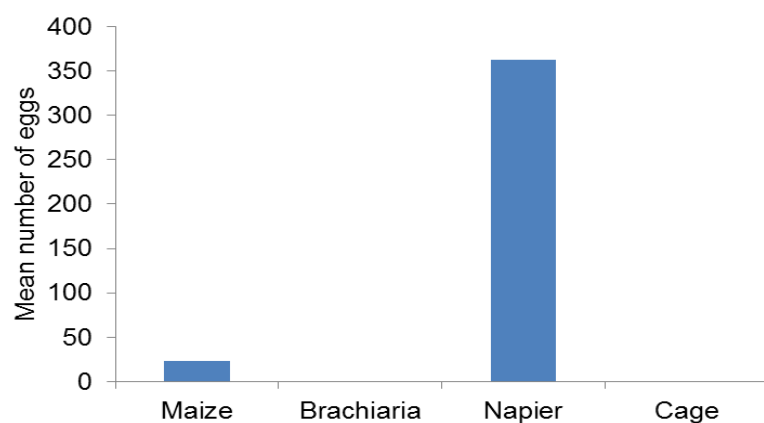


Figure 3.10: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

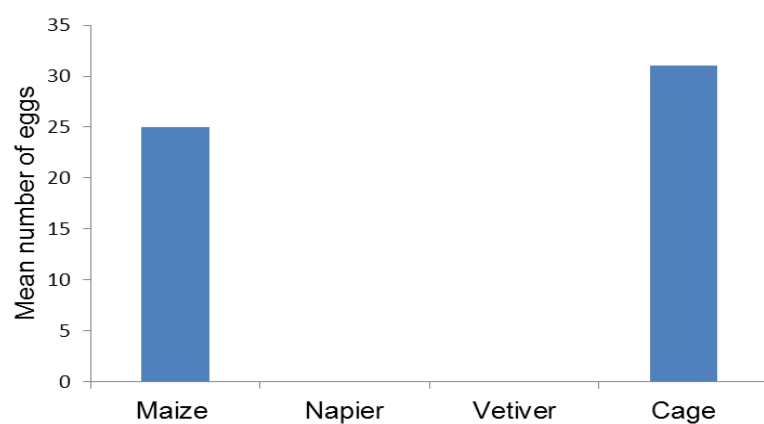


Figure 3.11: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

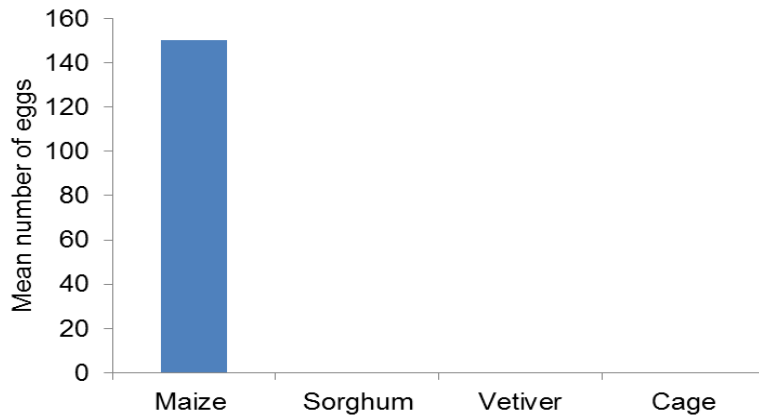


Figure 3.12: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

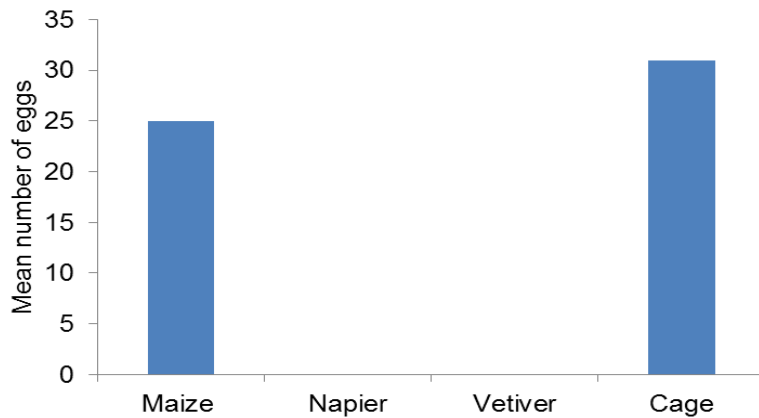


Figure 3.13: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

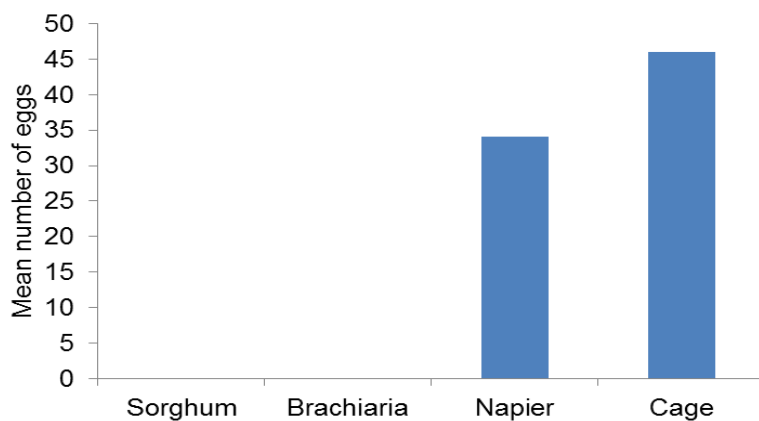


Figure 3.14: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

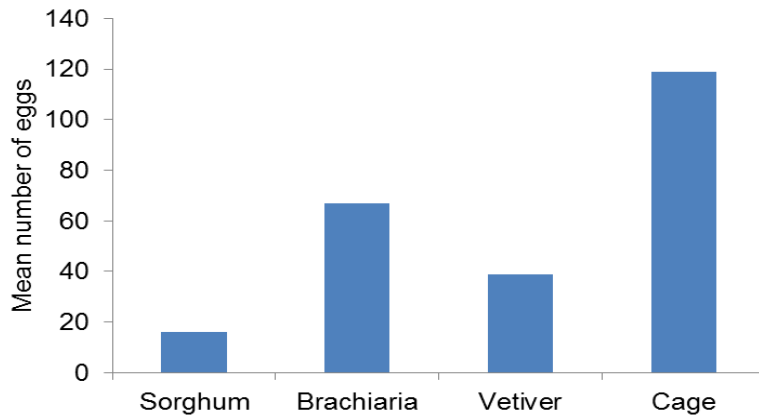


Figure 3.15: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

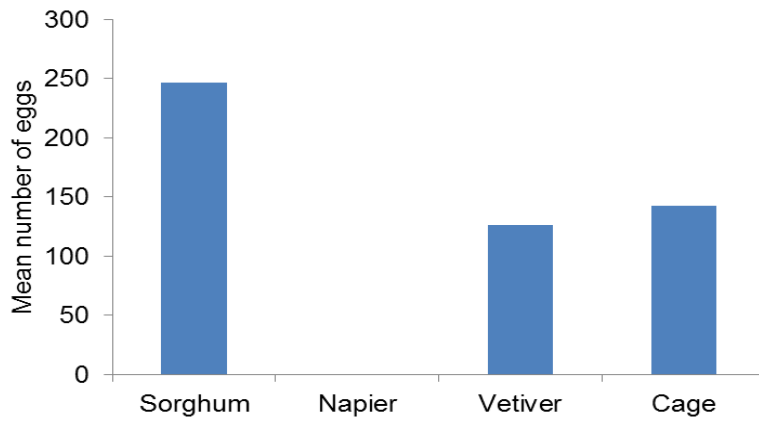


Figure 3.16: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests.

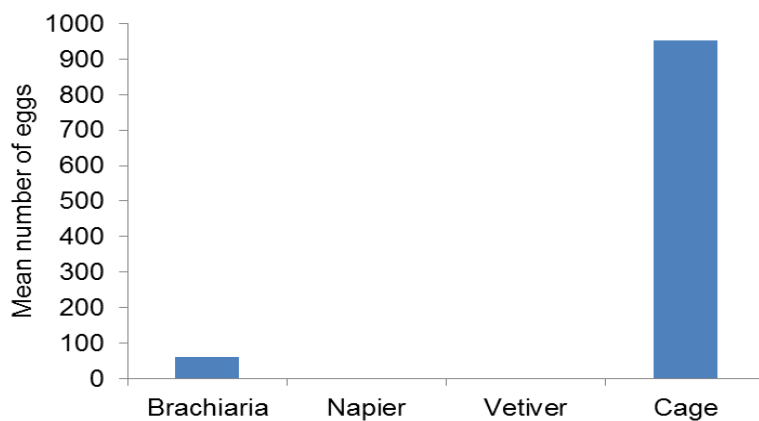


Figure 3.17: The numbers of eggs laid by gravid *Spodoptera frugiperda* moths on poaceous plant species in multi-choice tests

3.4. Discussion

Reproduction is one of the most energy consuming activities in an insect's life. The nutrients acquired by insects affect the body condition of both the larval and adult stages. Depending on the resources obtained, they can increase/decrease the developmental duration, increase/decrease adult longevity, and affect body size. When nutrient stress occurs during early life stages it may result in a decrease in the ability to attract and locate mates (Sauvion *et al.*, 2017). The quality and quantity of host plant tissue consumed by larvae is also important for larval growth, larval defense against natural enemies and it determines adult reproductive success (Sauvion *et al.*, 2017).

In this study, maize was by far the most preferred host plant of FAW larvae. Larval survival was higher and development faster on maize compared to the other plants used in the study. Sorghum was also suitable for the development of FAW larvae, but fewer larvae reached the pupal stage and the development time was significantly longer compared to maize. Very few larvae developed to the pupal stage when feeding on Napier grass and those that did had a significantly longer development time compared to larvae that fed on maize. This is in accordance with the findings of Khan *et al.* (2007), who reported *B. fusca* larval survival to be significantly lower and larval development time significantly longer on Napier grass compared to maize. Khan *et al.* (2006), reported the same tendency for the spotted stemborer, *Chilo partellus* (Lepidoptera: Crambidae) with larval survival significantly lower and development time two weeks longer on Napier compared to maize. In this study Brachiaria, Napier and Vetiver grass were found not to be suitable for the development of FAW larvae. Midega *et al.* (2011) reported larval survival of *C. partellus* to be much lower on Brachiaria and Napier compared to maize and sorghum, which is similar to the results observed for FAW in this study. It has been suggested that stemborer larvae survived better on preferred plants (i.e. maize and sorghum) due to these plant species having softer tissues (Khan *et al.*, 2006; Midega *et al.*, 2011). Taking this into account, the higher survival of FAW larvae on maize can possibly also be ascribed to the suitability of maize and sorghum leaf tissue as food to early instar FAW larvae.

Low nutritional quality or low digestibility of plant tissues are regarded as defense mechanisms of plants against herbivory (Williams, 1999). Other defense mechanisms of plants against herbivory include silica, tannins, lignins, hardening of the cuticle

tissue and a reduction in nutrient content (Chen, 2008; Mithöfer and Boland, 2012; Peterson, 2016). Plants that have reduced nutritional quality or low digestibility are typically unfavorable to herbivorous insect larvae as the larvae tend to grow slower, rendering them susceptible to predator attack for a longer period of time, as well as causing reduced fecundity in the adult stage (Williams, 1999; Hanley *et al.*, 2007; Mithöfer and Boland, 2012).

Maize was the most preferred host plant over sorghum, Brachiaria, Vetiver and Napier grass in this study, indicating that maize is preferred by FAW larvae for feeding. Many larvae did not choose, but were wandering inside petri dishes rather than choose Brachiaria, Napier or Vetiver grass. When maize was given as a choice, nearly all larvae preferred maize over the other plant species.

In a study conducted by Cheruiyot *et al.* (2018), *C. partellus* larvae also preferred maize leaf cuttings to Brachiaria leaf cuttings after 24 hours. First instar *C. partellus* larvae did not consume any Brachiaria plant tissue (Cheruiyot *et al.*, 2018), indicating a non-preference by *C. partellus* larvae for Brachiaria, similar to FAW in this study. Midega *et al.* (2017), demonstrated that maize fields surrounded by Brachiaria as a trap crop was effective at reducing FAW numbers inside the maize field. *Busseola fusca* (Lepidoptera: Noctuidae) larvae also preferred leaf cuttings of maize over Napier grass (Khan *et al.*, 2007). *Chilo partellus* larvae also prefer maize over Napier, Brachiaria grass (Midega *et al.*, 2011) and Vetiver grass (Van den Berg, 2006). Vetiver is an unsuitable host for first instar larvae, and as a result larvae migrated to maize plants (Van den Berg, 2006). The non-preference exhibited by larvae may be due to plant chemicals that are present, physical characteristics of the plant or poor nutrient quality (Cheruiyot *et al.*, 2018).

Several oviposition experiments were performed as part of this study, but an inadequate amount of data was collected. However other studies on oviposition suggest that FAW moths are not impartial to laying on non-host plants rather than host plants, and generally do not show a marked preference for host plants compared to other plants (Orsucci *et al.*, 2018).

Fall armyworm larvae are polyphagous, yet there are two genetic strains (the Corn strain and the Rice strain) that show different dietary preferences. It has been suggested that the difference in dietary preference has driven speciation between

these two strains (Nagoshi and Meagher, 2003; Machado *et al.*, 2008; Orsucci *et al.*, 2018). The Corn strain favors maize, sorghum and cotton (*Gossypium* spp.), while the Rice strain favors smaller grasses such as turf, pasture (*Cynodon dactylon*) and rice (*Oryza* spp.) (Nagoshi and Meagher, 2003; Machado *et al.*, 2008; Orsucci *et al.*, 2018). In oviposition studies conducted by Orsucci *et al.* (2018) moths from neither the Corn strain or the Rice strain showed preference for the expected host plants (maize or rice respectively). Many eggs (Corn strain = between 33% and 55%; Rice strain = 85%) were laid on the cages. Experiments show that the Corn strain was indeed specialized for maize, but were also able to recognize rice as a host plant, even though it is not its preferred host. There is no evidence, however, that rice is the preferred plant for the Rice strain, but larvae do survive slightly better on rice (Orsucci *et al.*, 2018). Larvae of the Corn strain gain their nutrients from the plant by overexpressing genes involved in detoxification, while the Rice strain gains nutrients from the plant by overexpressing secondary metabolite pathways that compensate for the lack of nutrients in rice plants (Orsucci *et al.*, 2018). Being better adapted, the Rice strain is able to complete its life cycle on pasture grasses that have a lower nutrient quality than maize (Orsucci *et al.*, 2018). Cross mating between these two FAW biotypes do occur, allowing for mixed strains to occur (Nagoshi *et al.*, 2006; Machado *et al.*, 2008). Since mixed strains seem to be more prevalent in South Africa, this could have been the reason for poor results obtained in the moth oviposition experiments.

Fall armyworm larvae are highly mobile and polyphagous (Chang *et al.*, 1985; Rojas *et al.*, 2018; Montezamo *et al.*, 2018), and will often disperse away from the plant that the gravid female has chosen as an oviposition site (Rojas *et al.*, 2018). The larvae are cannibalistic and cannibalism starts at an early age (2-4 day old larvae). Dispersal away from the oviposition site therefore decreases the chance of cannibalism and interspecific competition (Rojas *et al.*, 2018). For FAW larvae up to 180 plant species have been recorded as hosts (Castro and Pitre, 1988). Dispersing larvae are more likely to come into contact with plants that are host plants due to the high polyphagia exhibited by the larvae (Rojas *et al.*, 2018). As a result, a gravid moth's oviposition choice may be overridden by the dispersal behavior of larvae. The hypothesis for maximum embryo survival suggests that gravid moths choose their oviposition sites

based on other factors, such as parasitism risk or egg mortality (Refsnider and Janzen, 2010; Rojas *et al.*, 2018).

Midega *et al.* (2018) reported that FAW could be controlled by implementing a push-pull system where maize was intercropped with Greenleaf desmodium and Brachiaria cv Mulato II that was planted around maize fields. The reduced incidence of damage by FAW on maize was attributed to desmodium acting as a 'push' factor, emitting repellent volatiles and Brachiaria acting as a 'pull' factor, emitting volatiles that are attractive to female moths. Midega *et al.* (2018) also suggested that the increase in plant biodiversity resulted in a greater abundance, diversity and activity of predatory arthropods, further suppressing FAW numbers on maize.

Conclusion

Maize is the most preferred FAW larval host plant species of the poaceous plants evaluated in this study. Due to the low survival rate and non-preference of FAW for Brachiaria, Napier and Vetiver grass, these plant species have potential as trap crops. However, this will only be the case if these grasses are preferred by female moths for oviposition. The potential of mixed strains occurring on the African continent, and the highly mobility and polyphagy of larvae could explain the weak preference for known hosts for oviposition, with the moths often choosing unsuitable hosts. This study has shown that Brachiaria, Napier and Vetiver grass have the potential to be trap crops for FAW. Midega *et al.* (2017), has shown that Brachiaria paired with desmodium can be effective at controlling FAW, but the mechanisms need to be studied further. Gravid moths seem not to be particular about where they oviposit their eggs. Could this be due to the high mobility of the FAW larvae, allowing the larvae to make their own choice of host? Or maybe as suggested by Rojas *et al.* (2018), gravid moths make oviposition choices based on other factors such as parasitism risk or egg mortality.

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Chapter 4: *Spodoptera frugiperda* larval feeding preference on selected broad-leaf crop species

Abstract

Spodoptera frugiperda, or Fall armyworm (FAW), is known to be a polyphagous insect species, causing damage to a large number of cultivated plant species. FAW has attained pest status on numerous cultivated plants belonging to the family Poaceae, mainly on maize and sorghum. Although plants from this family are preferred, FAW is also becoming a pest on important broadleaf crops such as cotton and soybean, especially in areas where they are cultivated after maize. Larval feeding preference tests were done using the following crop host species: maize, sorghum, soybean, dry bean, cotton, sunflower and groundnut. No-choice, two-choice and multiple-choice bioassays were conducted in petri-dishes in which leaf material were provided to larvae. Larval response to the different crop species were recorded after 1, 4 and 24 hours for no-choice, and 24 and 48 hours for two- and multi-choice test. Maize and sorghum were significantly preferred over other hosts while groundnut was the least preferred plant species in two- and multi-choice tests.

Key words: crops, Fall armyworm, feeding preference

4.1. Introduction

Crop rotations and biological diversity have become important for the success of agricultural cropping systems. Crop rotation not only promotes soil fertility, but when managed correctly, it lowers the incidence of pests. Pest control by means of crop rotation works mainly by breaking the reproductive cycles of the insect, or by reducing migrating populations (Francis and Clegg, 1990; Meagher *et al.*, 2004). Generally the greater the difference between crop species in the rotation, the better the insect control (Francis and Clegg, 1990).

With the development of modern day agricultural systems that consist of vast areas of monoculture, people have unintentionally selected for insect pests that are adapted to these agricultural conditions (Sauvion *et al.*, 2017). *Spodoptera frugiperda* (Lepidoptera: Noctuidae), or Fall armyworm (FAW), is known to be a polyphagous insect species, causing damage to a large number of cultivated plant species (Casmuz *et al.*, 2010; Gouin *et al.*, 2017; Montezano *et al.*, 2018). It has attained pest status on numerous cultivated plants belonging to the family Poaceae, mainly on maize and sorghum, and although plants from this family are preferred, FAW is also becoming a pest on important broadleaf crops such as cotton and soybean, especially in areas where they are cultivated after maize (Pitre and Hogg 1983; Hardke *et al.* 2015; Gouin *et al.*, 2017). Although soybean, dry bean, cotton, sunflower and groundnut are listed as host plants of FAW, the host status of these non-poaceous crops in Africa is not known (Montezano *et al.*, 2018).

Knowing the host suitability of different crop species that FAW is capable of feeding on, including minor crops or crops that may sustain FAW populations during off seasons, is important in the context of pest management. If moths lay eggs on these crops or even non-crop plants where early instar development occurs on these plants adjacent to or within crop fields, larvae are capable of moving to cultivated crops after consuming their original host (Luginbill, 1928; Guagliumi, 1973). In addition, polyphagous feeding behaviour can allow build-up or maintain FAW populations outside of the primary cropping season or outside of cropping areas, contributing to increased pest pressure. For example, Favetti *et al.* (2017) reported that FAW infested pearl millet, *Pennisetum glaucum* (L.) r.Br. (Poales: Poaceae) during the dry season in Brazil, leading to high populations during the off-season.

Knowledge of which plants are potential hosts for FAW is an essential component of this approach. The aim of this study was to determine the preference of FAW for host plants that are also cultivated in the maize production region of Southern Africa, some of which are used in crop rotation systems or multi-cropping systems with maize.

4.2. Materials and methods

Insects

Spodoptera frugiperda larvae were collected from various areas in South Africa during 2018 and used to establish a rearing colony. Rearing was done in a rearing chamber at 26.5 ± 1 °C, RH $65 \pm 5\%$ and 14L:10D photoperiod. Larvae were reared individually in plastic containers (52 mm high and 30 mm in diameter) with a mesh infused lid and provided with fresh maize leaf tissue every 2 days. Once the moths emerged from the pupae, they were sexed and placed into 2 L plastic bottles containing moist crusher stones, cotton wool with a sugar water solution and a piece of maize consisting of the whorl and leaves following the methods described by Kruger *et al.* (2012). Eggs were collected daily and placed in small plastic containers (52 mm high and 30 mm in diameter) which were kept in a glass desiccator (150 mm diameter) in which RH was maintained at $70 \pm 5\%$ using a potassium hydroxide solution according to the method of Solomon (1951). The desiccators were also kept in the same rearing chamber as described above until neonates hatched from these eggs.

Bioassays

Plants were maize (DKC 80-10, cultivar), sorghum *Sorghum bicolor* (Poaceae) (cultivar: NS 5511), groundnut, (*Arachis hypogaea* L. (Fabales: Fabaceae) (cultivar: Akwa), soybean (cultivar: PAN 1583R), dry bean (cultivar: Star 2000), sunflower, *Helianthus annuus* L. (Asterales: Asteraceae) (cultivar: PAN7057) and cotton, *Gossypium hirsutum* (Malvaceae) (cultivar: Delta Opal. Seeds were planted in polystyrene cups (250 ml) (1 seed per cup) and watered daily.

The bioassays were conducted in petri dishes (12 cm in diameter) fitted with filter paper. Young leaves from the respective plants were used in this study. The leaves were placed on the filter paper which was kept moist to prevent desiccation of the leaf tissue. Choice tests conducted consisted of 15 petri dishes per host plant species (no-choice test) and 12 petri dishes per host plant combination (two-choice and multi-

choice tests). The no-choice test was done by placing one young leaf of a plant species in the center and one *S. frugiperda* neonate larva inside but to the side of a petri dish. For the two-choice test, two pieces of leaf tissue from different host plants were placed per Petri dish. One neonate larva was placed between the two leaf tissue pieces in the center of each petri dish. For the multi-choice test, one piece of leaf tissue from three different host plants was placed per petri dish. One neonate larva was placed in the center of each petri dish at an equal distance away from the respective leaf pieces.

The position of the larva inside each petri dish was recorded after 1, 4 and 24 hours in the no-choice tests and after 24 and 48 hours in the two-choice and multi-choice tests. The position of larvae in the petri dish was recorded as either on the host plant leaf or wandering and in choice tests with more than the one plant species, the plant species on which the larva occurred was also recorded. All experiments were conducted in a rearing chamber at 25°C, 65 ± 5% humidity and 14L:10D photoperiod.

The data was analysed using Statistica v13.3. The proportion of insects that made a choice was calculated, and data were analysed by means of a binomial distribution test. Insects that did not make a choice were not included into the analysis but were indicated on the graphs. The order of preference was calculated by pooling the data and calculating the percentage preference of each host plant species.

4.3. Results

There were no differences in the numbers of larvae on either sorghum or maize leaf tissue after 4 or 24 hours (Figure 4.1). Larvae provided with soybean leaf pieces as food wandered, but all these larvae settled on soybean after 24 hours (Figure 4.1). There were slightly more larvae present on dry bean leaf tissue after 24 hours than there were at 1 hour and 4 hrs. Fewer larvae were present on cotton at 48 hours compared to 1 hour and 4 hours (Figure 4.1). Larvae that were initially on sunflower also left and the number decreased after 1 hour (Figure 4.1). Significantly higher numbers of larvae were found wandering (i.e. made 'no-choice') in petri dishes containing dry bean, sunflower and cotton after 1, 4 and 24 hours (Figure 4.1).

The least preferred to most preferred species, indicated by larval numbers on the respective plant species after 1 hour were: dry bean < groundnut < soybean < cotton < sunflower < sorghum < maize. After 4 hours larval numbers indicated preference to

be: sunflower = soybean < dry bean < cotton < sorghum = maize. After 24 hours this preference was: sunflower < cotton < dry bean < groundnut < sorghum = maize.

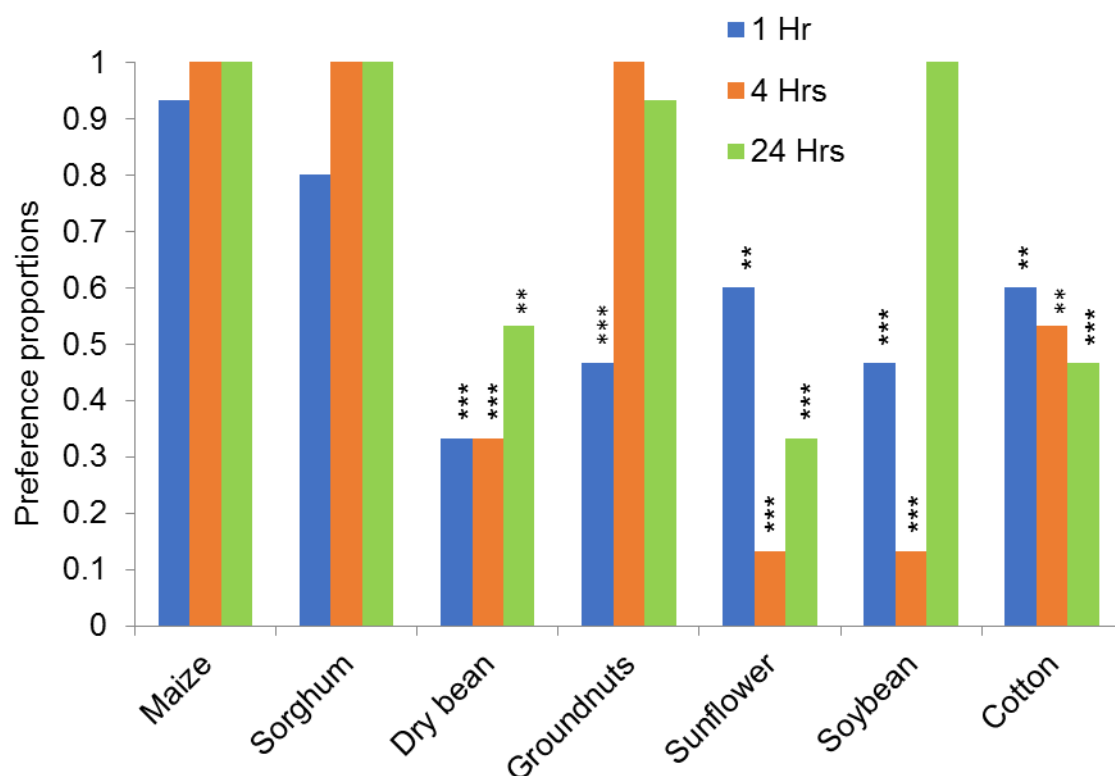


Figure 4.1: The response of *Spodoptera frugiperda* larvae in no-choice tests with maize, sorghum and non-poaceous plant species after 24 and 48 hours. Data over time were compared per plant species. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

In all two-choice tests, maize and sorghum was significantly more preferred than dry bean, groundnuts, soybean and cotton (Figures 4.2 and 4.3). Preferences for dry bean, soybean, sunflower and groundnut provided in two-choice tests, were similar after 24 hours (Figure 4.2). Dry bean was, however, significantly more preferred to cotton after 24 hours (Figure 4.2).

The least preferred to most preferred broad-leaf plants compared to maize at 24 hours were soybean < sunflower < cotton < groundnut < dry bean < maize < sorghum (Figure 4.2). The least preferred to most preferred broad-leaf plants compared to

maize at 48 hours were groundnut < sunflower < soybean < cotton < dry bean < sorghum < maize (Figure 4.3).

The highest incidence of larvae that did not make any choices after 24 hours was in the two-choice tests with dry bean and sunflower, followed by combinations of soybean and cotton, and dry bean and soybean (Figure 4.2). Combinations with maize and sorghum typically had low rates of 'no-choices' (Figures 4.2 and 4.3). The highest number of "no-choices" was observed in two-choice tests where groundnut and sunflower, and dry bean and soybean (Figure 4.3).

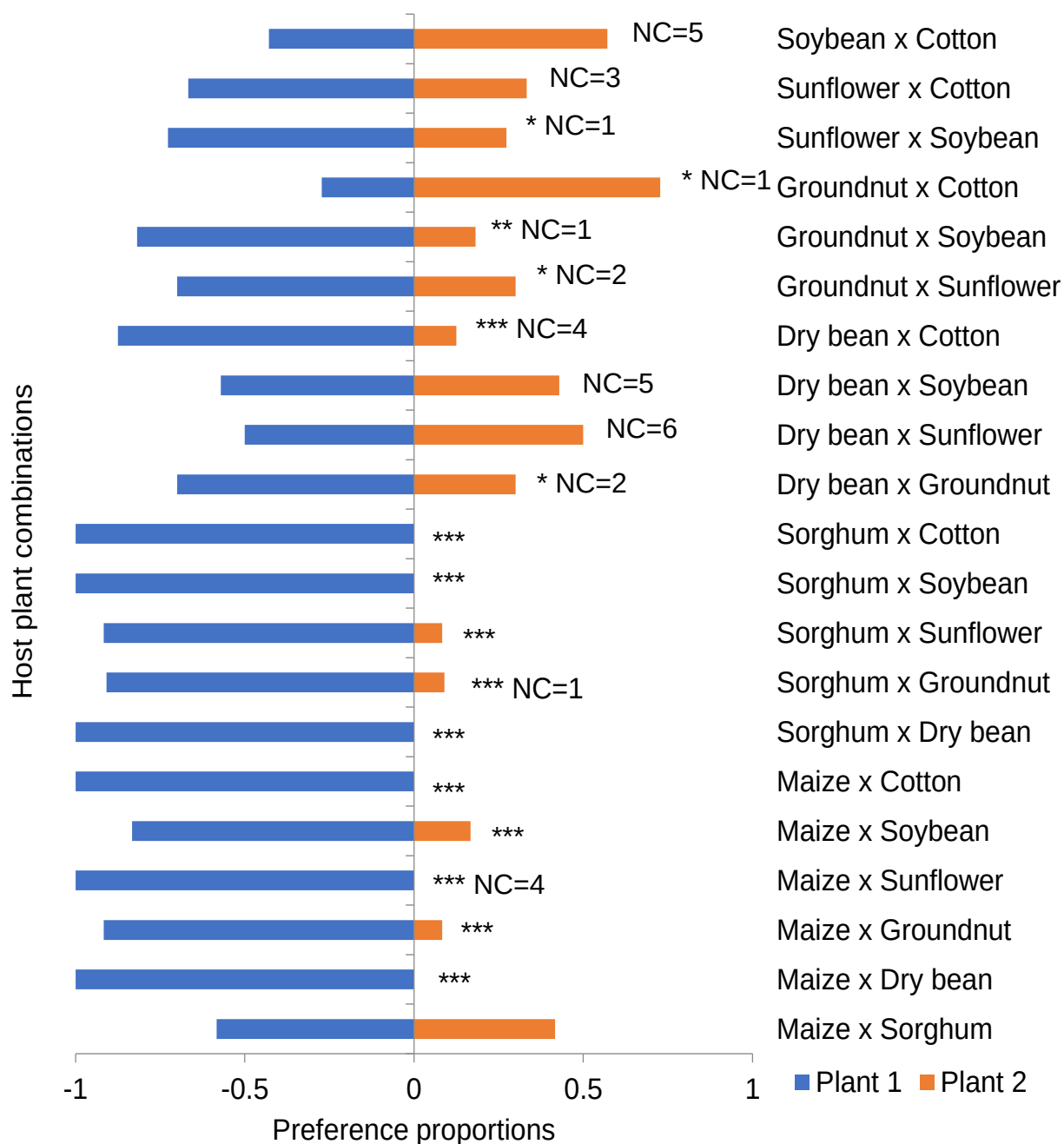


Figure 4.2: The preference of *Spodoptera frugiperda* larvae for different maize, sorghum and non-poaceous plant species in two-choice tests after 24 hours. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. 'NC' indicates the number of larvae (out of 12 per combination) that did not make a choice.

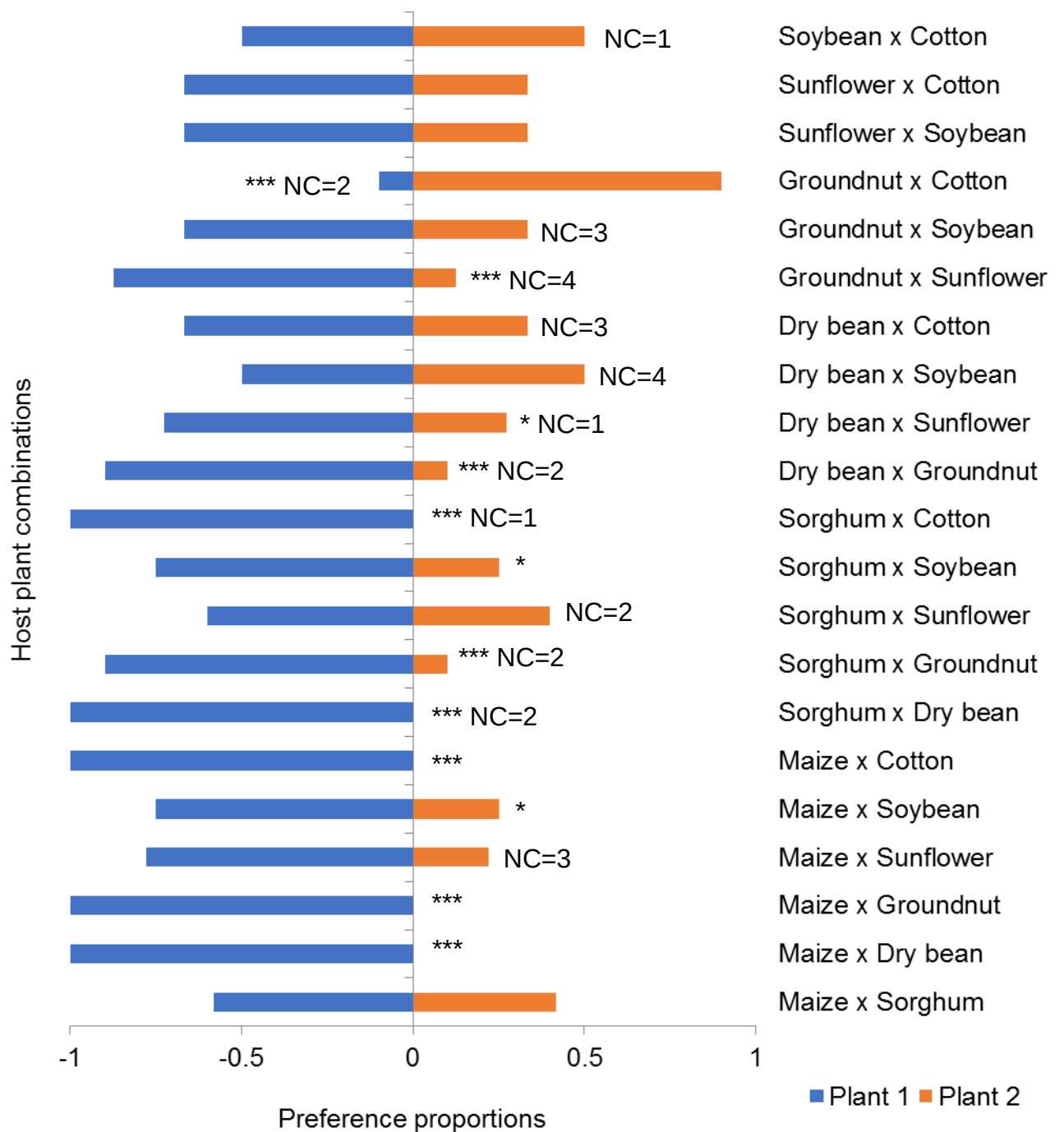


Figure 4.3: The preference of *Spodoptera frugiperda* larvae for maize, sorghum and non-poaceous plant species in two-choice tests after 48 hours. Significance indicated by * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$. 'NC' indicates the number of larvae (out of 12 per combination) that did not make a choice.

In multi-choice tests, maize was preferred over other hosts (Figures 4.4 and 4.5), except in the combination maize x dry bean x cotton at 24 hours (Figure 4.4). Except for combinations that included maize, sorghum was the most preferred host in the combinations: sorghum x dry bean x groundnut (Figures 4.4 and 4.5); sorghum x groundnut x sunflower (Figures 4 and 5); sorghum x groundnut x soybean (Figures 4.4 and 4.5); sorghum x groundnut x cotton (Figures 4.4 and 4.5). Sorghum was the most preferred host (excluding combinations with maize) in the combinations sorghum x sunflower x soybean and sorghum x sunflower x cotton at 24 hours (Figure 4.4), but not at 48 hours (Figure 4.5).

Excluding combinations with maize, dry bean was the most preferred host in the combinations: sorghum x dry bean x soybean at 24 hours (Figure 4.4), and sorghum x dry bean x sunflower and sorghum x dry bean x cotton at 48 hours (Figure 4.5).

Groundnut was not preferred over any other host plants in the respective combinations (Figures 4.4 and 4.5). Maize, sunflower was the most preferred host in the combinations: sunflower x soybean x cotton at 24 hours (Figure 4.4); dry bean x groundnut x sunflower and groundnut x sunflower x soybean at 24 and 48 hours (Figures 4.4 and 4.5); and combinations groundnut x sunflower x cotton, dry bean x sunflower x cotton and sorghum x sunflower x soybean at 48 hours (Figure 4.5). Soybean was the most preferred host in the combinations: sorghum x soybean x cotton, dry bean x sunflower x soybean, dry bean x soybean x cotton and groundnut x soybean x cotton at 24 and 48 hours (Figures 4.4 and 4.5); and sunflower x soybean x cotton at 48 hours (Figure 4.5). Cotton was the most preferred host in the combinations: sorghum x dry bean x cotton at 24 hours (Figure 4.4); and dry bean x groundnut x cotton at 24 and 48 hours (Figures 4.4 and 4.5).

The least preferred to most preferred broad-leaf plants compared to sorghum and maize at 24 hours were groundnut < dry bean < cotton < soybean < sorghum < sunflower < maize (Figure 4.4). The least preferred to most preferred broad-leaf plants compared to maize at 48 hours were groundnut < cotton < dry bean < soybean < sunflower < sorghum < maize (Figure 4.5).

The combination sorghum x dry bean x soybean had the highest number of larvae that did not make a choice (Figures 4.4 and 4.5).

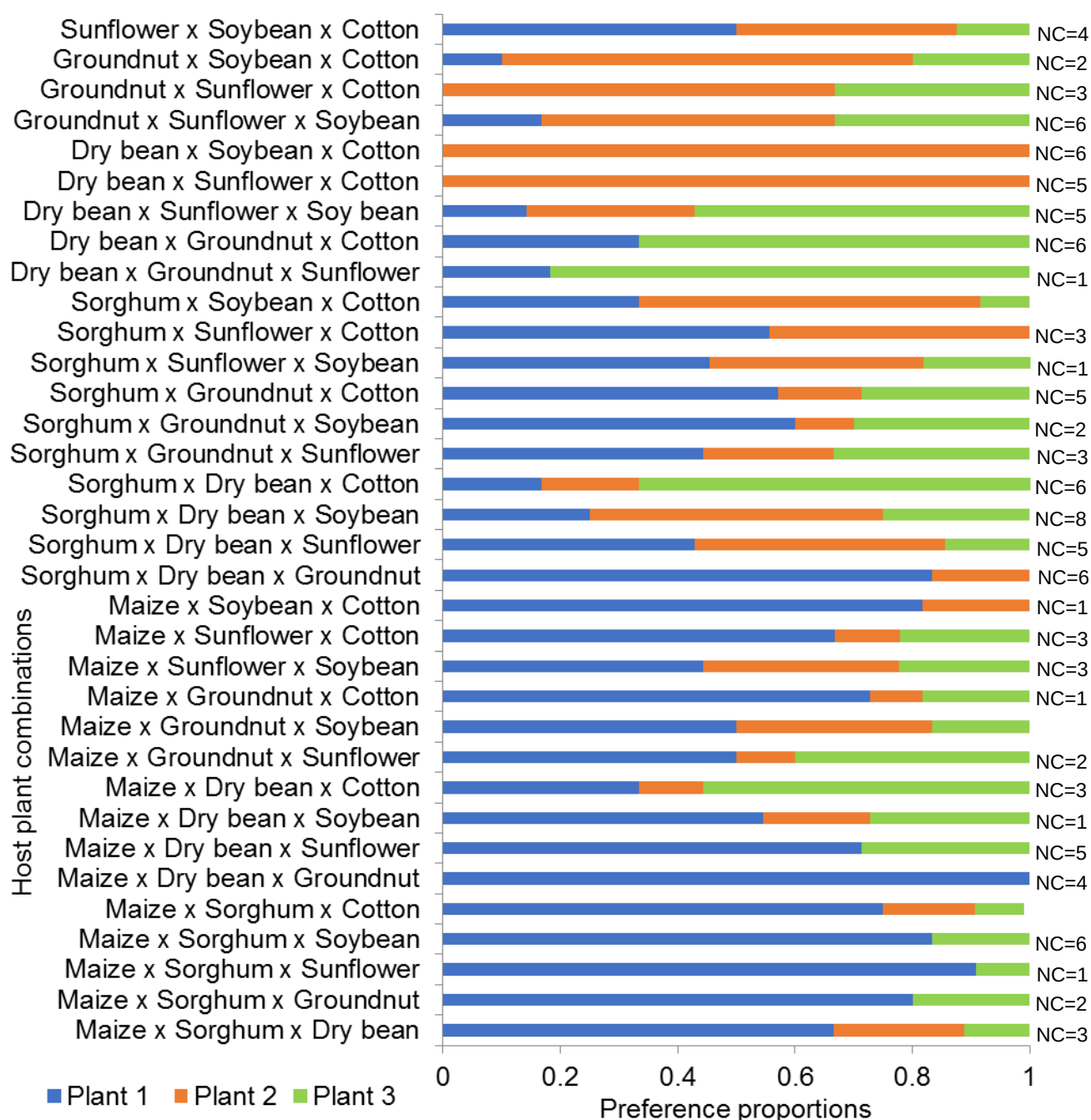


Figure 4.4: The preference of *Spodoptera frugiperda* larvae in multi-choice tests with maize, sorghum and non-poaceous plant species after 24 hours. Significant differences in preference are provided in table 4.1. 'NC' indicates the number of larvae (out of 12 per combination) that did not make a choice.

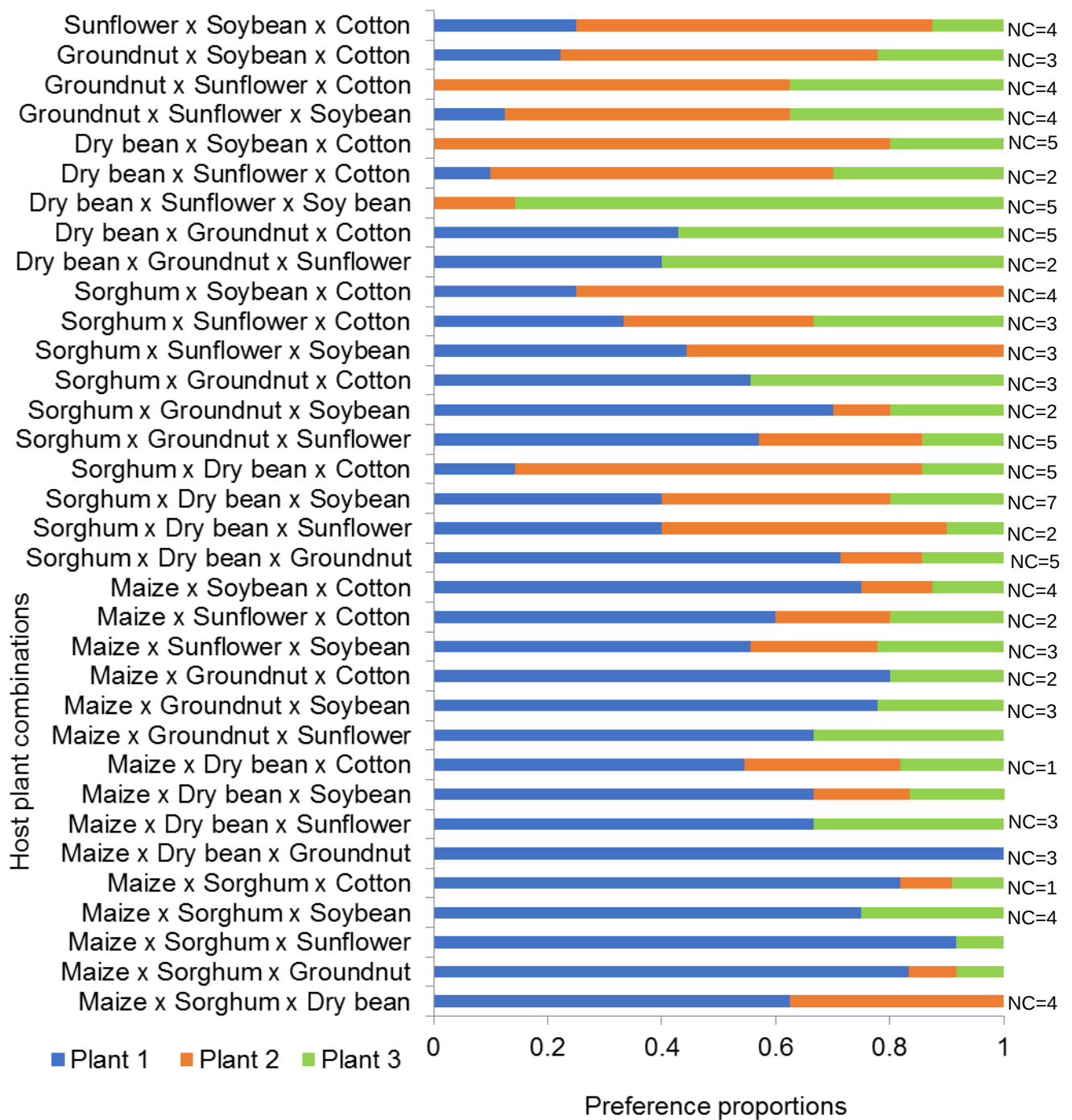


Figure 4.5: The preference of *Spodoptera frugiperda* larvae in multi-choice tests with maize, sorghum and non-poaceous plant species after 48 hours. Significant differences in preference are provided in table 4.1. 'NC' indicates the number of larvae (out of 12 per combination) that did not make a choice.

Table 4.1: Results of the binomial distribution test of *Spodoptera frugiperda* larval multi-choice feeding preference tests with host plant species after 24 and 48 hours.

Plant combinations	Specific combinations	P-value 24 Hrs	P-value 48 Hrs
Maize x Sorghum x Dry bean	Maize x Sorghum	NS	NS
	Maize x Dry bean	*	**
	Sorghum x Dry bean	NS	NS
Maize x Sorghum x Groundnut	Maize x Sorghum	***	***
	Maize x Groundnut	*	***
	Sorghum x Groundnut	NS	NS
Maize x Sorghum x Sunflower	Maize x Sorghum	***	***
	Maize x Sunflower	***	***
	Sorghum x Sunflower	NS	NS
Maize x Sorghum x Soybean	Maize x Sorghum	***	***
	Maize x Soybean	**	*
	Sorghum x Soybean	NS	NS
Maize x Sorghum x Cotton	Maize x Sorghum	*	***
	Maize x Cotton	**	***
	Sorghum x Cotton	NS	NS
Maize x Dry bean x Groundnut	Maize x Dry bean	***	***
	Maize x Groundnut	***	***
	Dry bean x Groundnut	NS	NS
Maize x Dry bean x Sunflower	Maize x Dry bean	***	**
	Maize x Sunflower	NS	NS
	Dry bean x Sunflower	NS	NS
Maize x Dry bean x Soybean	Maize x Dry bean	NS	*
	Maize x Soybean	NS	*
	Dry bean x Soybean	NS	NS
Maize x Dry bean x Cotton	Maize x Dry bean	NS	NS
	Maize x Cotton	NS	NS
	Dry bean x Cotton	NS	NS
Maize x Groundnut x Sunflower	Maize x Groundnut	NS	**
	Maize x Sunflower	NS	NS
	Groundnut x Sunflower	NS	NS
Maize x Groundnut x Soybean	Maize x Groundnut	NS	***
	Maize x Soybean	NS	*
	Groundnut x Soybean	NS	NS
Maize x Groundnut x Cotton	Maize x Groundnut	**	***
	Maize x Cotton	*	**
	Groundnut x Cotton	NS	NS
Maize x Sunflower x Soybean	Maize x Sunflower	NS	NS
	Maize x Soybean	NS	NS
	Sunflower x Soybean	NS	NS
Maize x Sunflower x Cotton	Maize x Sunflower	*	NS
	Maize x Cotton	NS	NS
	Sunflower x Cotton	NS	NS
Maize x Soybean x Cotton	Maize x Soybean	**	*
	Maize x Cotton	***	*
	Soybean x Cotton	NS	NS
Sorghum x Dry bean x Groundnut	Sorghum x Dry bean	**	*
	Sorghum x Groundnut	***	*
	Dry bean x Groundnut	NS	NS
Sorghum x Dry bean x Sunflower	Sorghum x Dry bean	NS	NS

	Sorghum x Sunflower	NS	NS
	Dry bean x Sunflower	NS	NS
Sorghum x Dry bean x Soybean	Sorghum x Dry bean	NS	NS
	Sorghum x Soybean	NS	NS
	Dry bean x Soybean	NS	NS
Sorghum x Dry bean x Cotton	Sorghum x Dry bean	NS	*
	Sorghum x Cotton	*	NS
	Dry bean x Cotton	*	*
Sorghum x Groundnut x Sunflower	Sorghum x Groundnut	NS	NS
	Sorghum x Sunflower	NS	NS
	Groundnut x Sunflower	NS	NS
Sorghum x Groundnut x Soybean	Sorghum x Groundnut	*	*
	Sorghum x Soybean	NS	*
	Groundnut x Soybean	NS	NS
Sorghum x Groundnut x Cotton	Sorghum x Groundnut	NS	*
	Sorghum x Cotton	NS	NS
	Groundnut x Cotton	NS	*
Sorghum x Sunflower x Soybean	Sorghum x Sunflower	NS	NS
	Sorghum x Soybean	NS	*
	Sunflower x Soybean	NS	*
Sorghum x Sunflower x Cotton	Sorghum x Sunflower	NS	NS
	Sorghum x Cotton	*	NS
	Sunflower x Cotton	*	NS
Sorghum x Soybean x Cotton	Sorghum x Soybean	NS	*
	Sorghum x Cotton	NS	NS
	Soybean x Cotton	*	***
Dry bean x Groundnut x Sunflower	Dry bean x Groundnut	NS	*
	Dry bean x Sunflower	**	NS
	Groundnut x Sunflower	***	**
Dry bean x Groundnut x Cotton	Dry bean x Groundnut	NS	*
	Dry bean x Cotton	NS	NS
	Groundnut x Cotton	**	*
Dry bean x Sunflower x Soybean	Dry bean x Sunflower	NS	NS
	Dry bean x Soybean	NS	***
	Sunflower x Soybean	NS	**
Dry bean x Sunflower x Cotton	Dry bean x Sunflower	***	*
	Dry bean x Cotton	NS	NS
	Sunflower x Cotton	***	NS
Dry bean x Soybean x Cotton	Dry bean x Soybean	***	***
	Dry bean x Cotton	NS	NS
	Soybean x Cotton	***	*
Groundnut x Sunflower x Soybean	Groundnut x Sunflower	NS	NS
	Groundnut x Soybean	NS	NS
	Sunflower x Soybean	NS	NS
Groundnut x Sunflower x Cotton	Groundnut x Sunflower	**	**
	Groundnut x Cotton	NS	NS
	Sunflower x Cotton	NS	NS
Groundnut x Soybean x Cotton	Groundnut x Soybean	*	NS
	Groundnut x Cotton	NS	NS
	Soybean x Cotton	*	NS
Sunflower x Soybean x Cotton	Sunflower x Soybean	NS	NS
	Sunflower x Cotton	NS	NS
	Soybean x Cotton	NS	*

Significance indicated by *P<0.05; **P<0.01; ***P<0.001 and NS = no significant difference.

4.4. Discussion

Spodoptera frugiperda is known to prefer plants from the Poaceae family (Castro and Pitre, 1988; Montezamo *et al.*, 2018). This accounts for the high larval preference for maize and sorghum observed in this study and concurs with results by Pitre *et al.* (1983).

Larvae in the no-choice experiments showed a preference of >40% on all the host plants at 24 hours, highlighting the extent of polyphagy in FAW (Perez-Zubiri *et al.*, 2016; Meagher *et al.*, 2004). This is most likely an important survival strategy for FAW, since it is a migrating species. Polyphagy may allow FAW to travel through areas where other more preferred host plants might not be available (Johnson, 1987). Poorer hosts such as cotton, dry bean, groundnut and sunflower may cause a reduction in insect health, making them more susceptible to insecticides, viruses, parasitoids and predators, and as a result decreases survival rate. Richter *et al.* (1987) found that pupae of FAW larvae reared on soybean were smaller and had a mortality rate as high as 94% before adult emergence. This was ascribed to the stress of consumption of a less suitable host. Ritcher *et al.* (1987) also reported that FAW larvae reared on maize or sorghum were less susceptible to a nuclear polyhedrosis virus (NPV).

The 2-choice tests showed that soybean was not preferred by FAW larvae when given a choice, yet more larvae preferred soybean with time. No-choice tests showed a high preference for soybean as time elapsed. This demonstrates that soybean is not a preferred host of FAW, but will be utilized as a host if other more preferred host plants are not available (Ritcher *et al.*, 1987). Therefore, in systems where maize or sorghum is intercropped with soybean, the preference for soybean should remain low. In maize-dry bean combinations, larvae exhibited a significantly lower feeding preference for dry beans. FAW larvae reared on kidney beans (*Phaseolus vulgaris*) showed higher rates of cannibalism than larvae reared on maize due to the differences in nutrition provided by the respective hosts (Raffa, 1987). It has been suggested by Mensah and Sequeria (2004), that maize could be intercropped with beans to help control the movement of FAW larvae. This was also suggested by Van Huis *et al.* (1981) who reported 20 – 30 % less damage to maize where maize was intercropped with beans. A similar conclusion was drawn by Ryder (1968) who reported that maize intercropped with sunflower was less severely attacked by FAW and had higher yields than

monoculture maize. This study demonstrated that in the presence of other hosts, sunflower was a less preferred choice.

This study indicated that cotton was not a preferred host plant of FAW larvae. A study by Luttrell and Mink (1999) indicated that FAW had the potential to damage cotton fruiting structures comparable to *Heliothis zea* (Lepidoptera: Noctuidae) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and that larvae had similar survival rates on these crop species and maize. However, the larvae used in the latter study were third instar larvae, which are stronger and more tolerable to unfavorable host plant conditions. The larvae used in this study were neonate larvae and more susceptible to unfavorable host plant conditions. The poor host status of cotton for FAW was highlighted by Nagoshi *et al.* (2007) who reported that infestations of cotton occurred mainly during the fruiting stages when FAW larvae moved from surrounding maize fields, or, in one case, FAW was found in cotton when no maize fields were present in the area (Nagoshi *et al.*, 2007).

It is suggested that when any of the high-valued host crops mentioned in this study, except for maize and sorghum, are used as a cash crops in areas where FAW populations persist, these two crops can be used as trap crops. By planting maize or sorghum around less favored cash crops, maize/sorghum could act as a barrier (Figure 4.6), in the same way that Napier grass acts as a barrier against female stemborer moths (Midega *et al.*, 2005) and FAW moths (Midega *et al.*, 2018).

Groundnut has been shown to be a less preferred host plant, especially in the two-choice and multi-choice tests. Campbell and Wynne (1980) suggested that FAW only infested groundnut fields when pest pressure is very high, suitable grasses are not available, or due to the excess grasses inside groundnut fields. They concluded that groundnut plants had mechanisms to resist FAW larval feeding through non-preference or antibiosis.

It is possible that maize and sorghum crops may act as refuges for overwintering populations of FAW. Although farmers will protect these crops by means of insecticide applications, abandoned or neglected fields after the harvest may become refuges that sustain pest populations. It is suggested that there should be an immediate destruction of these crops and any other preferred host plants after the harvest

(Knipling, 1980). Knipling (1980) also suggested the removal of any other non-crop plant species that could act as a refuge for FAW.

Migration to other host plants from maize or sorghum, generally only occurs when the population on these hosts has reached its threshold, the plants become no longer suitable as the season progresses or the suitable plants do no longer occur in the area (Pitre and Hogg, 1988; Nagoshi *et al.*, 2007). Cowpea and sunnhemp also have the potential to reduce the FAW population numbers due to increased development time and high larval mortality on these crops (Meagher *et al.*, 2004). This study similarly suggests that broadleaf crops such as soybean, sunflower, dry beans and groundnut are poor hosts of FAW and may also suppress its population numbers.

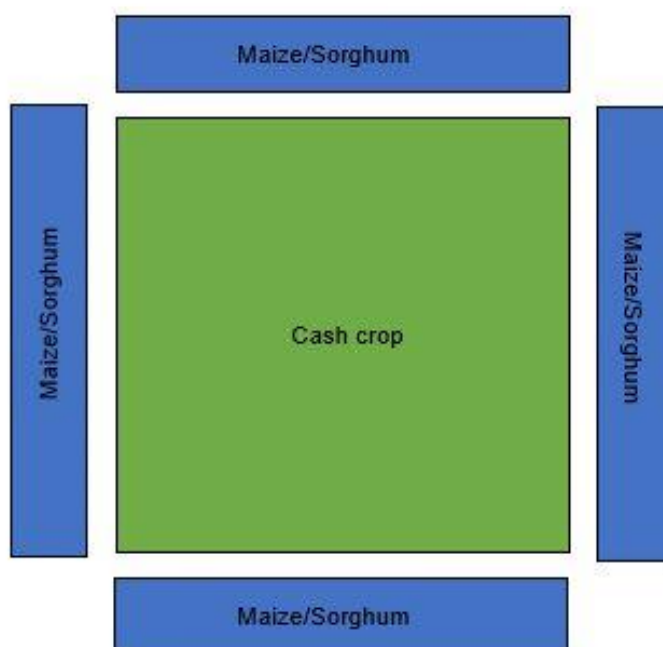


Figure 4.6: Diagram illustrating the possible arrangement of maize or sorghum as a perimeter trap crop.

Conclusion

Maize and sorghum were most preferred as host plants by FAW larvae. Therefore, because the other host plants are less preferred, maize and sorghum could be used as companion crops or trap crops in habitat management systems.

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Chapter 5: Discussion

5.1. The push-pull system

Midega *et al.* (2018) recently released a paper stating that a push-pull system used by subsistence farmers in Africa for the control of stemborers, namely *Busseola fusca* (Fuller) (Noctuidae) and *Chilo partellus* (Swinhoe) (Crambidae) is also effective against the FAW. In this system *Brachiaria* cv Mulato II is used as the 'pull' component, or the trap crop, while Greenleaf desmodium (*Desmodium intortum*) is used as the repellent intercrop, the 'push' component in the system (Midega *et al.*, 2018). It has not been established why this method is effective in controlling FAW, only that it is effective.

Other wild grasses such as Vetiver grass (*Vetiver zizanioides*) and cultivated grasses such as Napier grass (*Pennisetum purpureum*) have also been successfully used in the control of *C. partellus* and *B. fusca* (Van den Berg, 2006; Midega *et al.*, 2011; Cheruiyot *et al.*, 2018) and therefore may also be able to be used as trap plants towards controlling FAW.

5.2. The role of volatiles

Plants are able to synthesize a large diversity of volatile organic compounds (VOCs). Volatile organic compounds facilitate the plants interaction with its environment. The VOCs can attract pollinators, act as seed dispensers and protect the plant from herbivores, parasites and pathogens (Dudareva *et al.*, 2013).

Volatile compounds were collected via entrainment of intact plants (maize, sorghum, Napier, *Brachiaria* and Vetiver). The collected volatiles were analysed by means of gas chromatography (GC) and three main components occurring in all four plant species were identified *viz.* decanal, squalene and limonene.

Green leaf volatiles emitted by plants may play a key role in host location by gravid female moths (Birkett *et al.*, 2006). It was discovered by Birkett *et al.* (2006), that Napier grass did indeed contain a number of volatile compounds that are associated with host location by female stemborer moths. The findings of this study were in accordance with results published by Sparks (1979), Schmelz *et al.* (2001) and

Chamberlain *et al.* (2006) that Napier grass and maize emit higher volumes of volatiles during the evening (scotophase) which coincide with the time female stemborer moths oviposit their eggs. It is thought that stemborer moths make use of these large volumes of volatiles released during scotophase to locate suitable host plants (e.g. maize) on which to oviposit (Chamberlain *et al.*, 2006). In a study conducted by Malo *et al.* (2004), it was shown that FAW tend to respond to a wide variety of different volatile components. This suggests that female FAW moths may not be sensitive to volatiles from particular host plants. Female FAW moths laid eggs random on any plant or surface provided showing no clear oviposition preference which is in agreement with the results reported by Orsucci *et al.* (2018).

FAW larvae are highly mobile and will typically disperse away from the oviposition site (Chang *et al.*, 1985). Larvae may be sensitive to host plant volatiles, which would help them locate suitable host plants. Rojas *et al.* (2003) demonstrated by means of Y-tube olfactometer assays how FAW larvae typically selected host plants that have older damage symptoms compared to plants with recent damage.

5.3. FAW plant preferences

Polyphagous insects have a definite advantage over specialist insects in terms of host finding and survival. The advantages include a decreased search time, a buffer against ever-changing environments and the ability to spread over larger areas (Janz and Nylin, 1997). The plants on which insects choose to feed directly affect the growth and development of the insect, which in turn directly affect their performance. Host plant quality also determines the extent of the success of the insect's reproductive strategies as well as their fecundity (Shahout *et al.*, 2011; Sauvion *et al.*, 2017). Low nutritional quality or low digestibility of plant tissues are regarded as a defence mechanism of plants against herbivory (Williams, 1999) since reduced nutritional quality or low digestibility is typically unfavourable to herbivorous insect larvae. Larvae tend to grow slower on poor host plants, rendering them susceptible to predator attack for a longer period of time, as well as causing reduced fecundity of females (Williams, 1999; Hanley *et al.*, 2007; Mithöfer and Boland, 2012).

One of most energy consuming activities of an insect's life is reproduction. The quantity and quality of nutrients obtained from host plant tissues during the larval stages affects larval growth and developmental duration, body size, ability to defend themselves against natural enemies, adult longevity and reproductive success. If nutrient stress occurs during early life stages it may result in a decrease in the ability to attract and locate mates (Sauvion *et al.*, 2017). Plants that are typically favoured by stemborer moths for oviposition, but are unsuitable for the development of the larvae, make ideal trap crops (Shelton and Nault, 2004; Khan *et al.*, 2007). In this study, Napier grass, Vetiver grass and Brachiaria grass have been found to be poor hosts for the survival and development of FAW larvae, and should therefore in theory act as successful trap crops if the moths prefer these plants as oviposition sites. Unfortunately FAW moths seem to be impartial when it comes to choosing an oviposition site, often ovipositing seemingly at random as was demonstrated by this study as well as by Orsucci *et al.* (2018). The two *S. frugiperda* host strains occur in mixed populations on the African continent (Nagoshi *et al.*, 2006; Machado *et al.*, 2008). It may also have affected the results on oviposition preference in this study.

Since host plant selection is made on behalf of the larvae by the adult, a positive correlation is expected between adult host preference and larval performance, but this is not always the case (Berdegué *et al.*, 1998; Rojas *et al.* 2018). In the case of FAW, larvae that are highly mobile suggest that they have the potential to locate and choose their own hosts (Berdegué *et al.*, 1998). Their cannibalistic nature further motivates the need for FAW larvae to disperse away from the oviposition site. The polyphagous nature of FAW allows the larvae to easily come into contact with suitable hosts when dispersal takes place (Rojas *et al.*, 2018). The result is that the gravid moth's oviposition site choice may be unimportant since the larvae will disperse from the vicinity (Refsnider and Janzen, 2010; Rojas *et al.*, 2018).

5.4. FAW and the push-pull system

The important question is how and why the push-pull system, described by Midega *et al.* (2018), functions in suppressing FAW numbers in maize fields. Firstly, it has been established by Orsucci *et al.* (2018) and by this study that FAW moths are impartial to where they oviposit their eggs, therefore the role of volatiles in the trap crop

(Brachiaria, Napier and Vetiver grasses) is null and void. This was further supported by the fact that the larvae disperse away from the oviposition site.

It is more likely that plants such as Brachiaria, Napier and Vetiver act as a barrier around fields, preventing FAW moths from entering the crop field (Finch and Collier, 2012). In Kenya, Napier grass grows taller than maize and acts as a barrier for stemborer moths, preventing them from reaching the maize field (Finch and Collier, 2012). These grasses can also be intercropped with maize to act as a barrier for migrating FAW larvae and disrupt their host location (Hokkanen, 1991; Shelton and Nault, 2004; Cook *et al.*, 2007). Intercropping reduces pest density within crops by disrupting host location. In addition, enhanced natural enemy abundance due to vegetation diversification may lead to increased herbivore mortality due to predation and parasitism (Hokkanen, 1991; Shelton and Nault, 2004; Cook *et al.*, 2007).

5.5. Potential of FAW infestation of maize, sorghum and selected non-poaceous crops

FAW has attained pest status on numerous cultivated plants belonging to the family Poaceae, mainly on maize and sorghum. Although plants from this family are preferred, FAW is also becoming a pest on important broadleaf crops such as cotton and soybean, especially in areas where they are cultivated in rotation with maize (Pitre and Hogg, 1983; Hardke *et al.*, 2015; Gouin *et al.*, 2017). Although soybean, dry bean, cotton, sunflower and groundnut are listed as host plants of FAW (Montezano *et al.*, 2018), the host status of these non-poaceous crops in Africa is unknown.

Larval feeding preference tests conducted in this study using maize, sorghum, soybean, dry bean, cotton, sunflower and groundnut confirmed maize and sorghum as the most preferred crop hosts. Groundnut was the least preferred plant species in both two- and multi-choice tests.

Knowing the host suitability of different crop species that FAW is capable of feeding on, including minor crops or crops that may sustain FAW populations during off seasons, is important in the context of pest management. If moths lay eggs on these crops or even non-crop plants where early instar development occurs on these plants adjacent to or within crop fields, larvae are capable of moving to cultivated crops after

consuming their original host (Luginbill, 1928; Guagliumi, 1973). In addition, polyphagous feeding behaviour can allow build-up or maintain FAW populations outside of the primary cropping season or outside of cropping areas, contributing to increased pest pressure.

Poorer hosts such as cotton, dry bean, groundnut and sunflower may cause a reduction in insect health, making them more susceptible to insecticides, viruses, parasitoids and predators, and as a result, decreased survival rates (Richter *et al.*, 1987). These crops can be planted in rotation with other crops that are more severely affected by FAW to lower their population density to more manageable levels. Preferred crops can also be intercropped with less suitable crop hosts to establish a barrier that influences migrating larvae. Ryder (1968) reported that maize intercropped with sunflower was less severely attacked by FAW and that it had higher yields than monoculture maize fields. Mensah and Sequeria (2004) suggested that maize could be intercropped with beans to contribute to increasing mortalities of larvae that moved between different host plants.

Cotton, although not typically affected by neonate FAW larvae, may become infested by older, stronger larvae that have the potential to migrate to cotton fields should maize fields become over utilized by FAW, unsuitable or removed (Nagoshi *et al.*, 2007). Similarly, Campbell and Wynne (1980) reported that groundnut only became infested by FAW when pest pressure in neighbouring fields were very high, when no other suitable hosts were available, or when a large amount of excess grass was prominent in groundnut fields. It is suggested by this study that broadleaf crops such as soybean, sunflower, dry beans and groundnut are poor hosts of FAW, and that these can be used as a cultural control mechanism to suppress population numbers.

Maize and sorghum are the most preferred hosts of FAW (Pitre *et al.*, 1983; Gouin *et al.*, 2017). Migration to other host plants generally only occurs when the population on maize or sorghum has reached its threshold, the plants become no longer suitable as the season progresses or the suitable plants do no longer occur in the area (Pitre and Hogg, 1988; Nagoshi *et al.*, 2007). It is possible that winter plantings of maize and sorghum crops in areas such as the Mpumalanga lowveld or parts of the Limpopo province, may act as refuges for overwintering populations of FAW in South Africa. Although farmers will protect these crops by means of insecticide applications,

abandoned or neglected fields after the harvest may become refuges that sustain pest populations. It is suggested that there should be an immediate destruction of these crops and any other preferred host plants after the harvest (Knipling, 1980). When any of the high-valued host crops mentioned in the study, besides maize and sorghum, are used as a cash crops in areas where FAW populations persist, maize and sorghum can be used as trap crops. By planting maize or sorghum around less preferred cash crops, maize or sorghum could act as a barrier and trap crop, reducing the movement of larvae into the cash crop fields.

5.6. Future studies

The push-pull system uses Greenleaf desmodium as a repellent intercrop (Midega *et al.*, 2018). It is unclear if Desmodium repels FAW larvae and moths, and further studies should be conducted to determine if Desmodium does indeed play a role in deterring FAW. To better understand the oviposition behaviour of FAW, research should be conducted to determine if and how plant kairomones influence the host finding and orientation behaviour of gravid FAW moths. It has been suggested by Rojas *et al.* (2003) that tactile cues play an important role during oviposition and therefore warrants further research.

Conclusion

FAW moths seem to be impartial to where they oviposit. Moths typically respond to a large variety of volatile compounds suggesting that they are not primarily reliant on volatile cues to locate suitable host plants for oviposition. Attributes of the larvae such as cannibalism, high mobility and polyphagy suggest that larvae migrate away from the oviposition vicinity and could possibly use volatile cues to find their own host plants. Push-pull systems using Brachiaria, Napier and Vetiver grass may work as a trap or barrier strategy, limiting the numbers of FAW moths that lay their eggs on maize. Cash crops such as cotton, sunflower, groundnut and dry bean could be used in crop rotation systems or as intercrops to reduce the extent of FAW infestation on the main crop.

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