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Habitat Management using Stimulo-Deterrent Diversion techniques to decrease infestation of sugarcane by *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

Alistair Barker

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfillment of the requirements for the Degree of Master of Science

Johannesburg, 2008

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree in any other University.

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Abstract

Despite research focused on the control of *E. saccharina* Walker (Lepidoptera: Pyralidae), it remains the most destructive pest of sugarcane in South Africa and therefore a shift in the approach to the control of this insect was required. Habitat management techniques were employed through trials to understand the mechanisms used by insect pests in their host plant oviposition behaviour. Indigenous and beneficial non-crop plants, which could be used to attract insect pests away from sugarcane were identified and tested. An African grass, *Melinis minutiflora*, shown to be repellent to lepidopteran stemboring pests, was used in field trials in sugarcane.

Eldana saccharina was shown to be fairly indiscrimate in choosing host plant species. Oviposition trials showed that females made no choice for host plants oviposition based on the volatiles released by those plants. Females showed no preference for males over test plants. But did consistently move and make a plant choice more often than male moths. Moths were not attracted by the volatile stimuli of a host plant and the availability of cryptic sites might be a factor that influenced ovipositing females to choose a host plant.

Field trials tested the repellent action of *Melinis minutiflora* against *E. saccharina* and were shown to be more effective over a big field with space for *M. minutiflora* to establish thick undergrowth alongside a field plot. The other field sites showed no significant effect from *M. minutiflora* intercropped into treatment plots, or a slight negative effect. Later sugarcane planting times in relation to the grass planting time was a possible reason for the positive result in only two field sites allowing the grass to grow and establish before the sugarcane competed with the grass for sunlight.

A cost benefit analysis of planting a hectare of sugarcane with *M. minutiflora* showed an economic benefit linked to reduction in *E. saccharina* infestation. Field sites with low population pressure from *E. saccharina* would not yield the economic benefit of planting this grass. There was no significant loss in the height, density or sucrose yield (ERC% cane) between control and treatment plots in the field plots due to the presence of *M. minutiflora.* Comparison of weed biomass between treatment and control plots showed a significant reduction in the treatment plot where *M. minutiflora* out-competed the weeds already present. This grass was advantageous in the sugarcane field as it sometimes reduced moth infestation but did not significantly compete with sugarcane and showed weed suppressing potential.

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Chapter 1:
General Introduction

1.1. Introduction

Much research has been focused on the control of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) since it invaded sugarcane in South Africa in the 1940's and then again in the 1970's (Carnegie, 1973; Carnegie, 1974). Since the 1970's, research into the control of this indigenous African sugarcane stalk borer has centred on the more conventional methods such as chemical, mechanical, cultural and biological control. In addition, the identification of resistant cultivars is an important aspect of stemborer control with ongoing research being done to further develop the use of resistant varieties (Keeping, 2006). Despite these efforts, *E. saccharina* remains the most destructive pest of sugarcane in South Africa (Keeping, 2006), and therefore a shift in the approach to the control of this insect was required. As *E. saccharina* is indigenous, it was hypothesised that it could potentially be managed by using natural components of its indigenous habitat, such as other insect species. Increasing the proportion of these indigenous components, especially in large-scale sugarcane fields, will help develop a more biodiverse agro-ecosystem. This has been part of cultural control of pests practiced on a smaller scale, by farmers in Africa for many years (Van den Berg *et al*., 1998).

Manipulation of the agro-ecosystem has not been attempted in sugarcane before. This project was designed to try to understand the mechanisms used by insect pests in their host plant oviposition behaviour, and to identify indigenous and other beneficial non-crop plants, which could be used to attract insect pests away from sugarcane. In addition this project tests the use of a previously identified African grass, shown to be repellent to lepidopteran stemboring pests, in sugarcane.

1.2. Insect Control

1.2.1 Chemical Control

Chemical control forms the basis of modern insect pest control, especially on large-scale farms growing a cash crop (Van den Berg and Nur, 1998). However, problems relating to chemical use, particularly in Africa, include economic, social and health issues (Abate *et al*., 2000). Most farmers in South Africa are either small or medium scale growers,

cultivating less than one hectare up to 10 hectares, and are not able to afford chemical sprays to control pests (Kfir *et al*., 2002). Therefore there is a move towards control methods that are easy to implement and additionally do not have an adverse effect on the environment (Van den Berg *et al*., 1998). Insects may evolve resistance to chemical pesticides, therefore alternative control strategies such as cultural or biological control may be required (Hokkanen, 1991).

1.2.2 Biological Control

Biological control programmes for *E. saccharina* were started 25 years ago and are still considered one of the major options for the control of this pest in sugarcane (Conlong, 1994, Conlong and Kasl, 2000).The cryptic nature of this insect prevents insecticides from reaching the pest (Conlong, 2001), but suitable biological control agents can reach the caterpillars in sugarcane stalks. Biological control of sugarcane pests has been attempted in affected countries of Africa; South Africa, Kenya, Tanzania, Zambia, Benin, Cameroon, Ghana and the Ivory Coast (Conlong, 2001). The identification of arthropod predators (Leslie, 1988), parasitoids (Carnegie and Leslie, 1979), nematodes (Spaull, 1990) and genetically engineered bacteria (Herrera and Thompson, 1989), prompted their use in attempts to reduce the population size of stemborer pests (Conlong and Kasl, 2000). Classical biological control is generally the first attempt in biological control of a specific pest. Early biological control programmes by the South African Sugarcane Research Institute (SASRI) involved searching for indigenous egg parasitoids and larval parasitoids in the wetland sedges which *E. saccharina* naturally inhabits (Conlong and Kasl, 2001).

1.2.2.1 Classical biological control

Classical biological control is the importation of natural enemy organisms from the country of origin of the pest species, for release against the pest species in its new range. Classical biological control is not used for the control of *E. saccharina* because it is an indigenous insect, and its natural enemies occur naturally in the same area, but not necessarily in the same plants. However, Conlong (1990) argued that as *E. saccharina* expanded its host range into sugarcane from its natural host sedges relatively recently; natural enemies should be available from the indigenous host plants. These could be mass reared and released against the pest on the new host plant, sugarcane. The other approach possible is to introduce exotic parasitoids of similar pest stalk borers and assess which of

them attack *E. saccharina* in laboratory surveys (Conlong, 1994). There is of course a danger of introducing non-host-specific agents that could shift hosts, particularly when target populations are reduced and thereby affect the indigenous Lepidoptera populations. Exotic parasitoids of sugarcane pests were tested in a laboratory for host specificity before being released into the field (Carnegie and Leslie, 1979). Parasitoids that were not host specific were not released.

1.2.3 Cultural Control

Cultural control is one of the earliest agricultural methods used to manage insect pests, diseases, nematodes and weeds in crop systems (Hoy and McGawley, 1998). For centuries, since man first started farming and obtaining food through agriculture and before modern technologies provided chemicals for control of pest plants and insects, farmers used cultural control and generally ecologically beneficial farming practices in their fields.

Cultural control involves the manipulation of the agroecosystem in a way that will render it unfavourable to pest species, and is the most relevant and economically feasible method of control available to mostly resource poor farmers in Africa (Van den Berg *et al*., 1998). The manipulation of the crop and associated land has been applied to the farming of maize since the introduction of this crop onto the African continent (Kfir *et al*., 2002; Van den Berg *et al*., 1998).

Cultural control is currently implemented in the sugarcane industry in a number of ways for the control of *E. saccharina*.

- Preharvest burning removes eggs and young larvae on the dead plant material and some on or inside the stalks. However, many larvae survive in the soil, then move into the cut stalk and continue to develop, increasing damage after the next ratoon (Leslie, 1994).
- Cutting sugarcane below ground level at harvest time reduces re-infestation from eggs and larvae left behind in the stalk base (Carnegie, 1973).
- Placing a heap of soil over the cut sugarcane stools helps reduce the survival of individuals left in the stalks (Carnegie, 1973).

- Adding silicon, in the form of calcium silicate, to fields of sugarcane at planting provided greater protection against *E. saccharina* attack in susceptible, water-stressed sugarcane (Kvedaras *et al*., 2006).

Most infestation and damage by stemborers occurs when the farmers leave the sugarcane in the field after maturity to increase sucrose levels, before the sugarcane is harvested. The cultural practice of harvesting young sugarcane at 12 months in high pest population areas and 15 months in areas with lower pest populations, has been implemented (Leslie, 1994). However, there are economic penalties, in reduced sucrose yield, for harvesting sugarcane early. Harvesting young sugarcane does not stop other stemborers, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), as these pests feed on young to medium aged crops (Girling, 1972; Girling, 1978). These insects are not major pests of sugarcane in South Africa.

1.2.4 Resistant crops

Plant resistance to herbivore damage is not strictly a cultural control method as it requires specific breeding and research to develop and is a fairly recent advancement in agriculture. Resistant varieties are important for control of stemborer, *E. saccharina*, in South African sugarcane, and field based sugarcane variety resistance to *E. saccharina* has been assessed in South Africa since 1979 (Keeping, 2006). Integrated management of this pest on sugarcane has focused on combining varietal resistance, biological control, chemical control and crop management. Resistant varieties give intrinsic pest control to sugarcane that does not usually involve environmental factors and is generally compatible with other methods of insect control (Bosque-Perez and Schulthess, 1998), and is equally effective against low populations of pests as it is in fields with higher population levels (Kfir *et al*., 2002). Varietal resistance can be complimentary with other cultural control methods, and habitat management practices should not reduce the effectiveness of resistant varieties and may enhance it further.

1.3. Habitat management

For centuries small scale farmers in Africa have used methods of habitat management, such as intercropping or mixed cropping (Khan *et al*., 2000) to reduce the risk of crop

failure, achieve higher yields and improve the soil fertility (Kfir *et al*., 2002). This includes the selection of specific plants for introduction into the agricultural environment, which will have a beneficial effect on the crop plants. Habitat management can reduce the pest insect populations in the current crop or can be beneficial to the crop in the same soil in the following season (Kfir *et al*., 2002). Methods of habitat management such as green manuring, fallow cropping, managing planting dates and harvesting dates, and intercropping provide the most direct benefit to the crops (Van den Berg *et al*., 1998). The primary objective in using habitat management in sugarcane is to prevent pests from getting onto sugarcane.

1.3.1 Methods of Habitat Management

1.3.1.1 Green manuring

Green manures are plants grown within a cash crop primarily for erosion reduction, weed suppression and recycling excess nutrients in the soil (Prasifka *et al*., 2006). The practice of green manuring, ploughing green crops into the soil, has been practiced for thousands of years (Anonymous, 2000) and can add benefit to a field by increasing soil organism biodiversity, structure and soil water relationships (Tillman *et al*., 2004). Green manures provide food for micro-organisms, which increases nutrients which in turn encourages more natural enemies to visit the crop (Rhodes, 2005; Tillman *et al*., 2004). This project uses aspects of green manuring in the experimental field setups.

1.3.1.2 Rotating crops

Crop rotation is also an ancient cultural control method used in habitat management and is recommended for pest control where pests tend to remain close to sites where their larvae pupate (Hokkanen, 1991). The host crop is followed in the next season by a non-crop or the soil is left fallow, so that any pests that may have over wintered are faced with no hosts in the following season. This is particularly effective for pest species with a small host range and limited dispersal (Van den Berg *et al*., 1998). *Eldana saccharina* has limited mobility; larvae move from hatching sites along the leaf and up the stalks, and disperse short distances through dry litter (Leslie, 1993). Many farmers cannot rotate their crops due to shortage of available land (Tefera, 2004). Additionally sugarcane is generally regrown in the following year by ratoon crop, which after a number of years, if fields are rotated, would therefore need to be ploughed out and replanted as sets, at additional cost.

1.3.1.3 Planting and harvesting dates

Adjusting planting and harvesting dates is an important means of pest control in Africa (Abate *et al*., 2000) because the starting numbers of pests species are usually reduced early in the next season. Planting early in the season is an effective control method against stemborers on sorghum and maize, practiced in parts of Africa (Abate *et al*., 2000). This strategy is effective because these faster growing crops can be ready to harvest at the time when the stemborers are approaching peak populations so the infestation levels are low. If these crops are planted later, so that they mature later, the stemborer population will already be established and more damage will be done. Harvesting dates need to be arranged in response to population peaks in the pest species. *Eldana saccharina* has two peaks in moth populations in approximately April and November each year (Carnegie and Leslie, 1990). At these times the main crop should be at an age where it can be harvested or germinating so that the moth peak occurs at a time when the sugarcane is not that attractive to *E. saccharina*.

Harvesting of sugarcane in sections allow parts of the crop to become refugia for natural predators of the insect pests that infest the protected crop (Hossain *et al*., 2001). These small sections are allowed to overwinter in order to maintain natural predator populations in the crop.

1.3.1.4 Intercropping

Intercropping is the planting of more than one species of plant with dissimilar heights or different growth times in the same field to maximise the use of space in that field (Adeniyan and Ayoola, 2007). Intercropping has been used by subsistence farmers to increase crop yields but also to suppress weeds and reduce pest populations (Abate, 1991). Intercropping has led to insect pest suppression although farmers do not necessarily grow specific intercrops to exploit this effect (Kfir *et al*., 2002). Intercropping is used to increase productivity of crops planted together in limited land resources. The intercrop enhances the yield of the associated crop, when crops are matched correctly. This increase in plant biodiversity can increase the insect populations both pest and natural enemy species. Field studies in Africa have been conducted to identify the best combination of intercrops for reducing stemborer populations (Kfir *et al*., 2002). The benefit of

intercropping, along with increased biodiversity in a monocropped field, is that intercrop plants provide shelter and alternative food sources, such as nectar, for natural enemies of the pest insect (Abate, 1991). Khan *et al.*, (2001) found a significantly higher level of infestation by stemborers in maize fields without intercrop plants than fields with an intercrop.

Trap crops are within the scope of intercropping and are defined as plant stands which are used for the purpose of attracting; diverting, intercepting and/or retaining targeted insects in order to reduce damage to the main cash crop (Shelton and Badenes-Perez, 2006). Related to trap cropping is mixed cropping, planting two or more different crops in the same field that have a reciprocal interaction. Mixed cropping systems have advantages additional to those given by trap crops, including repelling insect pests, increasing natural enemies in the field, suppression of weeds by shading with mixed canopies or allelopathy and improved productivity of land (Altieri and Nicholls, 2004).

Improved land use is achieved by employing plants that have uses additional to attracting natural enemies or repelling pest species, usually as a fodder crop. By introducing these plants into the crop fields, areas of open ground for weeds are reduced and thereby competition with the cash crop is reduced (Altieri and Nicholls, 2004).

Work in Kenya on maize involved planting native African grasses that are highly attractive to ovipositing female stemborers, around maize fields to decrease pest populations (Overholt *et al.*, 2003). Intercropping cassava with maize in Nigeria was found to reduce larval numbers of *E. saccharina*, *B. fusca* and *S. calamistis* by approximately 50% when compared with monocrop maize (Kaufmann, 1983).

Khan *et al.*, (1997a) found intercropping maize with molasses grass, *Melinis minutiflora* Beauv. (Poaceae) caused a decrease in damage to the maize plants by *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) from 39.2% to 4.6%. Intact *M. minutiflora* releases volatile components containing (E)-4, 8-dimethyl 1, 3, 7 nonatriene, (Khan *et al.*, 2000), which has been implicated as a plant distress signal which recruits predators and parasitoids to the damaged plant and surrounding crop (Kfir *et al.*, 2002). 'Nonatriene' is also released by stemborer damaged maize (Kfir *et al.*, 2002).

When choosing a plant species to intercrop for pest control benefits, and yield advantages, a plant that is repellent to ovipositing females of the pest is ideally required. If the pest is

repelled by these plants they will not lay their eggs on the cash crop associated with them (Kasl, 2004). Another benefit of intercropping is that female insects will spend more time searching for a suitable host plant amongst the intercrops and so oviposit fewer eggs on the actual host (Skovgård and Päts, 1996). At the next level of intercrop selection, if the intercropped plants stimulate the pest larvae to feed on them and not disperse off onto neighbouring plants (Kasl, 2004), then they will act as a sink for the pest. Plants that act as sinks for pests or reduce larval survival are termed dead end crops (Shelton and Badenes-Perez, 2006).

1.3.1.5 Plant Volatiles

Insects are known to respond to chemical signals from other individuals of the same species, (pheromones) and chemicals from other species (allomones) that are detected in the environment (Turlings *et al*., 1990). Volatile chemical cues released by plants play an important part in allowing insect pests to find their host plants at a distance (Bruce *et al*., 2005). These particular volatiles are naturally emitted from the plant and not caused by feeding or damage to the plant material. If the insect receives the correct combination of sensory cues, a particular plant can be recognised as a potential host and these insects are attracted to them. When the wrong combination of cues is perceived, this plant is regarded as a non-host plant and can be avoided by the insect (Bruce *et al*., 2005). Herbivorous insects need to process numerous sensory inputs, from olfactory and gustatory cues, to physical information such as plant colour, shape and texture to find potential hosts (Bruce *et al*., 2005). These volatiles may also help predatory insects to find their herbivorous prey. Semiochemicals also come from sex pheromones, larval frass and volatiles emitted by the host plants (Mbata *et al*., 2004) and when released by plants under attack from insect herbivores they are particularly helpful to parasitoids (Turlings *et al*., 1990; Verkerk *et al*., 1998), which use volatiles when foraging for hosts (Dicke and van Loon, 2000, Turlings *et al*., 1990). In certain cash crops, parasitoid attractive volatiles emanate from stemborer-damaged stalks, and frass produced by feeding larvae (Havill and Raffa, 2000). The whole plant produces these volatiles even when only a portion of the plant is being damaged by herbivory.

1.3.1.6 Attractive and repellent plants

One method of pest management utilises plant volatiles in a habitat management system by planting non-crops that are attractive, and or plants that are repellent to the pests, in or near the crop fields. This strategy known as "push-pull", has been used successfully by cotton farmers (Pyke *et al*., 1987). Miller and Cowles (1990) coined the term 'stimulo-deterrent diversion', (SDD) for push-pull plants and used SDD to protect onions from onion flies by attracting (pulling) gravid, female onion flies away from the onion crops using onion culls while at the same time adding an additional push pressure using a feeding deterrent and a toxin (Miller and Cowles, 1990).

Push-pull systems involve manipulating the sensory perception or the behaviour of the pest away from the protected host (push) and towards an attractive alternate source; (pull) which removes it from the crop system. This is achieved by introducing volatiles, which make the host organism, in this case cotton, unattractive so that the pest, *Heliothis* spp (Lepidoptera: Noctuidae), will leave the crop to find an alternative host, which emits a more attractive volatile than the cotton field. Often this alternative is a killing lure which may prevent full development of the pest species on it (Pyke *et al*., 1987).

Potential push or pull plants are first tested in the laboratory or in field trials for their effectiveness to attract or to repel pest species when planted with a cash crop.

Khan *et al*., (2003) exposed a test organism (pest) to the non-host plant volatiles, along with volatiles from the host plant, in an olfactometer setup. Olfactometer trials are a simple method used in evaluating the behavioural responses of insects to volatiles (Beerwinkle *et al*., 1996). Test organisms are placed in a container with arms or exits. The volatile to be tested is drawn through one arm and clean air is drawn through the other arm. The insect is released at a position where it can sample both airflows and select between them (Beerwinkle *et al*., 1996). Alternately the trial can be done on a whole-plant scale where the test plant is setup in a cage with a control species (often the established host plant), and the insects are allowed to make a choice between them. Test plants that attract the pest organism more often than host plants are characterised as pull plants. Test plants that are chosen by the insect significantly less often than the host plants are labeled push plants.

Smart *et al.,* (1994) showed that the pea and bean weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae) could be controlled with a push-pull strategy by using semiochemical baited lures in plots. An antifeedant to reduce damage on these host plants and an aggregation pheromone were successfully used to divert the pest away from the crop to areas of alternate plants chosen by the pest, that could be discarded (Smart *et al.,* 1994). Khan *et al.,* (1997a) showed that *Melinis minutiflora* planted as a mixed crop, in a field of maize, acted as a push plant to stemborers while attracting the stemborer parasitoid, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), which further reduced stemborer infestation.

In a push-pull project setup by the ICIPE (International Centre of Insect Physiology and Ecology), stemborers were attracted away from the main maize crop by a border crop of *Sorghum sudanense* Stapf. (Sudan grass). The approach relied on a carefully selected combination of companion crops planted around and between the maize plants for manipulation of the agro-ecohabitat of pests and their natural enemies (Van den Berg, 2003). The trap crop, or pull plant, must be more attractive to the pest species than the protected crop, preferably over a long period of time. This attraction would need to be at the critical time of growth of the crop so as to pull the pest species away from the protected crop when it is most vulnerable to infestation (Hokkanen, 1991).

1.4. Sugarcane

Sugarcane (*Saccharum* sp.) is a rigid upright grass grown commercially for the production of sugar, which is obtained from the sap of mature stalks. Sugarcane has been grown along the coast of KwaZulu-Natal for more than a century (Atkinson, 1980, Van der Bank *et al.*, 1990). Originally found in Papua New Guinea and surrounding regions (Osborn, 1964), it is now grown worldwide in tropical and subtropical areas. In South Africa sugarcane is an important agricultural crop in regions of KwaZulu-Natal and Mpumalanga for both large and medium scale growers as well as individual small scale growers. The hard upright stem is fed on by larvae of stemborers from the Pyralidae or Crambidae (Overholt, 1998). The larvae of these moths are major pests of commercially grown sugarcane. Sugarcane was established in South Africa in 1847 (Osborn, 1964), where it flourished, but also gathered a number of pests, among which the indigenous stalk borer, *E. saccharina* is considered the most destructive (Keeping and Meyer, 2002).

Crop loss estimates are difficult to determine as the yield from sugarcane is dependent on climate, sugarcane variety and growing conditions of the field (Conlong, 1994). However, it is generally accepted that *E. saccharina* reduces the yield of sugarcane by approximately 0.1% of the total yield for every 1% stalks bored (Smaill and Carnegie, 1979) or 1% loss in useable sugar for every 1% internodes bored by the pest insect (King, 1989). This damage is determined from sugarcane field surveys and farmers are able to estimate the loss of sucrose yield from the number of nodes damaged in a representative sample of surveyed stalks (Conlong, 1994).

1.5. *Eldana saccharina*

Eldana saccharina is a stem boring insect pest of sugarcane, indigenous to areas of Sub Saharan Africa and naturally found in wetland sedges (Cyperaceae) (Conlong, 1994). Walker first described *E. saccharina* in 1865 from sugarcane in Sierra Leone (Conlong, 1994). It has since been collected in several graminaceous crops (Atkinson, 1980; Conlong, 2001) for more than a hundred years (Carnegie, 1974). *Eldana saccharina* was noticed as a pest of sugarcane in South Africa in 1939 in Umfolozi Flats (Carnegie, 1973; Dick, 1945). Populations of *E. saccharina* in sugarcane in South Africa then declined in the 1950's. However in 1973, a field at Umfolozi was discovered to be too damaged to be worth harvesting (Carnegie, 1974). *Eldana saccharina* populations have since increased dramatically and spread to other regions down the coast of KwaZulu-Natal (Conlong *et al*., 1988).

The life cycle of *E. saccharina* has been well documented by many researchers studying this pest in maize and sugarcane (Carnegie, 1974; Conlong, 1994; Dick, 1945; Girling, 1972; Girling, 1978; Waiyaki, 1968). The wetland sedges, *Cyperus papyrus* L. (Cyperaceae) and *C. dives* Delile (Cyperaceae) are the indigenous hosts of the larval stage of *E. saccharina* (Conlong and Kasl, 2001). The larvae are also able to bore into other crops such as *Sorghum bicolor* L. (sorghum), *Eleusine coracana* Gaertn. (millet) and *Zea mays* L. (maize) (Girling, 1972). A non-crop grass, *Pennisetum purpureum*, in the family Poaceae has been shown by Kaufmann (1983) to harbour eggs and egg batches from *E. saccharina*, though the hatched larvae do not survive on this grass. The infestation of crop hosts such as sugarcane is assumed to have happened when areas of natural habitat, where the host plants of this species grow, were reduced by sugarcane replacing their indigenous

hosts (Atkinson, 1980). Agricultural practices which increase nutrient levels have also made sugarcane beneficial for the survival and spread of *E. saccharina* (Atkinson, 1980).

1.5.1 Manipulation of oviposition behaviour

Female *E. saccharina* moths oviposit eggs in the fold of dead leaves of sugarcane (Leslie, 1990) and may lay eggs directly against the sugarcane stalk at the base of a leaf sheath or between the stem and the soil at the base of the stalk (Dick, 1945). These cryptic locations chosen by the female have three sides that must touch the tip of the ovipositor (Sampson and Kumar, 1985). Laying eggs in these locations has the dual advantage in that the eggs are hidden from egg parasitoids and predators, and are unaffected by insecticides, which do not penetrate far into the sugarcane canopy (Leslie, 1994). Cage trials by Kasl (2004) showed female *E. saccharina* lay egg batches on any plant material available. Eggs were also laid on the cage netting, the ground around the plants, and under the edges of the pots. Larvae move from these locations and must therefore to some extent select a host plant. Leslie (1993) showed that larvae can move distances of up to 800 mm in the leaf litter and were collected from regions lower on the stalk than where the eggs were laid on the dead leaf material. Larvae are also highly mobile on *Cyperus* spp. plants and their movement is dictated by larval feeding sites. Larvae move up the plant from eggs laid on the leaves on *C. immensus* and down the plant from eggs laid on the inflorescences on *C. papyrus* (Atkinson, 1980). Dispersal by *E. saccharina* to adjacent stalks on sugarcane is possible by way of the tangle of leaves between the rows of sugarcane. This may be how local infestation spreads in the field (Leslie, 1993), where eggs are laid in batches of up to 600 but single sugarcane stalks rarely contain more than two larvae.

1.5.2. Economic loss to sugarcane by *E. saccharina*

Eldana saccharina is a serious problem in sugarcane because of the economic loss it causes particularly to “stand over” sugarcane (cane grown for more than 12 months) (Carnegie, 1974). Long term control of this pest in most affected areas has not yet been achieved at a level that is economically viable, below the economic injury level, which is the lowest population density of a pest that will cause economic loss (Stern *et al*., 1959). The cost of controlling the pest must be less than the profit gained from the increased yield of sugar available as a result of the control method. Goebel *et al*., (2005) calculated the economic injury level, at 7% SLR (stalk length red), which was equal to 54% SD

(stalk damage). If sugarcane stalks are damaged more than this percentage, they will not be economically viable to mill. The amount of stalk length red is directly related to how many nodes of the stalk are damaged. The nodes that have been damaged by a stemborer often have a secondary infection from a fungus *Fusarium sp.*, which causes the middle of the sugarcane stalk to go red.

1.6. Previous work in SDD on sugarcane

Kasl (2004) tested the repellent and attractive nature of a number of non-crop plants to *E. saccharina* Walker (Lepidoptera: Pyralidae) in field trials and in laboratory experiments. The main results from that study are as follows:

Eldana saccharina females preferably lay their eggs on sugarcane plants that are six months old or older (Kasl, 2004; Keeping, 1999). Although not significant, more than 50% of the eggs laid by *E. saccharina* were found on *Sorghum bicolor* L. (wild sorghum) and *Pennisetum purpureum* when placed with sugarcane in a caged, whole-plant experiment, compared to sugarcane only control cages (Kasl, 2004). Sugarcane placed in cages with *S. bicolor* and *P. purpureum* had fewer eggs laid on them than sugarcane alone in control cages. These two non-crop companion plants contained more eggs and egg batches than any other non-crop plant tested in the cage laboratory trials. These differences were not significant but these plants may still have potential as pull plants due to the high number of potential pest larvae removed from the crop by these two plants (Kasl, 2004).

Cyperus dives was the host plant significantly preferred by female moths for oviposition compared to sugarcane and *P. purpureum*. Larvae of *E. saccharina* fed preferentially on the green plant material of test plants, *C. dives* and sugarcane. Larvae may have a dispersal phase (Leslie, 1993) in which they move from the location where the eggs were laid. *Cyperus dives* and *C. papyrus* were the hosts most favoured by *E. saccharina* larvae (Kasl, 2004). However, *C. papyru*s and *C. dives* are wetland sedges requiring water in regular supply in order to grow. Therefore, these potential pull plants can only be used in fields of sugarcane that are close to a wet area such as a swamp, river or marshland.

Field trials in the current study had wetland areas with *Cyperus dives* growing in the vicinity at each of the field sites. These natural host plants were expected to balance out the ‘push’ from *M. minutiflora*, with a ‘pull’ towards these plants for *E. saccharina*.

Kasl (2004) showed that *Melinis minutiflora* was repellent to *E. saccharina* females in olfactometer tests in the laboratory. Thereafter fields were planted with *M. minutiflora* where the infestation levels were expected to decrease as a result of the plants' repellent effects. However, this was not shown when *M. minutiflora* was planted as a single barrier line outside the sugarcane. Damage by *E. saccharina* was significantly reduced in two out of five fields where *M. minutiflora* was intercropped. However, in three of the field sites no direct beneficial effect of *M. minutiflora* was seen in reducing the infestation when compared with control fields. The pattern and timing of planting and irrigation may be important factors affecting the success of this grass as a push plant (Kasl, 2004). If the grass is planted too late it may be shaded out and if it is planted too densely it may compete with sugarcane for soil nutrients and water.

Kasl (2004) showed that the parasitic wasp, *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) increased its parasitism of *E. saccharina* pupae when placed in cages with *M. minutiflora* and *M. nerviglumis* Franch (Poaceae) (Conlong and Kasl, 2001). Although shown above to be influenced by the presence of *Melinis sp.*, the wasps were unable to learn this association between the plants and presence of their host, when exposed to *E. saccharina* pupae prior to release into the cage (Kasl, 2004).

1.6.1. Potential push-pull plants

Selective breeding of sugarcane for higher sucrose content is thought to have caused a loss of the chemical signals normally released when it is subjected to herbivory; consequently indigenous parasitoids that use chemical signals to locate *E. saccharina* in *Cyperus* spp. are not attracted to sugarcane (Conlong and Kasl, 2001, Smith *et al.*, 2006). In addition, parasitoids are not adapted to associate the chemical signal from sugarcane with their *E. saccharina* hosts (Kasl, 2004).

Kasl (2004) investigated five plants with regard to their potential to induce volatile driven effects in insects on sugarcane (Pull plants, Table 1.1). The plant that was shown to have the most potential for stimulo-deterrent diversion because of its repellent nature to *E. saccharina, M. minutiflora,* was also tested by Kasl (2004), (Push plants, Table 1.1).

Table 1.1. Plants tested by Kasl (2004) as potential pull or push plants to *E. saccharina*, with common names, alternative uses, and their effect on *E. saccharina* in laboratory trials.

Mode of Action	Species	Common Name	Alternate Use	Effects
Pull Plants	*Zea mays* (L.) Graminaceae	Maize	Grain crop	Attractive
	Sorghum bicolor (L.) Poaceae	Wild sorghum	Grain crop, fodder, weed suppression	Attractive
	Pennisetum purpureum (Schumacher.) Poaceae	Napier grass	Fodder grass, erosion control, weed suppression, medicinal	Attractive
	Cyperus papyrus (L.) Cyperaceae	Papyrus	Building material, crafts	Attractive
	Cyperus dives (Delile.) Cyperaceae	Giant water grass	Cultural uses, Rhizome has medicinal uses	Attractive
Push Plants	*Melinis minutiflora* (Beauv.) Poaceae	Molasses grass	Fodder grass, weed suppression, medicinal uses	Repellent

The work done by Kasl (2004) has prompted further work on her findings and similarly the results from this current study will prompt further implementation of habitat management strategies into sugarcane fields.

Plants shown in Table 1.2 may be attractive or repellent to *E. saccharina* and were tested in this study by similar methods to those followed by Kasl (2004). *Pennisetum purpureum* is being tested again in the current study as a repeat of the methods followed by Kasl (2004) to see if similar results will be shown. Other plants in Table 1.2 have characters that could potentially be beneficial in habitat management strategies for *E. saccharina* infestation reduction in sugarcane.

Table 1.2. Plants to be tested in laboratory trials and field trials in this project as potential push or pull plants, common names and alternate uses of these plants

Mode of Action	Species	Common Name	Alternate Use
Potential Pull plant	*Vetiveria zizanioides* (L.) Nash Graminae	Vetiver grass	Soil erosion, slope stabilisation
	Coix lachryma jobi (L.) Poaceae	Jobes tears	Fodder grass, cultural uses
	Paspalum urvillei (Steud.) Poaceae	Vasey grass	Fodder grass
	Pennisetum purpureum (Schumacher.) Poaceae	Napier grass	Fodder grass, erosion control, weed suppression, medicinal
Push Plants	*Melinis minutiflora* (Beauv.) Poaceae	Molasses grass	Fodder grass, weed suppression, medicinal uses

1.7. Aims

The aim of this study is to test *Vetiveria zizanioides*, *P. urvillei*, *P. purpureum* and *C. lachryma jobi* in olfactometer and oviposition trials, in the laboratory for their potential as pull plants for *E. saccharina*. Field work aims to show *Melinis minutiflora,* identified as a push plant (Kasl, 2004), is effective when intercropped with sugarcane to repel *E. saccharina* away from this crop. The last aim of this study is to determine any potential, additional benefits these field tested plants may have for sugarcane agriculture such as weed suppression.

The knowledge of the potential effects of these "new" plants and established push plant, could give farmers more choices when implementing cultural control methods and making changes to the sugarcane farming environment.

1.8. Key questions

- Are *V. zizanioides, C. lachryma jobi, P. purpureum* and *P. urvillei* preferred to sugarcane for oviposition by *E. saccharina?*
- Are *V. zizanioides, C. lachryma jobi* and *P. urvillei* attractive to *E. saccharina* in olfactometer trials?

- Does *M. minutiflora* repel *E. saccharina* and reduce its infestation of sugarcane in field trials?
- Are there additional benefits and consequences to planting *M. minutiflora* in rows next to sugarcane?

1.9. Hypotheses

The attractive nature of non-crop plants, *V. zizanioides*, *P. urvillei* and *C. lachryma jobi*, to *E. saccharina* adult males and females for oviposition will be determined with the use of an olfactometer. The volatiles drawn from these plants will attract more moths than those from sugarcane.

The test plants, *V. zizanioides*, *P. urvillei*, *P. purpureum* and *C. lachryma jobi* are hypothesised to attract more gravid female *E. saccharina* and thereby more eggs and egg batches than sugarcane in ovipositional preference trials.

The intercropping of *M. minutiflora* into field sites is hypothesised to decrease damage to sugarcane by *E. saccharina* more than unmodified control plots.

1.10. Dissertation structure

This dissertation is divided into six chapters. The first chapter is a literature review and introduction to the project background. The next two chapters deal with laboratory work, covering the ovipositional preference of *E. saccharina* to various non-crop plants in cage trials and olfactometer trials testing the attractive or repellent nature of various non-crop plants. The fourth chapter reports the field trials on sugarcane with *M. minutiflora* intercropped as a repellent crop, and the resulting *E. saccharina* infestation. Chapter five deals with the weed suppressing properties of *M. minutiflora* and the competitive effects of *M. minutiflora* on sugarcane. The last chapter summarises the preceding chapters, draws conclusions from the study, and makes recommendations for farmers and possible future research in this area of habitat management.

Chapter 2:
Ovipositional preference of *E. saccharina* to various non-crop plants in cage trials

2.1. Oviposition by Insects

Female insects generally search for a specific location to lay their eggs, often on material that will become the food for the emerging offspring (McClure *et al.*, 1998), and will use many cues when searching for a suitable host plant. These cues can be chemical, visual, or tactile. Insect taste and odour receptors can detect and differentiate between the large range of chemicals saturating the environment and allow them to find food, mates and possible oviposition sites (Hallen *et al.*, 2006). The Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), will not oviposit in the absence of gustatory and olfactory stimuli (Justus and Mitchell, 1996). These moths were manipulated with extracts from *Chrysanthemum morifolium*, in olfactometry and oviposition trials by Liu *et al.*, (2006), and showed that treated host plants had fewer eggs laid by *P. xylostella* than host plants that were untreated (Liu *et al.*, 2006).

Oviposition site preference and performance of offspring are closely related when the female lays eggs on the host where the offspring must complete development (McClure *et al*., 1998). Larvae often have limited mobility to forage; therefore the choice of oviposition site by females is crucial to offspring survival. Oviposition sites chosen by females should coincide with sites of high nutritional value and/or low predation risk and competition pressure (Marchand, 2003). As an example, in certain families of Diptera, the smell of decaying organic material will attract females to oviposit (Noorman, 2001). In the Lepidoptera, *Heliconius erato phyllis* (Fabricius) (Lepidoptera: Nymphalidae), was shown by Kerpel *et al.*, (2006) to oviposit significantly more on plants enriched by nitrogen, which in turn led to decreased larval development time and increased adult size. Female moths make a choice for oviposition sites that are beneficial to their offspring. It can be assumed that the plant species with the most number of eggs laid on it, is the most preferred host and the plant with the second most eggs and egg batches is less preferred (Thompson, 1988).

2.1.1. *Eldana saccharina* oviposition

Female moths of *E. saccharina* are not strong flyers, only being able to fly a short distance to find suitable host plants and therefore usually lay their eggs close to adult emergence sites (Carnegie, 1974). Kasl (2004) found that *E. saccharina* female moths are fairly indiscriminate in their choice of oviposition sites when given a choice of potential host plants and sugarcane. In the field, Carnegie (1974) and Dick (1945) mentions that preferred oviposition sites include leaf sheaths and areas between the soil and stalk base, although they may lay on earth clods or residual plant material. Female moths prefer to lay eggs on dry leaf material rather than young green leaves (Atkinson, 1980; Atachi *et al*., 2005). Plants with more dry leaf material should be chosen more often for oviposition (Leslie, 1990) by moths with an inclination for cryptic oviposition (Carnegie and Smaill, 1982). Females are able to lay eggs up to two days after they are mated (Waiyaki, 1968), while unmated females can wait up to four days before laying sterile eggs (Girling, 1978). Eggs may be laid singly or in batches ranging from two up to 200 eggs (Teetes and Pendleton, 2001). Each batch on average contains 20 eggs (Carnegie, 1974) and a total of 450 - 600 eggs are laid in a female life time (Girling, 1978).

In a sugarcane field attacked by *E. saccharina*, most damage is found on the older plants (Conlong, 2001). Atachi *et al*., (2005) found when given a choice between wild grasses and maize (*Zea mays*) that *E. saccharina* laid more eggs on wild grasses, *Pennisetum maximum* Jacq., *Pennisetum polystachion* Linn., *Andropogon gayanus* Kunth., and *Sorghum arundinaceum* Desv. *Eldana saccharina* was shown by Bruce *et al*., (2004) to lay fewer eggs and egg batches in the presence of neem oil (*Azadirachta indica* A. Juss). When exposed to the fungus, *Fusarium verticillioides* Sacc. (Nirenberg) in maize, *E. saccharina* was shown by Ako *et al*., (2003) to oviposit more eggs and have higher larval survival than on controls where fungicides were applied. Clearly many factors can influence *E. saccharina* oviposition.

Egg dumping (Tallamy *et al*., 2002) occurs when females deposit all their eggs, without parental care and without placing them in a safe location to improve the success of hatching or subsequent survival. The female may lay all her eggs in an unsuitable location, or a non-host plant which is still more beneficial to her fecundity than not laying eggs at all. Egg dumping can be beneficial for some insects, like *E. saccharina*, that have precocial offspring that can find and feed on a wide range of host plants (Kasl, 2004).

2.1.2. Trap crops

Trap crops are often planted because they are a dead-end crop, on which the offspring of pests cannot survive (Shelton and Badenes Perez, 2006) and may be a sacrificial species for the pest to attack, in order to protect a more valuable resource, most often a cash crop (Foster and Harris, 1997). Pest insects are often killed with toxic pesticides, but in recent years a reduction in reliance on chemical methods has been prompted by a move to manage pests through behavioural manipulation of the insect (Foster and Harris, 1997) by planting trap crops next to a higher value crop (Shelton and Badenes Perez, 2006). Twenty one varieties of *P. purpureum* and *P. purpureum* x *P. glaucum* hybrids were used in bioassays to identify potential trap crops for the graminaceous stemborer, *Chilo partellus* (Van den Berg, 2006). Results from two-choice tests with maize showed that *P. purpureum* was preferred by *C. partellus* moths for oviposition. However, larval survival was higher on maize as very few larvae survived on the *P. purpureum* varieties (Van den Berg, 2006).

Knowledge of the behaviour and characteristics of the target insect is essential when arranging control strategies with trap crops (Hokkanen, 1991). *Eldana saccharina* has two moth peaks in the year when adult moth numbers are highest (Carnegie and Leslie, 1990). Trap plants need to be established in cash crop fields during major moth peaks; and at a plant age that is most preferred by the moth pest (Kasl, 2004). Trap crops will need to be most attractive when the sugarcane is most susceptible to attack. Kasl (2004) found that *E. saccharina* prefer to feed on mature plants of six months or older.

Moths take refuge in the canopy of plants during the day and only move around to call and mate in the evening (Carnegie, 1973). *Eldana saccharina* moths most often walk around plants, flying only if they are disturbed or threatened (Carnegie, 1974). *Eldana saccharina* staying in the canopy makes insecticides less effective in killing the adult life stage of this pest, as they have reduced exposure to the chemicals.

2.1.3. Previous work

Kasl (2004) conducted trials on the oviposition preferences of *E. saccharina*, which showed that *E. saccharina* preferred the natural host plants to sugarcane, and of all the test plants, *S. bicolor* and *P. purpureum* were found to attract the highest proportion of eggs when placed in a cage with sugarcane plants. *Sorghum bicolor* is a viable host for *E.*

saccharina, however *P. purpureum*, does not support larval growth, but was still apparently attractive to ovipositing female moths (Kasl, 2004).

Leslie and Keeping (1996) showed that when given a choice between varieties of sugarcane, female *E. saccharina* were selecting oviposition sites based on the variety. The sugarcane being resistant or susceptible may play a role in the effectiveness of trap crops to pull *E. saccharina* out of sugarcane fields. Varieties were ranked from most resistant to most susceptible based on the number of internodes damaged in field trials and in a pot trial. Established varieties were similar in their ranking in both field trials and pot trial (Leslie and Keeping, 1996).

Kasl (2004) suggested that plant architecture of dead leaves might play a role in ovipositional choice by females. Females rarely lay eggs on the flat green leaf material and prefer dead leaves, especially dead leaves with curled edges (Leslie, 1990; Mabulu and Keeping, 1999). A lack of suitable oviposition sites on the plant could force a female moth to lay eggs on other surfaces, even if these are not as attractive to the female.

2.1.4. Test Plants

Potential trap crops are identified and then tested in oviposition cage trials to determine whether they are more attractive to *E. saccharina* than sugarcane. The hypothesis being that sugarcane associated with these test plants will attract less oviposition, when compared with potential trap plants.

Vetiveria zizanioides L. (Poaceae) is a grass used extensively in erosion control (McCosh, 2001) and soil conservation in many rural areas of Africa (Van den Berg *et al.*, 2003) and has shown potential as a pest management tool. Preliminary studies claim that moths from stemboring pests prefer *V. zizanioides* to maize for egg-laying (Van den Berg *et al.*, 2003). *Vetiveria zizanioides* does not have a long stalk therefore stemborer larvae were found to infest the culms and midrib of leaves instead (Van den Berg *et al.*, 2003). The oils produced by this grass are aromatic and believed to be repellent to other insects and diseases (McCosh, 2001).

Coix lachryma jobi L. (Poaceae) was chosen for this study due to the fact that during field surveys, stemborers such as *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Chilo partellus* were found feeding inside the stalks of this plant (Dr D.E. Conlong, pers comm.). It is hypothesised that because this broad-leaf grass is favoured by other stemborers for feeding, *Chilo* sp. and *Sesamia calamistis*, (Khan *et al.*, 1997b) it might

also be a preferred site for laying eggs by *E. saccharina*, and later for feeding by hatched larvae of *E. saccharina*.

Paspalum urvillei Steud. (Poaceae), a weedy grass found surrounding sugarcane fields, was noticed by a work team during a field survey of sugarcane in 2004, to be heavily infested by *E. saccharina*. This grass was chosen by *E. saccharina* in the field, therefore it was tested in a laboratory setup.

Studies have been done on *P. purpureum* and its insect population reducing properties (Kaufman, 1983; Khan *et al*., 2006) and these properties will be useful in pest control if introduced into sugarcane agriculture to decrease pest damage. *Pennisetum purpureum* was used in ovipositional cage trials by Kasl (2004), in which *E. saccharina* showed a weak attraction for this plant. Low numbers of live *E. saccharina* larvae were found on the stalk and leaves of *P. purpureum* by Kaufmann (1983) because it produces a gummy substance (Kaufmann, 1983, Kfir *et al*., 2002) that kills the feeding larvae. Van den Berg *et al*., (2001) found that *P. purpureum* was a host plant to other stemborers, *B. fusca* and *C. partellus*.

2.2. Methods and Materials

A glasshouse experiment to test the ovipositional preference of mated female *E. saccharina* moths for different non-crop plants was conducted. Cage trials were used to test the repellent or attractive nature of mature plants of *V. zizanioides, P. urvillei, P. purpureum* and *C. lachryma jobi*, to *E. saccharina*. These non-crop plants were tested against two varieties of sugarcane, N21 and N11, as controls in the trials to determine their attraction to *E. saccharina*. Sugarcane variety N21 is a resistant variety and N11 is a more susceptible variety of sugarcane. All plants used in experiments, including both varieties of sugarcane, were six months old or older. All test plants were grown in shade houses on South African Sugarcane Research Institute (SASRI) property and watered every second day.

2.2.1 Test Insects

Eldana saccharina adult moths, both male and female, were supplied by the Insect Unit in the SASRI Entomology Department. *Eldana saccharina* adults were reared using standard rearing procedures as described by Conlong *et al*., (1988) and supplied on the day they emerged. A total of 450 males and 450 female moths were used in ten replicates of nine

combinations, and five moths of each sex were used in each replicate. All moths were virgins when introduced into the cages.

2.2.2 Experimental design

Six metal, box frames (dimensions 1m x 1m x 2m height) covered in green shade netting (30% shading), making up cages, were placed in a glasshouse with sand bags holding the netting down around the base of the cage. One test plant (*V. zizanioides, P. urvillei, P. purpureum* or *C. lachryma jobi*) and one sugarcane plant (variety N11 or N21), both older than six months, were placed approximately 0.4m from each other (Figure 2.1) in the middle of each cage. The position in the cage, of each plant, was randomised for front or back and left or right between replicates to remove any possible bias for position.

A platform of width 0.35m, made of Masonite (thickness 0.005m) cut to 0.5m in length was placed across the gap between the plants, on top of the plastic plant bags. Five emerged unmated adult *E. saccharina* female moths were introduced in a bottle into the middle of the platform, along with five adult male moths, at approximately midday. The bottle (diameter 0.1m) was inserted from below, into a hole in the Masonite platform. This bottle acted as a refuge for the moths during the day, and stayed in the board till egg surveys were done two days later. The platform allowed the moths to walk to either plant.

Male and female moths were left for two days to allow them to mate and the females to make a choice of oviposition site. Females will lay eggs on a substrate within 24 hours after mating (Carnegie, 1973). After two days plants were checked for eggs by removing each leaf with the leaf sheath and searching both abaxial and adaxial surfaces of the leaf and leaf sheath for eggs. Any eggs found were collected on the section of leaf they were laid, placed in a Petri dish and then counted under a microscope. The number of eggs and egg batches found on other surfaces in and around the cage were also collected and recorded. Other surfaces included the cage netting, the cage frame, the black soil bags, the floor, the Masonite board, the bottle and the soil at the base of the plant stalk.

All dead leaf material was separated from green leaf material. Dead leaf material was collected, dried to a stable mass in an oven at 70 °C for one week and weighed to see if there was a correlation between amount of dry biomass and number of eggs and egg batches. Initially dead leaf biomass samples were dried and weighed each day until their mass was identical two days in a row, which took a week.

The control consisted of two sugarcane plants, varieties N11 and N21, tested together in a cage. Moths were placed in the cage as described above. The number of eggs laid on both varieties was counted as above and compared against the results from the other test plant combinations. The mean number of eggs collected from each plant and on other surfaces was compared. After every trial, the cages were swept out before the next combinations of plants were placed in the cages.

2.2.3 Statistical analysis

Results were analysed with an Analysis of Variance (ANOVA) for the differences between the mean number of eggs laid on test plants, sugarcane and other surfaces in the cage. Where the assumptions of normality and homogeneity of variance were violated, Mann-Whitney U (Wilcoxon rank sum) tests were used to test for significance.

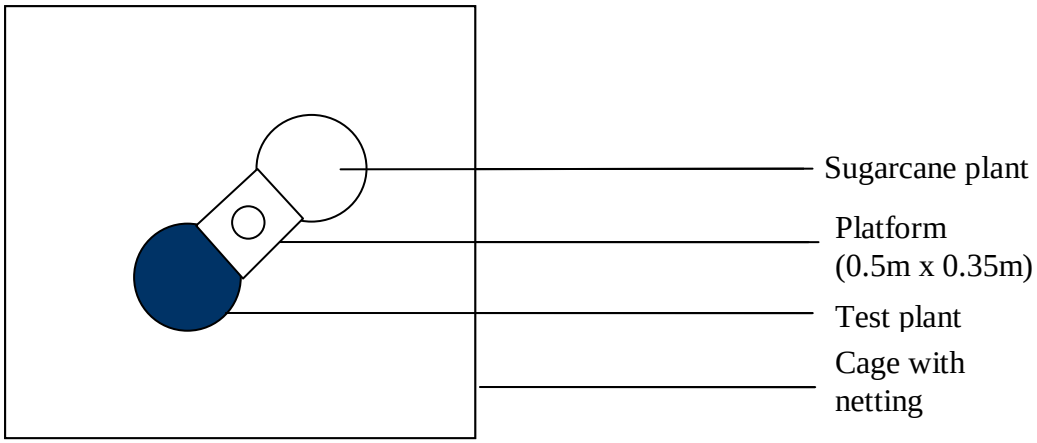


Figure 2.1: Plan view of experimental set-up for oviposition choice trials in cages. Position of plants in cage was changed front or back and left or right for each replicate.

2.3. Results

The mean number of eggs found on each plant in combination in cages is shown (Figure 2.2 – Figure 2.6) with the mean number of eggs found on other surfaces. *Eldana saccharina* laid eggs equally on cages surfaces and plant material. No preference for a test plant over sugarcane for oviposition was shown.

The control experiments with N11 and N21 varieties of sugarcane (Figure 2.2) showed no significant preference by *E. saccharina* ($F=1.98$, $df=2,27$, $P= 0.16$) for one sugarcane variety over the other.

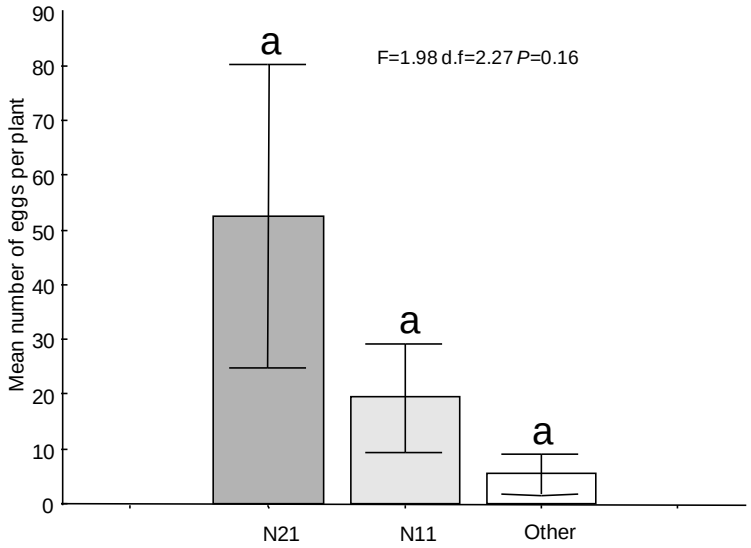


Figure 2.2. Mean number of *E. saccharina* eggs (±SE) found on the leaves of sugarcane varieties N11 and N21 in an oviposition choice cage trial, and eggs laid on other surfaces in the cage (Other). Columns with different letter above are significantly different from each other. (ANOVA) n=10

The mean number of eggs laid by *E. saccharina* in cage trials of combinations of N11 or N21 paired with *P. urvillei* (Figure 2.3), showed no significant difference (F=0.76, df=2,27, *P*=0.47). Therefore, there was no attraction for this trap plant over sugarcane shown by *E. saccharina*.

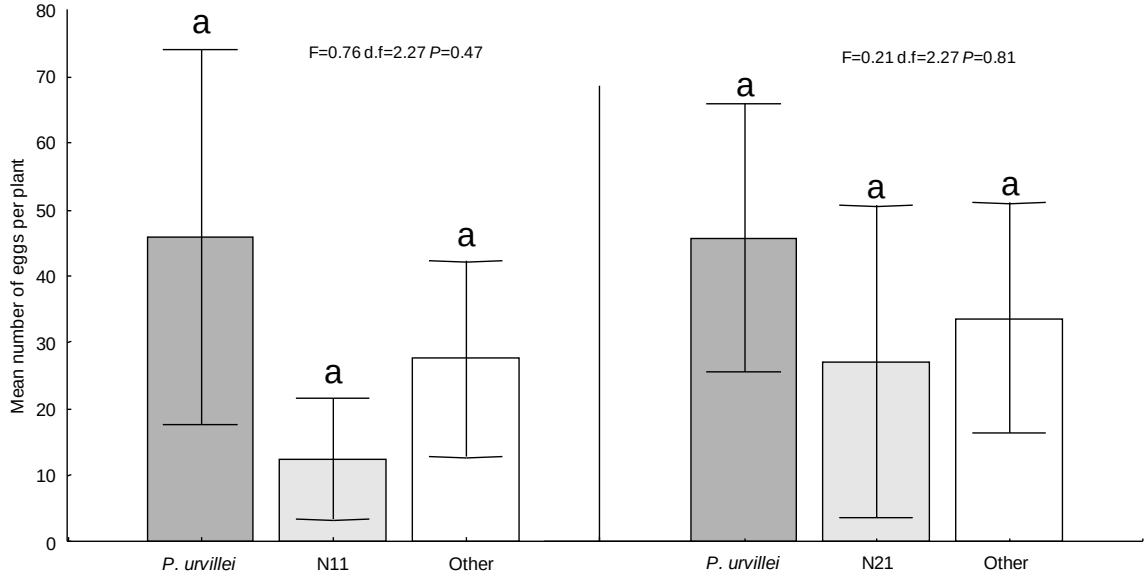


Figure 2.3. Mean number of *E. saccharina* eggs (±SE) found on the leaves of sugarcane varieties N11 and *P. urvillei* and N21 and *P. urvillei* in oviposition choice cage trials, and eggs laid on other surfaces in the cages (Other). Columns with different letter above are significantly different from each other (ANOVA) n=10.

The mean number of eggs laid by *E. saccharina* in cage trials of combinations of N11 or N21 paired with *V. zizanioides* (Figure 2.4) showed no significant difference (F=0.58, df=2,27, *P*=0.56) (F=0.36, df=2,27, *P*=0.70). Therefore, there was no attraction for this trap plant over sugarcane shown by *E. saccharina*.

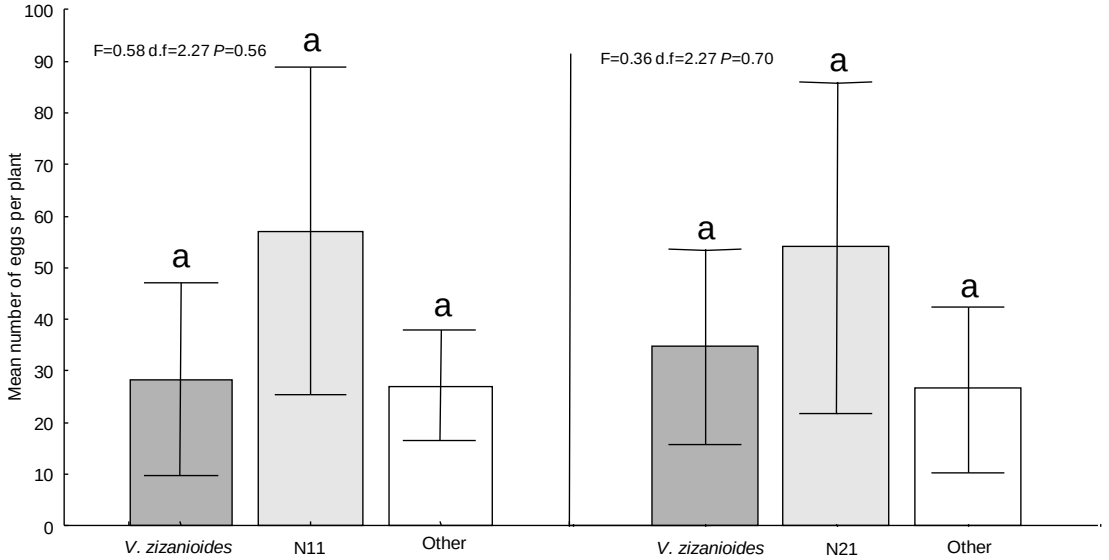


Figure 2.4. Mean number of *E. saccharina* eggs (±SE) found on the leaves of sugarcane varieties N11 and *V. zizanioides* and N21 and *V. zizanioides* in oviposition choice cage trials, and eggs laid on other surfaces in the cages (Other). Columns with different letter above are significantly different from each other. (ANOVA) n=10

The mean number of eggs laid by *E. saccharina* in cage trials of combinations of N11 or N21 paired with *C. lachryma jobi* (Figure 2.5) showed no significant difference (F=1.27,df=2,27, *P*=0.29) (F=0.99,df=2,27, *P*=0.38). Therefore, there was no attraction for this trap plant over sugarcane shown by *E. saccharina*.

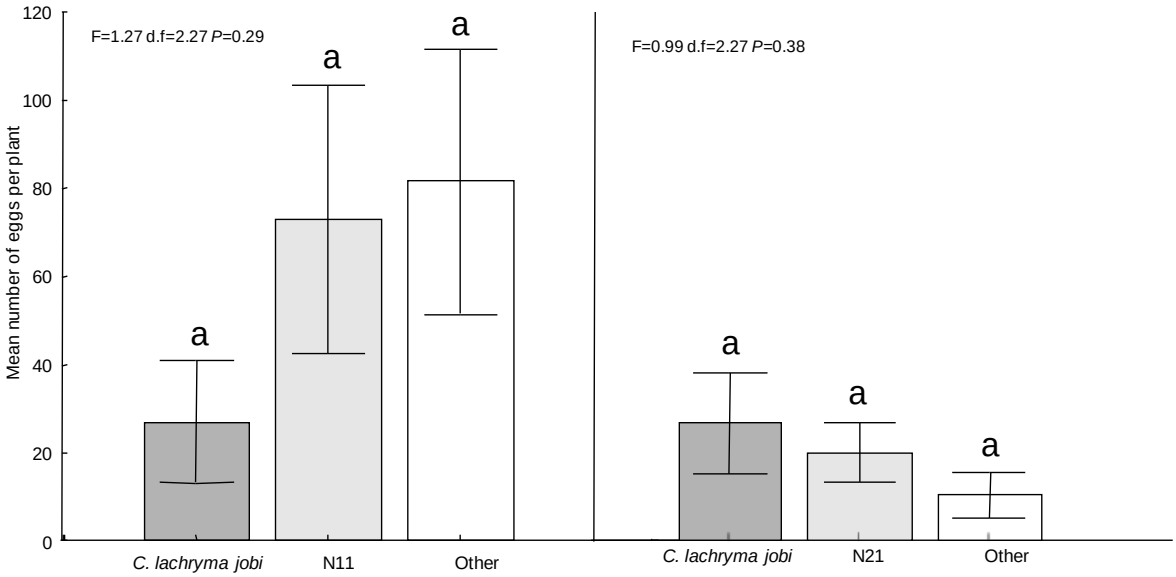


Figure 2.5. Mean number of *E. saccharina* eggs (±SE) found on the leaves of sugarcane varieties N11 and *C. lachryma jobi* and N21 and *C. lachryma jobi* in oviposition choice cage trials, and eggs laid on other surfaces in the cages (Other). Columns with different letter above are significantly different from each other. (ANOVA) n=10

The mean number of eggs laid by *E. saccharina* in cage trials of combinations of N11 or N21 paired with *P. purpureum* (Figure 2.6) showed no significant difference

(F=0.81,df=2,27, *P*=0.45) (F=0.30, df=2,27, *P*=0.73). Therefore, there was no attraction for this trap plant over sugarcane shown by *E. saccharina*.

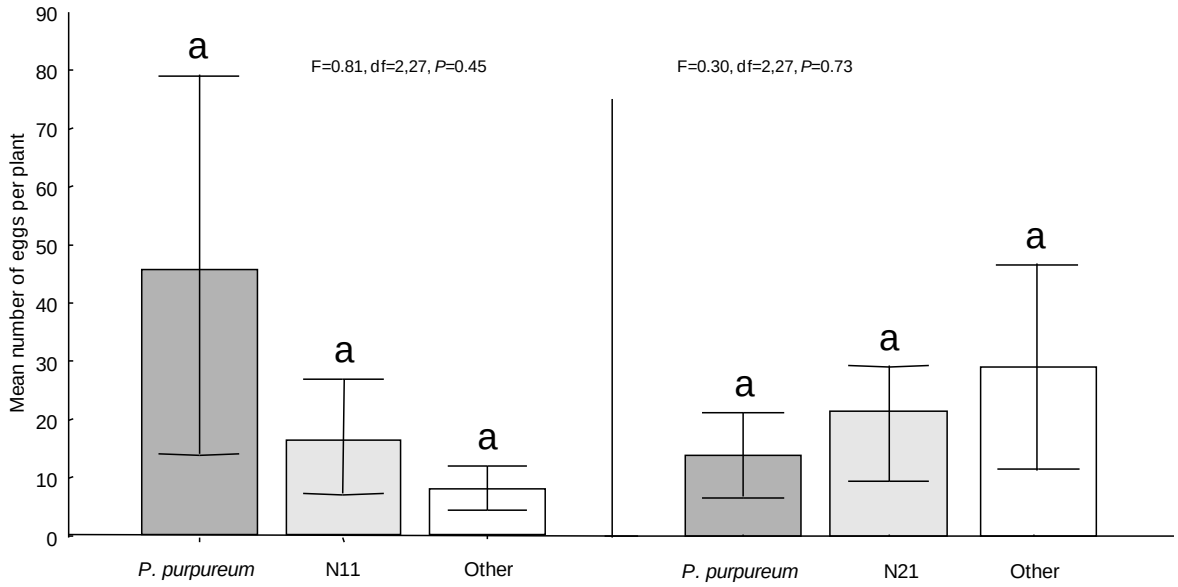


Figure 2.6. Mean number of *E. saccharina* eggs (±SE) found on the leaves of sugarcane variety N11 and *P. purpureum* and N21 and *P. purpureum* in oviposition choice cage trials, and eggs laid on other surfaces in the cages (Other). Columns with different letter above are significantly different from each other. (ANOVA) n=10

A relationship is shown between the number of eggs laid and the dry biomass of test plants or sugarcane. *Vetiveria zizanioides* had the highest mean number of eggs per plant, while N21 sugarcane has the highest mean dry biomass (Figure 2.7).

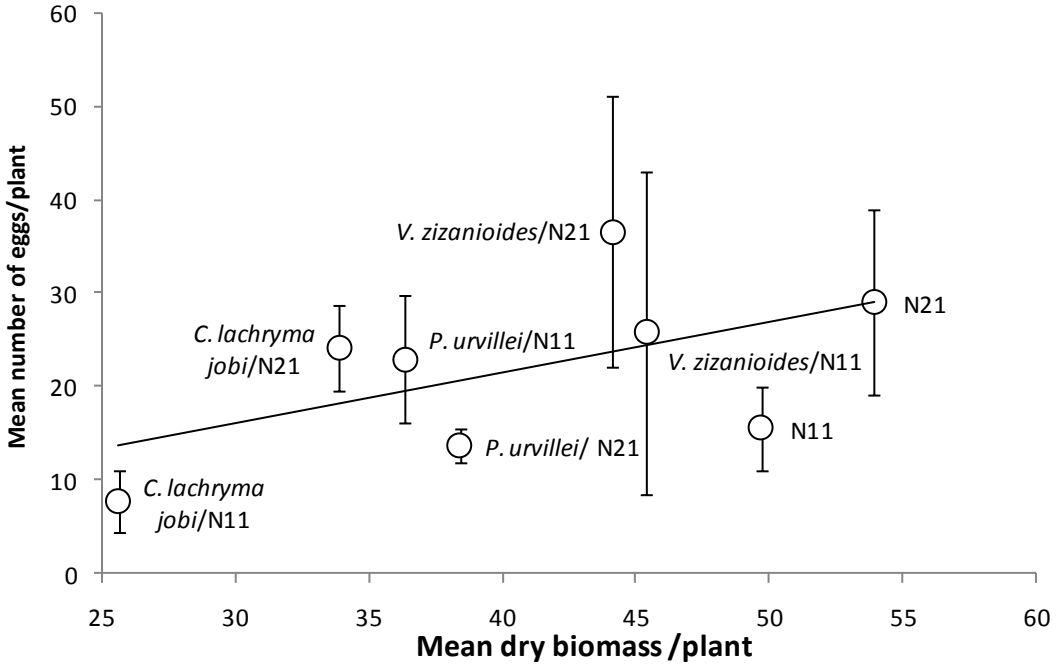


Figure 2.7. The relationship between biomass of dry material on test plants and mean number of *E. saccharina* eggs (±SE) collected from each plant. Circles represent mean values and bars show standard error. n=10 trials per plant species

2.4. Discussion

2.4.1 Mean egg numbers

Sugarcane variety N21 had a high number of eggs laid on it by *E. saccharina* compared to N11 sugarcane, in the control experiment (Figure 2.2). Although this difference was not significant, it does support results from oviposition trials by Mabulu and Keeping (1999) where N21 sugarcane was found to have the highest number of eggs in three experiments. This is an unusual result because N21 sugarcane is a supposed resistant variety (Leslie and Nuss, 1992). Resistance by varieties of sugarcane might only be shown when the young larvae first try to enter the stalk or start feeding on the leaves of these varieties. The external appearance and surface characteristics of N21 sugarcane do not deter female *E. saccharina* moths from ovipositing on these plants, despite N21 sugarcane being covered in hairs. Sosa (1988) found that the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae) did not oviposit on a pubescent sugarcane variety in two-choice tests with a glabrous variety. The hairs on the leaves of the pubescent variety adversely affected both oviposition and larval movements. The influence of hairs on the leaves of these test species was not examined but could play a part in explaining why *E. saccharina* did not oviposit more on test plants than on sugarcane (Sosa, 1988; Sosa, 1990).

Using oviposition cage trials, Kasl (2004) found that there was no significant attraction shown by *E. saccharina* for the test plants, *Sorghum bicolor*, *P. purpureum*, *Hyparrhenia dregeana, Bothriochloa insculpta* and *Panicum maximum* over sugarcane in terms of more eggs being laid on the leaves of test plants than on sugarcane. Kasl (2004) concluded that *E. saccharina* was fairly indiscriminate in where it oviposited. There was no attraction either way for sugarcane or for any other plant by *E. saccharina*.

Sugarcane and *P. purpureum* were tested in these experiments, the results support this statement made by Kasl (2004) that *E. saccharina* females do not make a definitive choice for one plant over another.

In terms of which of the test plants is most attractive to *E. saccharina* females for oviposition, *P. urvillei* had more eggs laid on it than the sugarcane plants in the same cage (Figure 2.3). *Vetiveria zizanioides* had the highest mean number of eggs per plant in relation to dry plant biomass (Figure 2.7). There were no other significant differences in mean number of eggs laid, between any plant pairs tested. The deviation around most of the mean number of eggs laid was wide for all test plants and sugarcane. There was no significant difference between the mean number of eggs laid on sugarcane and *V.*

zizanioides when tested in a cage trial against N21 and N11 respectively (Figure 2.4). The plant structure of *V. zizanioides* with 'V' shaped leaves bunched together at the base, and the edge of the leaf blades that curl towards the leaf margin, provides many cryptic oviposition sites at the base between the leaves. The chemical attractiveness of *V. zizanioides* may not be influencing the number of eggs laid on this plant, but rather female moths chose to lay eggs on the leaves and dry material of *V. zizanioides* because of the availability of cryptic oviposition sites. The highest mean number of eggs was found on *C. lachryma jobi* when tested in cage trials with the variety of sugarcane, N21 (Figure 2.5). Conversely *C. lachryma jobi* had the lowest numbers of eggs laid on the leaves and dry material when tested in cage trials with the other sugarcane variety, N11 (Figure 2.5).

Kasl (2004) showed *P. purpureum* to be the test plant most attractive to *E. saccharina* for oviposition, though not statistically significant, along with *Sorghum bicolor* L. (Poaceae) when tested against sugarcane in cage trials. This 'pull' potential of *P. purpureum* is not supported by the results of these cage trials. N21 sugarcane had an equal mean number of eggs compared to *P. purpureum*, (Figure 2.6) and although there were a higher mean number of eggs on *P. purpureum* when tested against N11 sugarcane (Figure 2.6), the size of the deviation around this mean explains why the result is not a significant difference (ANOVA, $F=0.81, df=2,27$, $P=0.45$). *Eldana saccharina* does not appear to show a specific oviposition preference for any test plants over sugarcane in these experiments. However, when given a choice between its natural sedge host *Cyperus immensus* C. B. Cl., sugarcane and a sedge non-host plant, *Cyperus latifolius* Pior, *E. saccharina* preferred the natural host (Atkinson, 1980). Therefore, it does show some host preference. The frequency of laying on unnatural surfaces was not significantly different from the frequency that the moths laid on sugarcane and *C. latifolius* (Atkinson, 1980), but similar to that found in these trials.

Many of the other surfaces such as the floor of the cage, plastic plant bag and cage netting that were available for oviposition had egg batches on them. These sites are not feeding sites for emerging larvae and female moths are therefore laying indiscriminately in these locations. The tactile stimulus for oviposition, three sides touching the ovipositor (Sampson and Kumar, 1985), can be found in the fold of the soil bag. Although these other locations had eggs, they were not placed in long rows as was seen in the rolled edges of the sugarcane leaves. The sites offering concealment for eggs laid by females suggest it is

a strong potential cue for oviposition. Cryptic site availability may be the overriding factor influencing the oviposition site preference of females rather than the volatile emissions of plants which are beneficial food plants for their offspring. The abundance of cryptic sites on potential trap crops could be measured for the role it may play in *E. saccharina* oviposition choice.

Eldana saccharina can survive in the wild without being host specific because it can feed on a variety of host plants including various crop plants and natural hosts (Atkinson, 1980; Conlong, 2001). Since *E. saccharina* is polyphagous (Atachi *et al.*, 2005), it has the ability to have a variety of potential hosts plants, non-specific oviposition choice and still survive in the wild.

Results in this experiment are similar to those found by Rebe *et al.*, (2004), which showed no significant difference in egg numbers laid by *C. partellus* on maize and two varieties of *S. bicolor* in two-choice oviposition trials. However, contrary to these results, *V. zizanioides* was found to be more attractive than maize to *C. partellus* in oviposition trials by Van den Berg *et al.*, (2003).

2.4.2 Biomass and mean egg batches

Kasl (2004) showed a correlation between amount of dry leaf material and number of eggs and egg batches laid on plant material. Ovipositing females have been shown to select plants with the greatest amount of dead leaf material (Atkinson, 1980; Leslie, 1990) which is supported by results of these trials (Figure. 2.7).

Vetiveria zizanioides attracted the highest number of eggs, but the dry biomass was not significantly different from *C. lachryma jobi* and *P. purpureum*. The high number of eggs laid on *V. zizanioides* may be influenced rather by the cryptic site availability on the plant. Dry plant biomass in most cases influences the number of cryptic oviposition sites (Mabulu and Keeping, 1999). This may explain why these plants are chosen by ovipositing females. Sugarcane leaf architecture allows for many cryptic oviposition sites, and the dry leaf biomass is the highest in sugarcane compared to other test plants. However, sugarcane did not attract significantly more eggs that other test plants.

Vetiveria zizanioides had a higher mean biomass than the other test plants but the mean number of eggs on *V. zizanioides* had a wide deviation from the mean. The relationship between dry biomass and mean number of eggs, found and described by Kasl (2004), is supported in this experiment. *Eldana saccharina* may have been influenced by the amount

of dead leaf material to lay eggs on these plants more than plants with lesser amounts of dead leaf material (Carnegie and Smaill, 1982).

Eldana saccharina female choice of test plants for oviposition does not show a specific preference for one plant over another. Female moths are not selective in where they lay their eggs which agrees with findings of Kasl (2004). Oviposited eggs can hatch on most surfaces and the hatching larvae are not confined by the surface on which they emerge because they have the ability to move to find food (Leslie, 1993). Larvae are able to survive and develop on a wider range of plants than are used by females for oviposition (Thompson, 1988). Female moths do not need to lay eggs directly on host plants because the larvae can move to suitable host plants.

Many oviposition studies are based on the idea that females will choose plants in a hierarchical order if exposed to two or more plants at the same time (Conlong *et al.*, 2007). The most favoured plant will have the most number of eggs on it; the next favoured the second most and so on (Thompson and Pellmyr, 1991). However these experimental designs do not take into account the individual preference within the population of females. The variation in oviposition preference is obscured by the composite distribution of eggs from many females. Competition between females for oviposition sites may reveal a more uniform distribution rather than a specific preference that could have been shown by an individual female (Thompson and Pellmyr, 1991).

Push pull systems rely on introduced plants being more attractive to the pest species than the cash crop in order to pull insects away to alternate host plants or more repellent to push them away from crop fields. The results in these trials do not support the use of any of the tested plants because *E. saccharina* was not shown to oviposit significantly more or less eggs on a test plant than sugarcane. If these plants were to be used in push pull systems in sugarcane fields, the females may still oviposit on these plants in roughly the same manner as in these trials, but larval movement between plants would allow *E. saccharina* larvae to choose a suitable host plant. Conlong *et al.*, (2007) showed that larvae have a clear preference for their natural host sedges over sugarcane in dual choice tests. In a sugarcane field where no significant preference for one plant is shown by the pest, the most abundant plant would probably be chosen most commonly, in this case sugarcane. This is one reason why strongly attractive or repellent plants need to be used as intercrops in push pull strategies. However because these experiments were conducted in the limited, enclosed

area of a glass house, they may have been swamped with volatile chemicals from the close proximity of all of the plants (Kasl, 2004).

2.4.3 Conclusions

This study has shown that female *E. saccharina* are not very particular about where to oviposit. If given a cryptic and secure location, irrespective of whether it is good for the eggs or the emerging larvae, the female may lay eggs in that place.

Biomass of dead plant material, though favoured above green plant material for oviposition (Mabulu and Keeping, 1999), may influence the number of eggs or egg batches laid by *E. saccharina*.

The results of these experiments show that females make no choice of host plants when they lay their eggs. Alternatively, it could be a cage effect that is hindering the female moths from behaving as our hypothesis states. A further study is needed where only the chemical cues followed by *E. saccharina* are tested away from other influences, to test plants attractive or otherwise to *E. saccharina,* based on the volatiles released by that plant. The next chapter deals with olfactometer trials of test plants and their attraction to *E. saccharina*.

Chapter 3:
Attraction of *Eldana saccharina* adults to non-host plants in olfactometer trials

3.1. Introduction

3.1.1 Volatiles

Many phytophagous insects use airborne volatiles emitted from plants to detect their hosts (Mitchell *et al*., 1991, Tingle *et al*., 1990) and may associate particular volatiles with particular host plants. Female moths in flight can use volatile plant chemical cues to find their hosts even in a mixed crop environment (Couty *et al*., 2006). Therefore, these chemicals can potentially be used in pest control strategies to influence pest populations in agricultural fields. Volatiles are generally used for long range host finding while other stimuli may be more important at close range. Landing on the correct plant, tasting it, and possible tactile responses are important cues to the diamondback moth, more so than detecting the correct plant volatile while in flight (Justus and Mitchell, 1996). Short range signals will tell the female where to oviposit, while longer range volatiles allow the moths to find areas that contain suitable hosts. Parasitoids are also known to use volatiles to find their host species (Ngi-Song *et al*., 2000, Mbata *et al*., 2004). These volatiles come from herbivore damaged host plants, larval frass, sex pheromones and volatiles from the host insect (Mbata *et al*., 2004).

Volatile emissions in response to herbivory may be qualitatively and quantitatively different to those volatiles emitted by the plant when not being fed upon (Dicke and van Loon, 2000). Parasitoids are not attracted to odours from uninfested maize or from artificially damaged maize plants (Potting *et al*., 1995). Volatiles that specifically express distress in the plant are not released from artificially damaged plant material, unless oral secretions from the pest species are applied to the damage sites (Turlings *et al*., 1990). Frass on host plants produced by larval feeding, though not as potent as the pest feeding volatiles (Potting *et al*., 1995), are an additional attractant to parasitoids, when tested against host plants without frass present (Smith *et al*., 2006).

3.1.2 Habitat management

One form of habitat management, for pest control, involves identifying non-host plants that emit chemicals that are repellent to the pest species, but are attractive to their parasitoids (Liu *et al*., 2006). In order to identify plants that have chemical properties that will

influence the behaviour of pest species, trials need to be run with pest species choosing for or against the chemical volatiles of potential non-host plants. These plants will theoretically be intercropped with sugarcane to repel the pest species *E. saccharina* or attract it away from sugarcane towards another plant. If *E. saccharina* can be pulled away from sugarcane, by a more attractive plant, the number of adults landing on sugarcane will be decreased which in turn will reduce the larval infestation. A mechanism was needed to test the response of stemborers to test plants.

In the previous chapter, plants that had potential to be attractive to *E. saccharina* were tested in oviposition cage trials to assess which plant the female moth preferred for oviposition. The short range cues, tactile, visual or gustatory appearance were tested in these trials. The long range attraction of all insects to a host plant or a host insect is chemical in nature and is tested using an olfactometer. The long range signals can be as effective in deterring or attracting moths, as the surface stimuli or short range cues. Therefore, the plants used in the cage trials were tested again in olfactometer trials.

Table 3.1: Insects tested in different experimental setups and tested volatile

Insects	**Experimental Design**	**Test source of Volatiles**	**Response**	**Author**
Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae)	Volatile dissolved in ethanol in a sand substrate in joined containers	Nookatone isolated from Vetiveria oil	Repellent	Zhu *et al*., 2001
Helicoverpa zea Boddie (Lepidoptera: Noctuidae)	Two and six choice olfactometers	Chemical baits extracted from *Gaura suffulta*	Attractant	Beerwinkle *et al*., 1996
Eldana saccharina Walker (Lepidoptera: Pyralidae)	Gas chromatography-electroantennography	*Eldanolide* extracted from abdominal hair pencil secretions	Attractant	Burger *et al.,* 1993
Plutella xylostella Linnaeus (Lepidoptera: Plutellidae)	Olfactometer	Extract of *Chrysanthemum morifolium* placed on host plant	Repellent	Liu *et al*.,2006
Heliothis subflexa Gn. (Lepidoptera: Noctuidae)	Flight tunnel bioassay	Extracts from host plant *Physalis* spp.	Attractant	Tingle *et al*., 1990

Pest insects are often tested in olfactometers although there are other methods, such as gas chromatography with electroantennograms for behavioural responses. Olfactometer studies using the whole animal are cheaper and simple to perform and if the animals are co-

operative, then good results can be obtained. Table 3.1 gives a summary of various studies of olfactory responses by a variety of insects to extracted volatiles.

3.1.3 Olfactometers

Olfactometers are basically 'yes' or 'no' devices to test a response either for or against a particular plant, volatile or stimulus. Olfactometers are made in various forms; mainly based on the Y-junction principle (Beerwinkle *et al*., 1996) which involves two odours being drawn from two sources through tubes which join together. The test subject is usually placed at this junction where it can make a choice between two sources at the same time. Other olfactometer setups can involve a wind tunnel or flight chamber where insects follow a volatile stream to an odour point source, into a chamber where they can be collected. This allows factors such as light, temperature, humidity, and air speed to be controlled to simulate ideal field conditions (Beerwinkle *et al*., 1996). Moths that are nocturnal will be influenced in their behaviour by the amount of light in the experiment while temperature also influences movement. Air speed can also affect the behaviour of the test insects, especially when searching for volatile odours (Messing *et al*., 2003).

Eldana saccharina choice behaviour for test plants was tested in a Y-tube olfactometer by Kasl (2004) but showed no significant responses and choices were largely found to be random. The high number of non-choices made by the insects in the Y-tube olfactometer trials did not show repellence by plants, except for *Melinis minutiflora*, which was chosen significantly less by *E. saccharina* adults than sugarcane variety NCo376. These results suggested that the insects were behaving unnaturally in an artificial choice environment.

3.1.4 Olfactometer studies

Different olfactometer setups allow experiments to be suited to specific test organisms (Table 3.1). The banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae), showed stronger discrimination to different odours in dual port olfactometers with continuous airflow, compared to a double pitfall olfactometer (Tinzaara *et al*., 2003). The insect in this experiment needed airflow to be attracted and did not respond as well when the air from the pitfall traps was still. Steinberg *et al*., (1992) found that when testing parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) in three olfactometer setups, a glass flight chamber gave the best results. Wasps need to fly to find their host insects; other insects like *E. saccharina* are assumed to mostly crawl between host plants. Successful results for a beetle were collected from Y-track olfactometer experiments by Nakamuta *et*

al., (2005). Death feigning beetles, vine weevil *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae), which would normally be unresponsive if handled in a typical olfactometer were setup in a special Y-tube olfactometer that allowed the beetles to enter the olfactometer by themselves. This yielded positive response results and preference to one pheromone over another was shown (Nakamuta *et al.,* 2005).

3.1.5 Olfactometer problems

There are problems that may be encountered when setting up an olfactometer for specific test subjects. Firstly airflows mixing in the turbulent air at the confluence of the Y-tubes restrict the ability of small insects to differentiate the different odour plumes, in an olfactometer setup (Vet *et al*., 1983). The confined space inside a Y-tube or a four chamber olfactometer is not suitable for an insect that detects odours and volatiles during flight. Flying insects should be tested in wind tunnel setups or large flight chamber experiments. In many olfactometer experiments a 'choice line' is drawn a short distance down both arms of the two-way olfactometer. When crossed by the test subject it indicates that the organism has made a choice (Vet *et al*., 1983). Individuals in these experiments are often stressed and the first opportunity to escape may be down one of the arms of the olfactometer, therefore it is the escape response that is being tested instead of a choice of a specific volatile.

Given the above problems with olfactometers, the question needs to be asked in this study, which setup is the best olfactometer for *E. saccharina*? *Eldana saccharina* is nocturnal, has a short adult life, crawls and does not fly well (Atkinson, 1980). *Eldana saccharina* has been shown to be troublesome in previous choice type experiments, by Kasl (2004) and this study (Chapter 2). As a result, a new approach was taken, in which moths were exposed to volatile odours in a box olfactometer.

The box olfactometer (Beerwinkle, 1996) (Figure 3.1) allowed for the release of moths in a dark, enclosed space suited to the nocturnal, cryptic nature of *E. saccharina*. The box is larger than the Y-tube olfactometer tube, so moths can move around in the dark and choose the most attractive odour, but can change its choice before moving down an arm, if it encounters a more attractive volatile.

3.1.6 Olfactometer tested plants

The same test plants used in cage trials are tested again in the olfactometer trials. These plants have volatiles that are attractive or repellent to *E. saccharina* and are being tested to determine their influence on pest insect behaviour. All tested plants are in the Graminaceae, the same family as maize and sugarcane.

The oils produced by *V. zizanioides* are aromatic and repellent to termites (Zhu *et al*., 2001) and diseases (McCosh, 2001). *Vetiveria zizanioides* was also found in studies to 'trap' maize stemborers and stop them moving onto maize in a nearby field (McCosh, 2001). Vetiveria oil from the roots also has applications in soap and cosmetic industries and is used as an anti-microbial and anti-fungal agent.

Coix lachryma jobi was chosen for this study because it has been found infested by stemborers in the field. Ary *et al*., (1989) found *C. lachryma jobi* to contain a protein inhibitor of gut amylase in locusts. This could be important in the protection of crops from insect feeding if this protein inhibitor works in moths of stemboring pests. *Coix lachryma jobi* is also aromatic and has medicinal properties.

Paspalum urvillei can be used by farmers as animal fodder, so there are additional benefits if it can influence insect behaviour and is accepted by farmers.

3.1.7 Female mate choice

Most lepidopteran sex pheromones are produced by the females of a given species and elicit a response by male moths (Foster and Harris, 1997). *Eldana saccharina* chemical and auditory mating calls are produced by the abdominal pencil hairs and tymbals on the tegulae of the males (Bennett *et al*., 1991). These calls are attractive to both male and female, *E. saccharina* moths (Burger *et al*., 1993). However, it is not known if the male chooses the host plant to call from, then females stay on that plant to oviposit, or if the males first attract the female moths to them on any plant, mate, then females make their own host choice for plants to oviposit on. Female moths may be attracted to a possible host plant from a long range, then once on that plant, the female searches for the calling males who are attracting females at the short range. The choice of host plants by males should therefore be included in olfactometer trials.

The male sex pheromone, known as eldanolide, is attractive to other male *E. saccharina* causing an aggregation of males around the calling male (Burger *et al*., 1993). Aggregating

behaviour of *E. saccharina* forming male calling groups increases the strength of the signal to attract more females (Bennett *et al*., 1991).

3.1.8 *Eldana saccharina* in sugarcane

Sugarcane is usually planted as sets, sections of stalk with one node, in rows roughly 150 cm wide, across a field. Double stalks are sometimes planted in the soil along the row to increase sprouting potential. After two or three weeks stalks sprout and form a dense line of sugarcane in each row. The spaces between rows are wide enough to walk between and use to intercrop other plants in a push-pull system.

Female *E. saccharina* in sugarcane move from one plant to another from the end of a leaf of one plant to a new leaf on the next plant, crawling all the way (Leslie, 1990). Eggs are oviposited on any dead leaf material along the way between these plants. While moving between plants, there is potential for other chemical stimuli to be introduced that will disrupt or deter the female from staying on the sugarcane. The potential of intercrops to have this effect on female *E. saccharina* moths was tested in this study.

3.2. Methods and Materials

The graminaceous plants, *V. zizanioides, P. urvillei* and *C. lachryma jobi* used in cage trials (Chapter 2), were also tested in box olfactometer experiments, to assess their long range attractiveness to *E. saccharina*.

3.2.1 Experimental Design

A box olfactometer (200 x 300 x 250 mm) (Figure 3.1) was constructed out of opaque PVC (Polyvinyl Chloride). Two plastic tubes coming out of the base of the box, 30mm from sides and front, each held a collecting bottle wedged onto the bottom. Funnels, made of paper and replaced for each replicate, were placed inside the top of the plastic tubes (diameter 40mm) to stop the moths from returning to the main chamber and to channel moths into collecting bottles (diameter 100mm). In the hole (diameter 40mm) in the base of the collecting bottle, a rubber stopper was fitted. A glass tube (inside diameter 10mm) was pushed through the rubber stopper and the end of the glass tube was covered with gauze, to prevent moths moving from the collecting bottles onto the test plants. The other end of the glass tube was pushed over latex tubing, (inside diameter 10mm, length 1000mm), which was then connected to a molded PVC attachment. This PVC attachment fitted on either side

of a hole cut in a nylon oven bag, identical to the ones used by Kasl (2004). Nylon bags (width 500mm, open on both ends, cut to required length) enclosing all plant parts and were tied closed on one end but sealed loosely around the base of the test plant stalk on the other end of the bag. A computer fan attached to the back of the box, drew air from plant bags through the box (Figure 3.1, E), at a speed of 3.6km/h which allowed all volatiles coming off of the plants in the experiment to be drawn through the olfactometer, past the adult moths. A PVC lid of the same material as the sides and base of box was sealed with petroleum jelly to the top, so that air was only pulled into the box through the choice tubes and out of the box through a filter gauze (Figure 3.1, D).The whole setup allowed easy disassembly for servicing and cleaning.

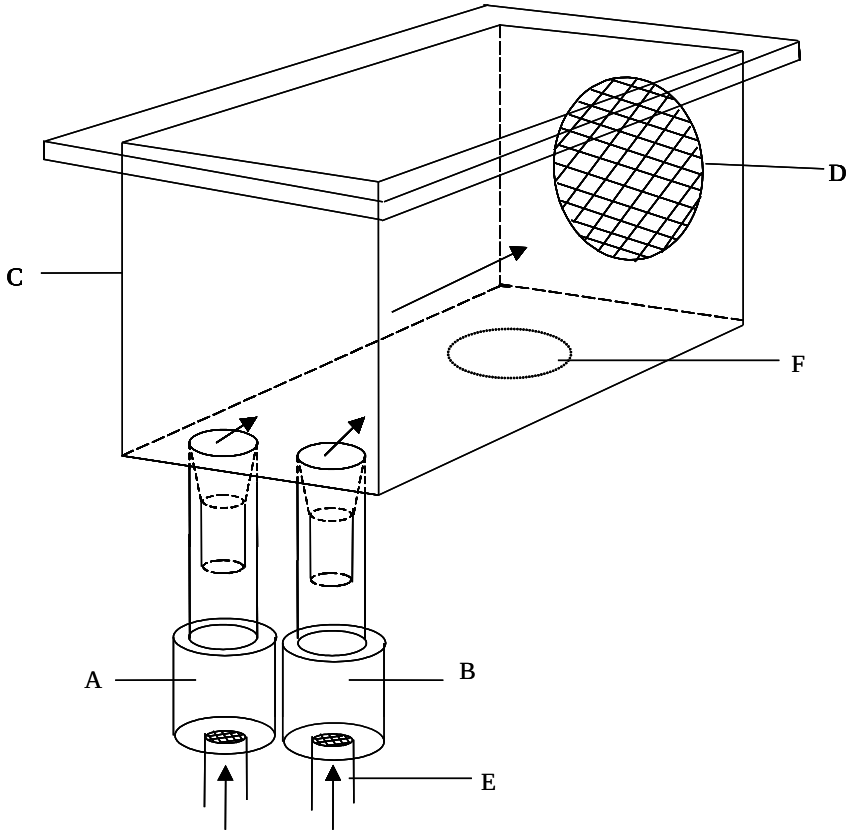


Figure 3.1: Schematic diagram of two-choice olfactometer apparatus (Beerwinkle *et al*., 1996). Collecting bottles (A, B). Insect exposure chamber (C). Air extraction port filter pad (D). Arrows indicate flow of air from test plants, through a glass tube (E). Moths were released in the middle of the box (F).

Five males and five female moths, just emerged and unmated, were placed together inside the main box (Figure 3.1, F) at midday and the lid sealed on top so they were in the dark. The moth preferences for a non-crop plant were tested against sugarcane and compared with control experiments. Moths were allowed to make a choice between two stimuli (Table 3.2), or make ‘no choice’ and stay in the box. Experiments were run over two nights allowing the moths to mate on the night after female emergence, if males were placed with

females. The males and/or female moths were collected 24 hours later, in the collecting bottle of the treatment that they chose. Moths were allowed to mate in the box setup so that females, (which in these trials are assumed to be the sex that chooses host plants), could make a choice for a plant from one of the collecting chambers. A blank was prepared in the same way as for the test plants, without a plant being tied in the nylon bag. Trials were also run with each test plants and sugarcane being tested against five male moths (Table 3.2). Only virgin adult female moths were released in the box and therefore did not mate before making a choice. This gave an indication of the preference of females for males or for the plant odours. The test plants placed in nylon bags were: Sugarcane variety N11, *V. zizanioides, P. urvillei, C. lachryma jobi*.

Table 3.2: Combinations of test plants, blanks and males, run in olfactometer trials for choice of *E. saccharina* in two-choice trials

Choice 1	**Choice 2**	**Moths being tested**
Sugarcane variety N11	*Vetiveria zizanioides*	Males and females
Sugarcane variety N11	*Paspalum urvillei*	Males and females
Sugarcane variety N11	*Coix lachryma jobi*	Males and females
Blank	Sugarcane variety N11	Males and females
Blank	*Vetiveria zizanioides*	Males and females
Blank	*Paspalum urvillei*	Males and females
Blank	*Coix lachryma jobi*	Males and females
Male moths	*Vetiveria zizanioides*	Females only
Male moths	*Paspalum urvillei*	Females only
Male moths	*Coix lachryma jobi*	Females only
Male moths	Blank	Females only
Male moths	Sugarcane variety N11	Females only
Blank	Blank	Males and females

All combinations in trials were replicated ten times and the mean number of male and/or female moths that made each choice was calculated.

The number of moths collected in plastic bottles after each trial were counted and recorded as well as the number of moths of each sex that stayed in the box and made ‘no choice’. Moths that did not move out of the box, into one of the collection bottles were counted as a ‘no choice’ and this percentage was used in the calculation of the probability that moths chose to move to a particular arm based on the volatiles released from there.

After each run the olfactometer boxes, lids, latex tubes, attachments and collection bottles were washed in warm soapy water and rinsed thoroughly to remove any trace chemicals left by the previous individuals or plant volatiles. Test plants were alternated between the left hand side and right hand side of the olfactometer between each replicate performed to ensure no bias toward one side over the other. Plants used were six months or older and grown in the shade house on the SASRI farm, Mount Edgecombe. Three different trials were run at a time in three identical olfactometers. Plants to be tested in combinations were mixed across the three olfactometers until all combinations were repeated ten times.

3.2.2 Statistical Analysis

Significant differences between each of the main effects (i.e. Plant combinations, Sex, Plant combinations/Sex) and their interactions were established using an analysis of variance (ANOVA; Genstat Version 8.0). A Sidak pairwise multiple comparison test (Genstat Version 8.0) was used to quantify significant differences. When there were significant differences in the combinations, Sidak tests were used to determine which individual result showed significant difference or whether the combined combinations were significantly different together in comparison to other combinations. The probability of moths making a choice against making 'no choice' was tested with a Student's T-test at 5% significance. A T-test was performed on the grouped data of female choice, by comparing the choice of assumed mated females - those placed in the box with males- to virgin females - those placed alone in the box and choosing a plant volatile against male moths. Where the assumptions of normality and homogeneity of variance were violated, Mann-Whitney U (Wilcoxon rank sum) tests were used to test for significance.

3.3. Results

Moths, both male and female, did not show a statistically significant preference for volatiles from any plants, male moths or for a blank arm except in trials involving *P. urvillei* and sugarcane and the trial involving *C. lachryma jobi* and male moths. Mated male and female moths showed no significant response to *V. zizanioides* or N11 sugarcane. However significantly more female moths chose to move into one of the arms of the setup than made a 'no choice' and remained in the box ($F=0.029$, $df=2,45$, $P=0.05$). Male moths made 'no choice' significantly more than female moths ($F=$, 0.036, $df=2,45$, $P=0.05$) (Figure 3.2).

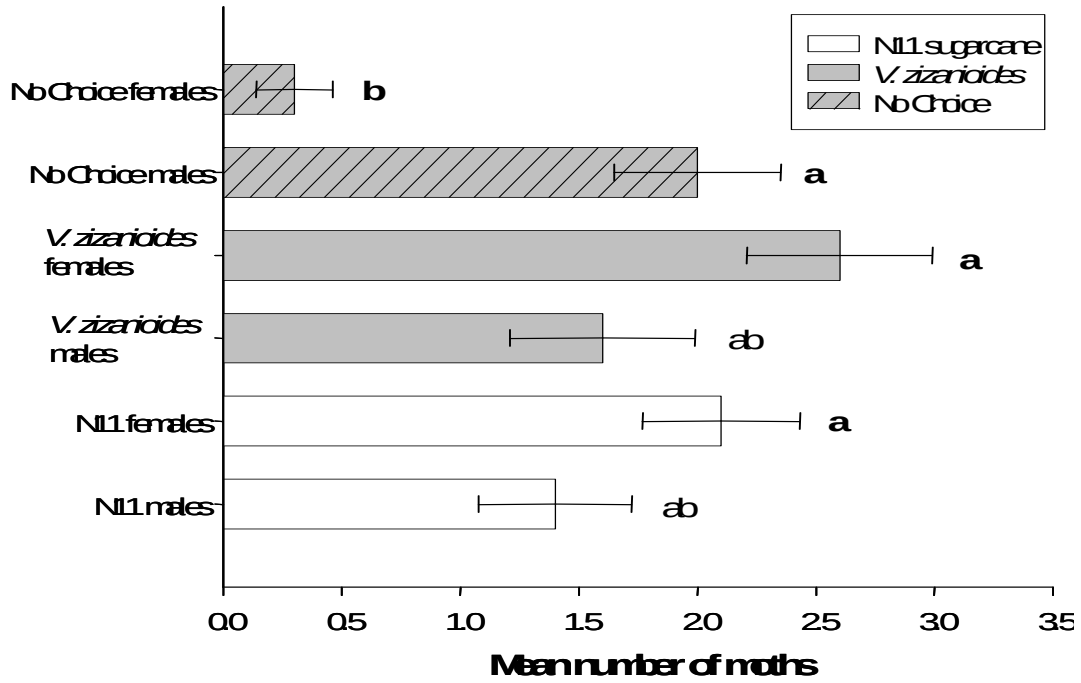


Figure 3.2. *Eldana saccharina* female and male responses to an odour choice between N11 sugarcane and *V. zizanioides*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

Mated male and female moths showed no response to *V. zizanioides* alone. There was no significant difference in the number of moths that made a choice for *V. zizanioides*, for a blank, or made 'no choice' (Figure 3.3).

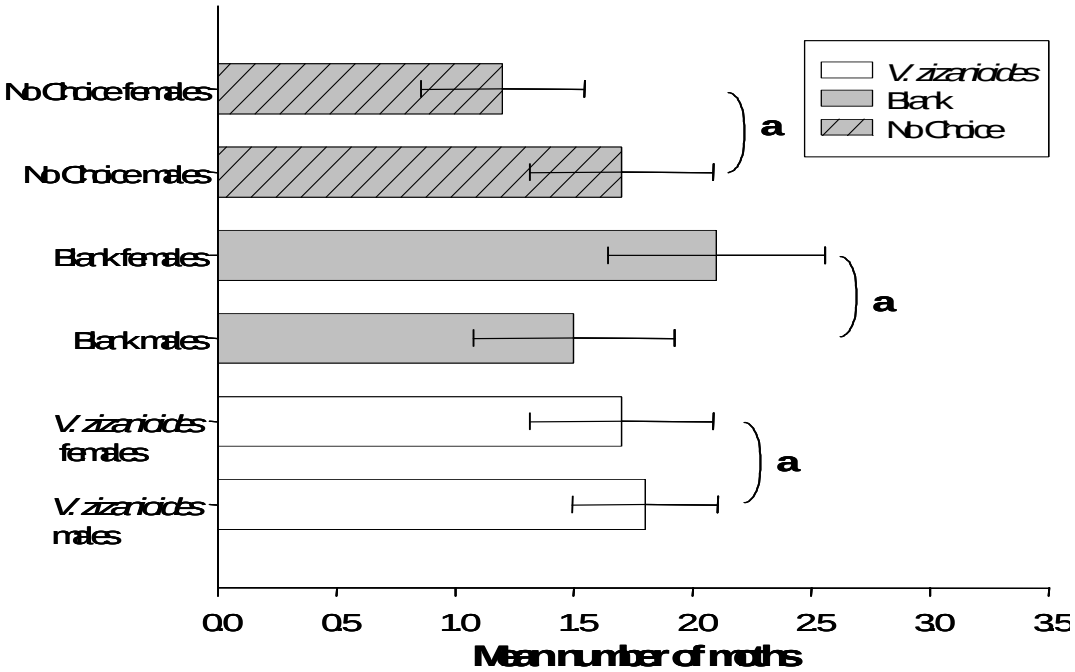


Figure 3.3. *Eldana saccharina* female and male responses to an odour choice between *V. zizanioides* and a blank arm, in a box olfactometer. Main effect means (±SE) bracketed together, followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

Mated male and female moths showed no significant response to *C. lachryma jobi* or N11 sugarcane. Significantly fewer females made 'no choice' and stayed in the box than females that chose the arm containing *C. lachryma jobi* (F=0.026, df=2,45, *P*=0.05) (Figure 3.4).

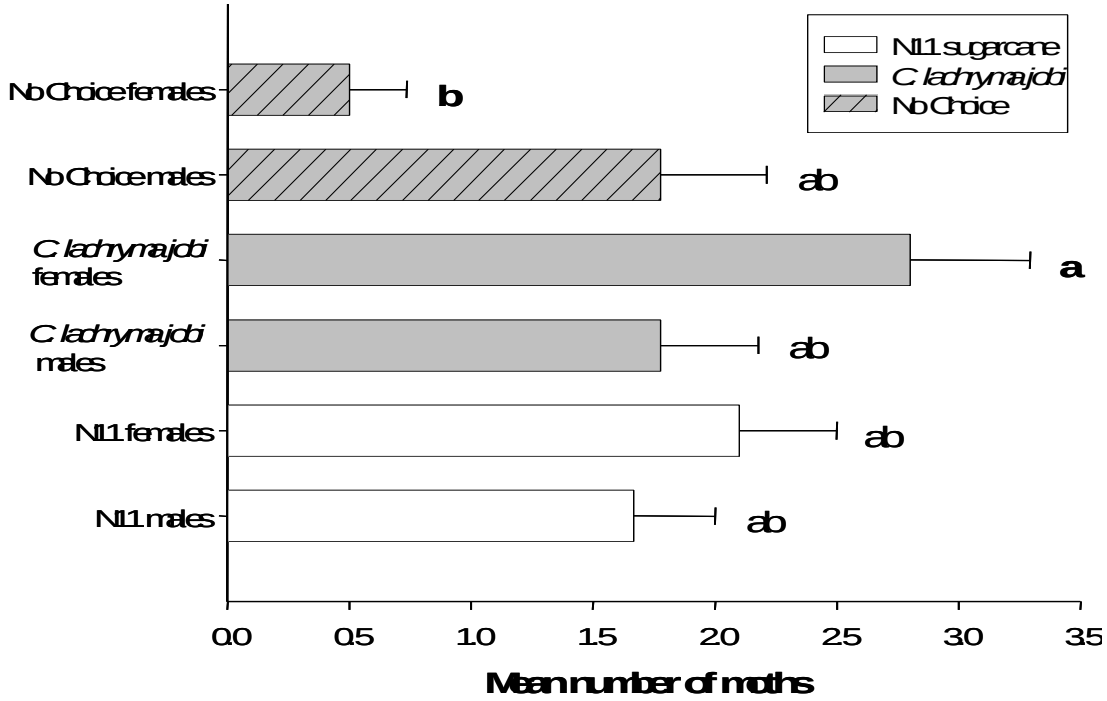


Figure 3.4. *Eldana saccharina* female and male responses to an odour choice between N11 sugarcane and *C. lachryma jobi*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, *P*<0.05) n =10 trials of 5 males and 5 females

Mated male and female moths showed no significant response to *C. lachryma jobi* alone. There was no significant difference in the number of moths that made a choice for *C. lachryma jobi*, for a blank, or made 'no choice' (Figure 3.5).

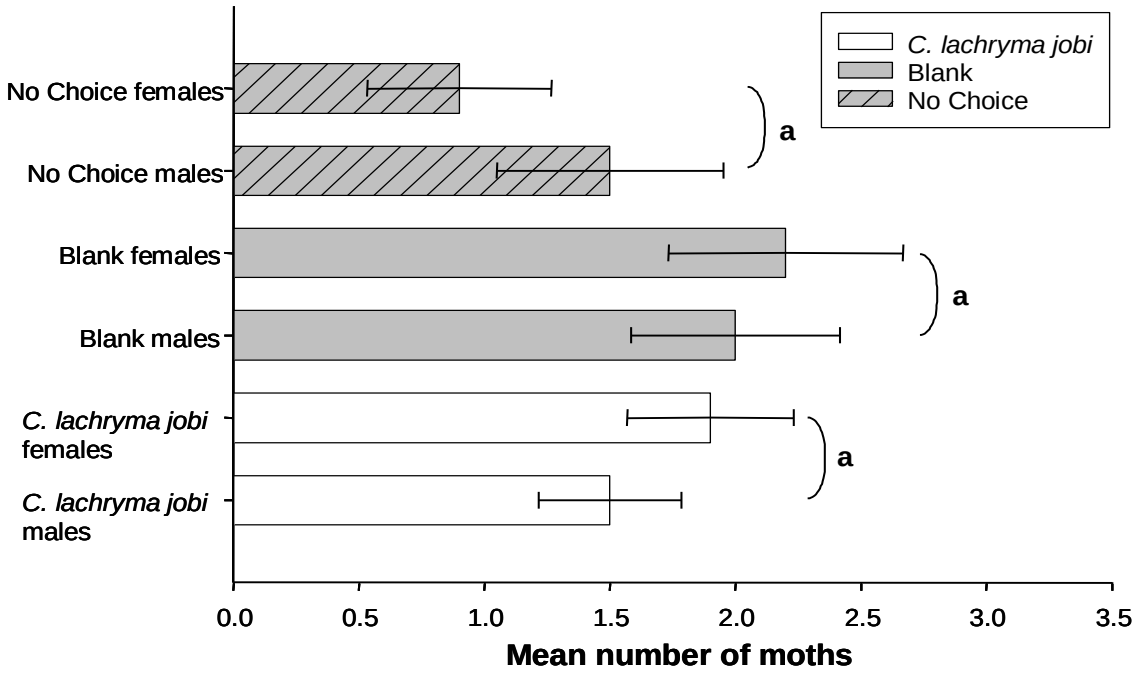


Figure 3.5. *Eldana saccharina* female and male responses to an odour choice between *C. lachryma jobi* and a blank arm, in a box olfactometer. Main effect means (±SE) bracketed together, followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

Female moths showed a significant response to N11 sugarcane over *P. urvillei* (F=0.036, df=2,42, $P=0.05$) (Figure 3.6). There was no significant response by mated male moths. There was also no significant response by male and female moths to remain in the box and make 'no choice'.

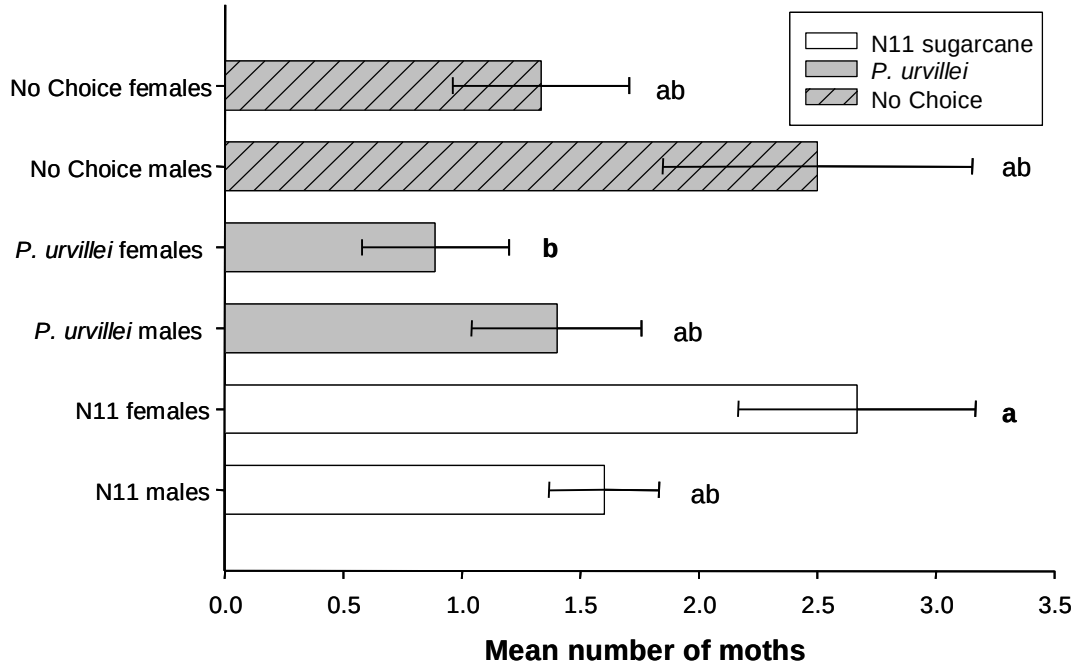


Figure 3.6. *Eldana saccharina* female and male responses to an odour choice between N11 sugarcane and *P. urvillei*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

Mated male and female moths showed no response to *P. urvillei* alone. There was no significant difference in the number of moths that made a choice for *P. urvillei*, for a blank, or made 'no choice' (Figure 3.7).

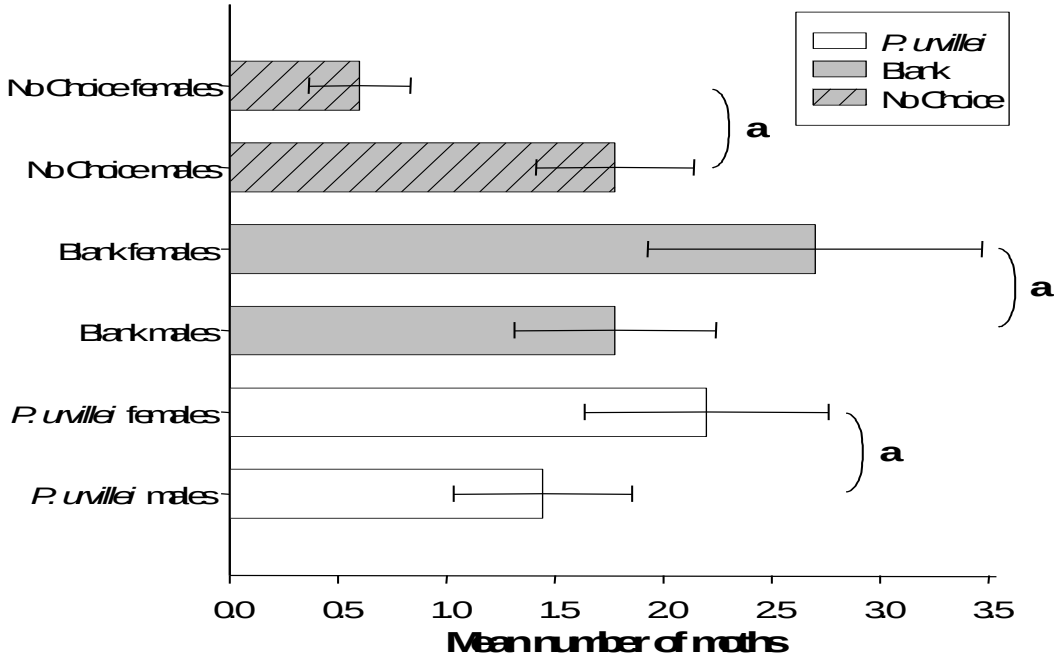


Figure 3.7. *Eldana saccharina* female and male responses to an odour choice between *P. urvillei* and a blank arm, in a box olfactometer. Main effect means (±SE) bracketed together, followed by different letters are significantly different (ANOVA, $P<0.05$), n =10 trials of 5 males and 5 females

Virgin female moths showed no significant response to *V. zizanioides*, male moths or to remaining in the box and making 'no choice' (Figure 3.8).

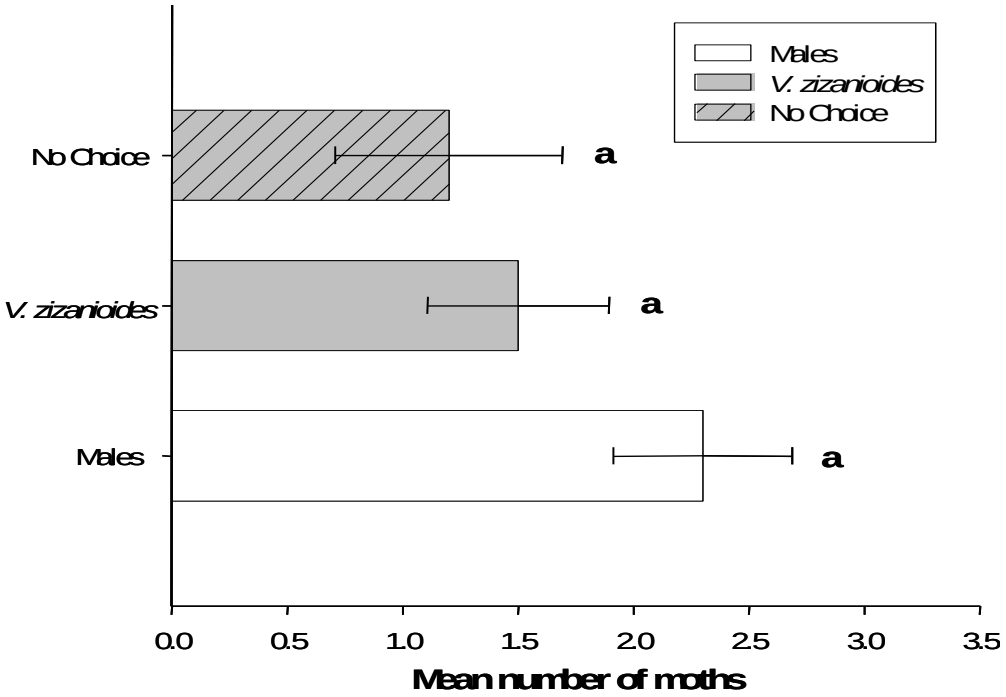


Figure 3.8. *Eldana saccharina* virgin female responses to an odour choice between *E. saccharina* males and *V. zizanioides*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 unmated females

Virgin female moths showed a statistically significant response to male moths over *C. lachryma jobi* and staying in the box and making a no-choice (F=0.005, df=2,18, *P*=0.05) (Figure 3.9).

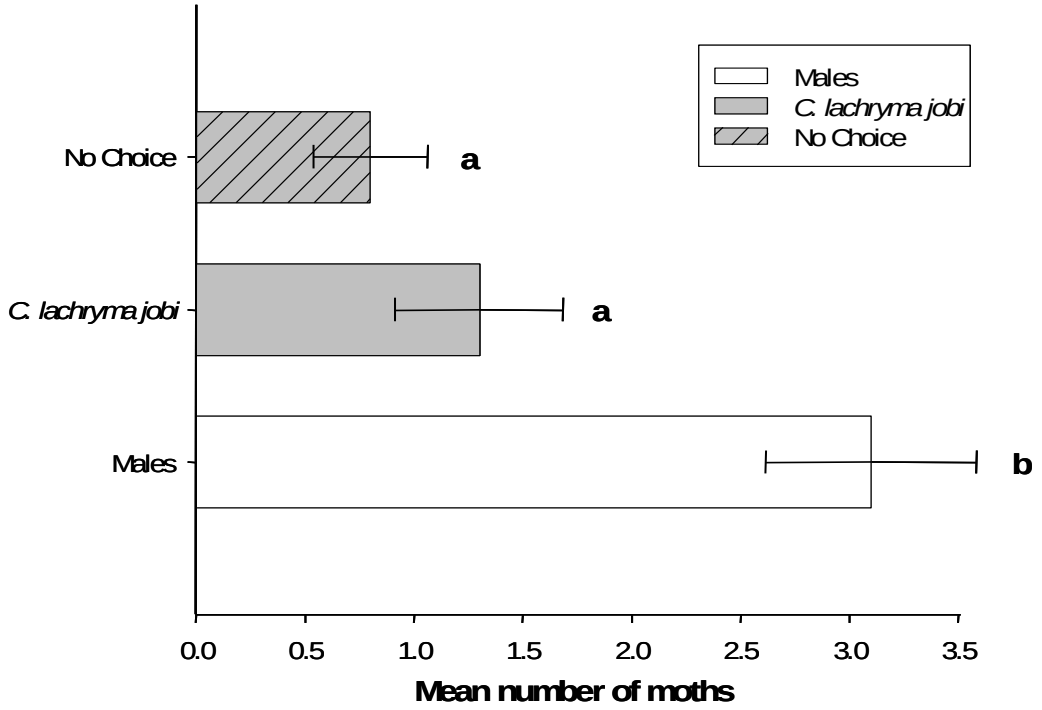


Figure 3.9. *Eldana saccharina* virgin female responses to an odour choice between *E. saccharina* males and *C. lachryma jobi*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, *P*<0.05) n =10 trials of 5 unmated females

Virgin female moths did not show a significant choice between either *P. urvillei* or male moths. Significantly fewer virgin female moths remained in the box and made 'no choice' than females who chose to move out of the box (F=0.034, df=2,18, *P*=0.05)(Figure 3.10).

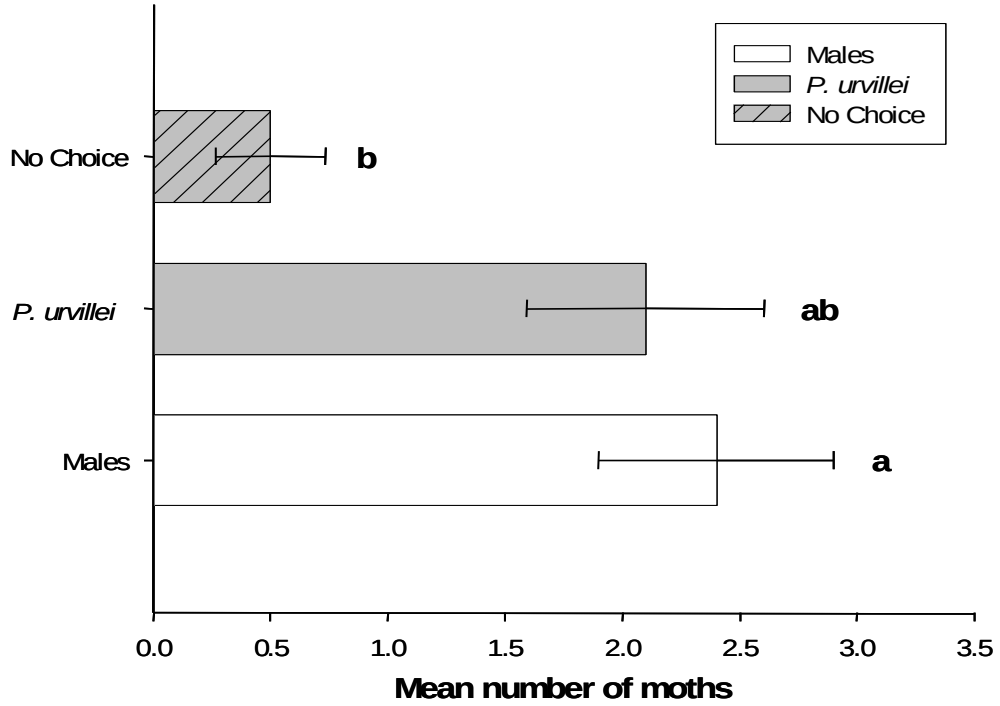


Figure 3.10. *Eldana saccharina* virgin female responses to an odour choice between *E. saccharina* males and *P. urvillei*, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 unmated females

Virgin female moths showed no statistically significant response to N11 sugarcane, male moths or to remaining in the box and making 'no choice' (Figure 3.11).

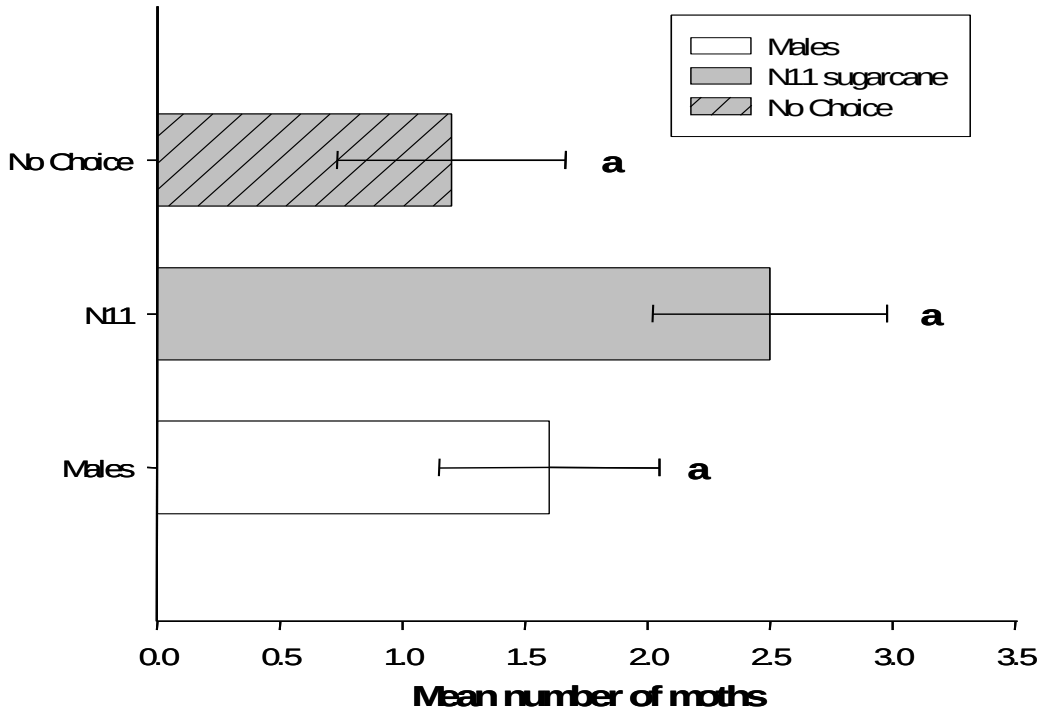


Figure 3.11. *Eldana saccharina* virgin female responses to an odour choice between *E. saccharina* males and N11 sugarcane, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 unmated females

Virgin female moths showed no significant response to male moths or to remaining in the box and making 'no choice' (Figure 3.12).

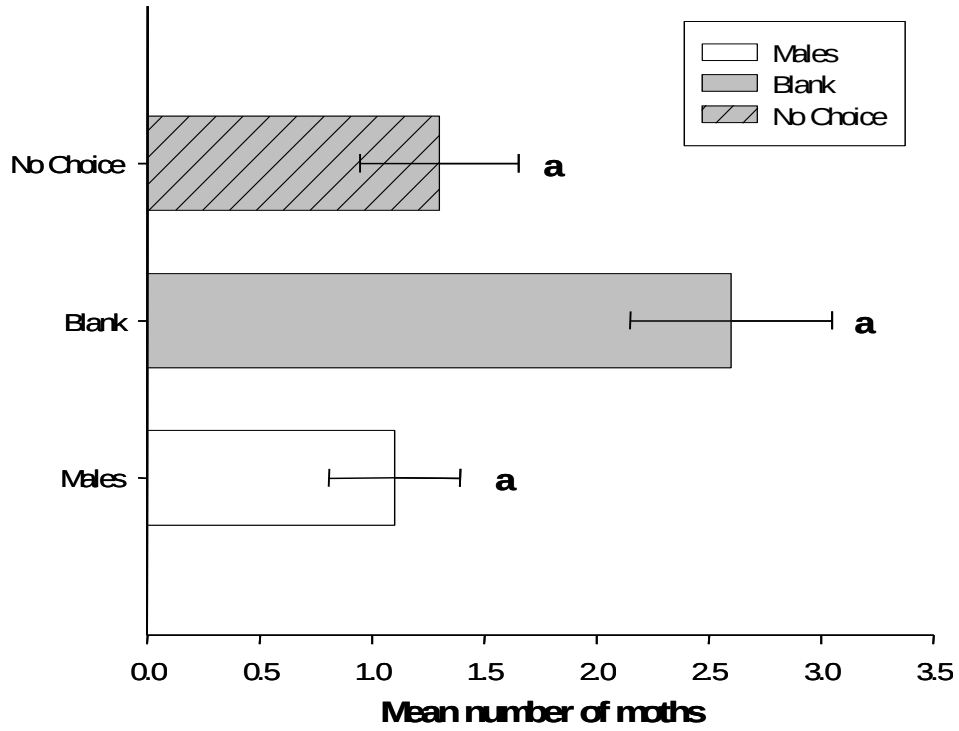


Figure 3.12. *Eldana saccharina* virgin female responses to an odour choice between *E. saccharina* males and a blank arm, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 unmated females

Mated female and male moths showed no response to one side of the olfactometers over the other. Mated male and female moths did choose the right arm significant more than remaining in the box and making 'no choice' ($F=0.008$, df=2,42, $P=0.05$) (Figure 3.13).

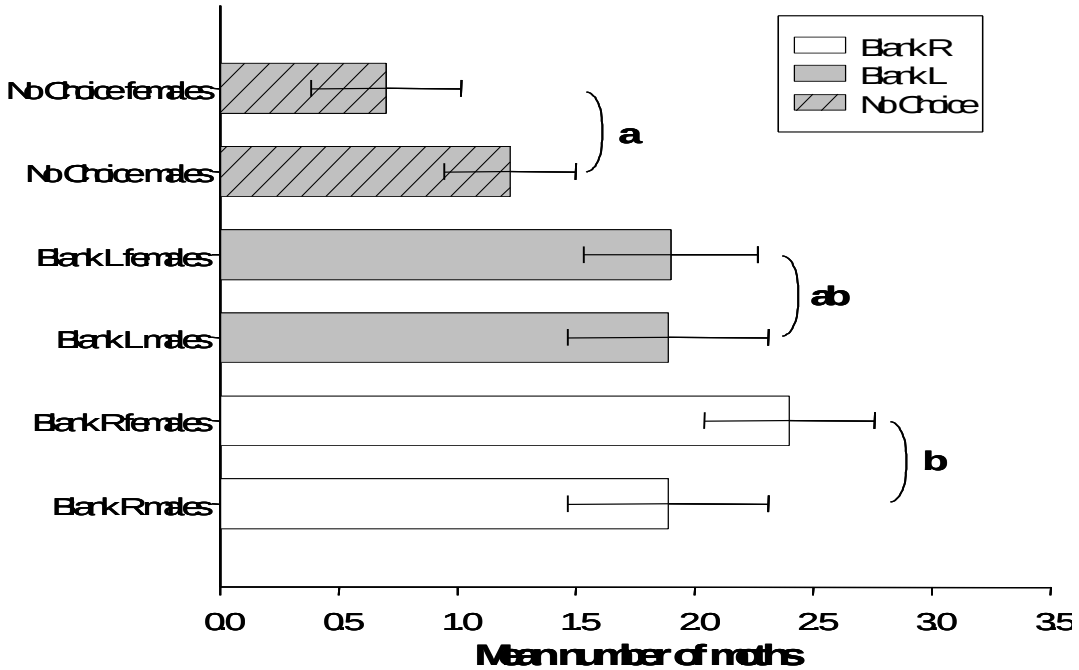


Figure 3.13. *Eldana saccharina* female and male responses to an odour choice of two blank arms, in a box olfactometer. Main effect means (±SE) bracketed together, followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

Mated male and female moths showed no significant response to N11 sugarcane. However significantly fewer female moths made a 'no choice' and remained in the box than chose N11 sugarcane ($F=0.034$, df=2,42, $P=0.05$) (Figure 3.14).

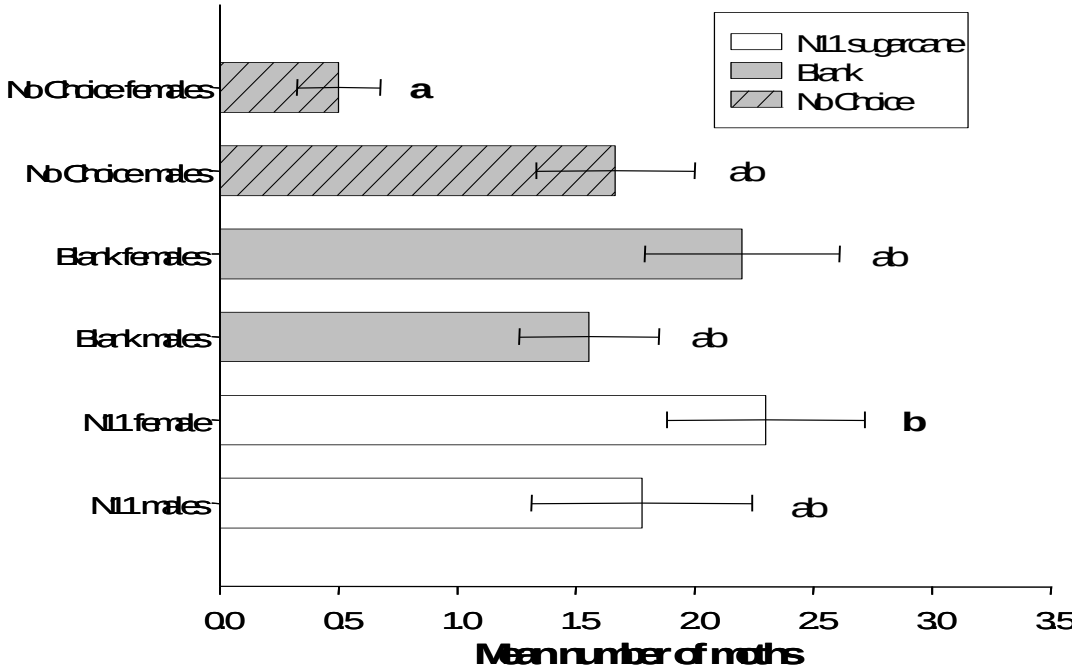


Figure 3.14. *Eldana saccharina* female and male responses to an odour choice between N11 sugarcane and a blank arm, in a box olfactometer. Means (±SE) followed by different letters are significantly different (ANOVA, $P<0.05$) n =10 trials of 5 males and 5 females

In these results, significance in different choices by male and female moths is shown on individual bars. In certain setups, the significant difference is only specific to the variation in the setup and not to the difference between males and female moth choices. Females moved out of the box olfactometers significantly more than the males indicating that females may make the choice for the host plant rather than males. Certain plants at the end of an arm in experimental setups were moderately attractive to both male and female moths and these plants are of interest.

N11 sugarcane and *P. urvillei* is the only combination where the proportion of 'no choice' made by male and female moths was not significantly different from the proportion of males and females that made a 'choice' to move out of the olfactometer box (Table 3.3). In most trials the moths made a choice for an arm, rather than a 'no choice' and remain in the box.

Table 3.3: The probability that male and female moths, combined, make a 'choice' between leaving the box and a 'no choice' by staying in the box when placed in olfactometer trials with two stimuli choices. (*) indicates significance at 5%

Probability that no choice = choice made	
Blank R + Blank L	0.001*
Blank + *V. zizanioides*	0.006*
Blank + *P. urvillei*	0.041*
Blank + *C. lachryma jobi*	0.016*
Blank + N11	0.016*
Males + Blank	0.001*
Males + N11	0.001*
Males + *V. zizanioides*	0.002*
Males + *P. urvillei*	0.001*
Males + *C. lachryma jobi*	0.001*
N11 + *V. zizanioides*	0.031*
N11 + *P. urvillei*	0.496
N11 + *C. lachryma jobi*	0.036*

3.4. Discussion

Significant choices made by female moths for odours are shown in only two combinations out of the 13 used in these trials. In the combination of sugarcane and *P. urvillei*, female moths chose the odour of sugarcane significantly more than the odour of *P. urvillei* (Figure 3.6). This could indicate that sugarcane is more attractive than *P. urvillei*, or it could be that *P. urvillei* was not attractive and the moths chose sugarcane because it was in the other arm of the olfactometer. Secondly in the preference for males versus the non-host plant *Coix lachryma jobi*, females chose males significantly more than *C. lachryma jobi* (Figure 3.9). Females may have been attracted to the males in this setup, or the *C. lachryma jobi* may have been repellent to the moths. There were no other examples with *C. lachryma jobi* in this study that could support either hypothesis, with a significant result.

There was no significant difference for a test plant over a blank arm with male and female moths (Figures 3.3, 3.5, 3.7 and 3.14) and no significant difference for male moths over blank arms shown by virgin females (Figure 3.12). Tested plants were not attractive enough to cause moths to choose a plant volatile over no volatile in the other arm.

The probability that moths preferred to move out of the box into an arm of the olfactometer rather than stay in the release box and make 'no choice' (Table 3.3) showed that in all combinations, save for one, the moths significantly chose to move into an arm rather than stay in the box. The one combination in which there was no significant difference was *P.*

urvillei tested against sugarcane (Figure 3.6). This one result showed that *P. urvillei* does not attract *E. saccharina* to cause the moths to move out of the box and make a choice for one of the arms. The previous result with *P. urvillei* and sugarcane also showed *E. saccharina* does not choose *P. urvillei*. There is a possible repellent effect from *P. urvillei* as *E. saccharina* has not chosen for it, and further study should be done on this grass. This does not explain why this insect was found in numbers on this plant in the wild. Chemical volatiles from the test plants may not play a role in the choice of this insect rather the tactile stimulus from this plant could be attractive to the moths in the field.

The preference of moths towards one side of the olfactometer was tested by running the olfactometers with blanks in both arms and the mean choice for the right arm was compared with the mean choice for the left arm (Figure 3.13). The results showed no significant difference between the arms therefore there was no bias for one arm influencing the plant choice in the olfactometers. All box olfactometers used in the trial were identical in their setup for the experiments. They were run under the same laboratory conditions and both genders of moths were released at the same time into the box. Therefore, no other influences causing different choices by moths in the replicates were present. The only factors different between each experiment was the combination of plant volatiles drawn through each of the boxes.

What is known about *E. saccharina* can help in understanding possible reasons as to why there was no clear preference for test plants shown. Male *E. saccharina* moths call by expressing pheromones, from both wing glands and abdominal pencil hairs (Burger *et al.*, 1993), and use sound (Bennett *et al.*, 1991) during calling. The interaction between moth genders and which gender makes a choice for the plant, are crucial to understanding the results from this study.

The first possible scenario that takes place in the olfactometer trial with both sexes, is that males call in the box, females respond and they mate, then females make a choice for a host plant. This is supported by the observation that females consistently moved out of the box more often than males (Figure 2). The cues being followed by the female are not related to the plants present in each choice chamber, as there is no significant preference shown for any plants by females or male moths.

Something else may influence the movement of female moths. The olfactometer results from mated females compared with unmated, virgin females showed a non-significant difference (Table 3.3). Mated females chose to move out of the box equally as often as virgin females. Mated females need oviposition sites and move to find one on a host plants. Virgin females did move out of the box into one of the arms even though they were not mated. However they did not significantly choose male moths over other plants or blank arms (Figure 3.8 - 3.12). This could be due to males not being able to lek or call properly within the confines of the holding containers. The females may also need the cues from both the plant and the males to attract them to a test plant. Alternately in this scenario females may choose the plant first and then look for males on that particular plant to mate with. Olfactometers do not lend themselves to test this concurrently with host plant choices.

Eldana saccharina is shown to be fairly indiscrimate in choosing host plant species. Kasl (2004) found in oviposition trials that no preference was shown by females as eggs were laid in many suitable and unsuitable locations in the cages. Chapter two of this study showed a similar result with female moths ovipositing indiscriminately around cages. Cryptic sites may be the main factor influencing ovipositing females to choose a host plant or just oviposition site. Female moths often oviposited in the corners of the olfactometer box, as well as at the bottom of the collecting chambers where the eggs were fairly protected.

Kasl (2004) found in olfactometer trials that females did not choose significantly for or against non-host plants. This is confirmed in these results in a different olfactometer setup using volatile cues from different potential host plants.

Beerwinkle *et al*., (1996) tested males of the noctuid, *Helicoverpa zea* in olfactometers and found that males responded significantly to plant odours from *Gaura longiflora* increasingly through the night. Males were able to choose the host plant by being attracted with plant volatiles. The second potential scenario in this trial is male moths make the choice of host plants based on the volatile cues in the environment. Once a suitable host plant that would be beneficial to its offspring is chosen and males have moved onto the plant, they would call to the females. Females being attracted to the males would supposingly mate and then oviposit on the “nuptial” plant without moving to another plant (Kasl, 2004). Females in this scenario would not be affected by plant volatiles and may

look rather for oviposition sites based on the architecture of the plant, and availability of cryptic sites. Pheromones are known to also attract other males to form a chorus or lek that calls together to attract females (Atkinson, 1981).

These results do not suggest that females followed male moths into whichever arm the males chose as females consistently moved more than males. The attractiveness of males in one arm and test plants in the other arm of an olfactometer showed females had no preference for males over test plants. In most trials, if the males were calling, it had no effect on the females or the males were not calling and the females made no specific choice for that arm of the olfactometer. Cristofaro *et al*., (2000) showed that beetles feeding on *Euphorbia sp.* did not choose a particular host plant based on the volatiles coming from this test plant but rather visual attraction is the more important stimulus. No significant results were seen in the beetle trial for a choice of one plant in an arm of the olfactometer over another plant. There is a possibility that the moths in the current study are also not attracted by the volatile stimuli of a host plant.

The preference of females towards males and male pheromones was not fully tested in these trials. Future studies should determine the influence of males calling in the canopy on female attraction to host plants. It is possible that the males were not able to effectively call for females in this experimental setup. In the same way that the cage trials in chapter 2 did not give significant results possibly because of the effect of being in a cage, the environmental setup was not realistic enough for a true behavioural analysis of these insects to be performed. A field setup would be the next option for a better reflection of *E. saccharina* behaviour and their attraction to potential host plants or repellence from non crop plants.

Chapter 4:
Intercropping sugarcane with *Melinis minutiflora* to reduce *Eldana saccharina* infestation

4.1. Introduction

Åsman (2002) says that it is important to do oviposition and host plant choices at different scales. The results in the laboratory with only one or two plants will not give the same results as a field trial where a female insect has a choice of a number of different plants in an environmental setup. Field trials are the next arrangement to test what was found in laboratory trials.

4.1.1 Intercropping

Intercropping, where two or more crops share the same piece of land for a part or all of a growing season (Parsons, 2003), is a traditional agricultural practice used particularly by small scale farmers (Van den Berg *et al*., 1998) to reduce crop losses from pathogen and insect infestations (Ma *et al*., 2007).

The Gatsby Charitable Foundation supports agricultural research and development, and in particular the work done on stemborer control strategies at the ICIPE. Projects run there on the habitat management of maize pests have had some good results when intercropping was introduced to reduce the negative impact of stemborers. Carefully chosen combinations of intercropped plants are planted around and among the maize plants to protect the maize plants by attracting and trapping stemborers. Invading adult moths are attracted to chemicals emitted by these intercropped grasses and instead of landing on the maize plants, the moths go towards the plants that are perceived as better for food or oviposition (Khan *et al*., 1997a). Good trap crops, used as pull plants in the fields are *Pennisetum purpureum* (Schum.) (Napier grass) and *Sorghum vulgare sudanense* (Pers.) (Sudan grass) (Kaufmann, 1983), while the push in this strategy was provided by *Melinis minutiflora* (Beauv.), (molasses grass) which emits chemicals that repel stemborers such as *C. partellus* away from the maize fields (Khan *et al*., 1997a). Other stemborers also repelled by *M. minutiflora* which makes it useful for several crops (Gohole *et al*., 2003; Khan *et al*., 2000).

Studies in Kenya (Midega *et al*., 2005) have shown that stemborer populations in maize were reduced by planting native African grasses, as borders around maize fields, which were attractive to ovipositing female moths. *Chilo partellus* and *B. fusca* were shown to prefer *S. vulgare sudanense* and *P. purpureum* rather than maize (Kfir *et al*., 2002, Overholt *et al*., 2003, Van den Berg *et al*., 2001). In West Africa, maize, millet and sorghum intercropping showed an 80% reduction in *C. partellus* infestation within 1 week after larvae hatched (Päts, 1992). Maize was intercropped with cassava in Nigeria and stemborer larval numbers were reduced by 50% compared to monocropped maize (Kaufmann, 1983). Of all the methods of intercropping, strip cropping has the most potential to increase crop yields by suppressing pest outbreaks, through increases in parasitoids attracted by the strip crop (Ma *et al.*, 2007). Strip cropping of wheat and alfalfa (*Medicago sativa* L.) has improved the effectiveness of biological control agents against wheat aphid (Ma *et al*., 2007).

Intercropping has uses other than insect control. Parasitic weeds such as *Striga hermonthica* (Del.) (striga weed) infests up to 40% of arable land in the savannah regions of Africa, causing large economic losses (Khan *et al*., 2001). *Striga hermonthica* germinates in the maize field as the cash crop begins to grow and competes with the maize plants for nutrients and water. Instead of growing roots, this parasitic weed draws its nourishment from the maize plants, weakening or even killing them (Gatsby occasional paper, 2005). Khan *et al*., (1997b), noticed that maize intercropped with *Desmodium uncinatum* (Jacq.) (Silver leaf) or *Desmodium intortum* (Mill.) had less striga weed infestations than maize in monoculture fields. *Desmodium uncinatum* is also a push plant for stemborers and had a reducing effect on stemborer populations in the field (Khan *et al*., 1997a; Khan *et al*., 2000).

4.1.1.1. Advantages of intercropping

Higher total yields are achieved with intercropping than with single crop yields, probably due to less intraspecific competition. It results in a more efficient use of resources in the environment, and additional weed control that the intercrop may establish by reducing available space for weeds. There is provision against crop failure and improved food quality provided by the variety in the crop field. Maize as a sole crop requires a larger area to produce the same yield as the maize component in an intercropping system, because the intercrop improves the yield of the main crop (Viljoen and Allemann, 1996).

4.1.1.2. Disadvantages of intercropping

Specific mechanisation is required to harvest plants of different heights or ages and only specific rows need to be harvested at a single time. There can be difficulty in controlling pests and weeds that may be introduced with the intercrop. The allelopathic effect of one crop on another can be harmful and drought stress can be aggravated at times of limited moisture. Cowpea as a sole crop requires a smaller area to produce the same yield, compared to its yield as an intercropping component, due to its poor ability to compete with maize (Viljoen and Allemann, 1996).

Sugarcane has great potential for intercropping, because it is planted in wide rows and takes several months to canopy, during which time solar energy, soil and rainfall, between the rows and around fields of sugarcane are wasted (Parsons, 2003). Plants with shorter maturing time that can be grown and harvested within 90 to 120 days are perfect for intercropping into sugarcane fields, provided there is additional benefit for the farmers planting this 'non-crop'; productivity of this land will be increased. The shorter maturing crops can be planted with sugarcane but harvested before the sugarcane reaches maturity and therefore do not compete with sugarcane when it is increasing sucrose yield. Areas of the sugarcane field such as the edge of roads, irrigation lines, borders of field waterways are all ideal for longer maturing intercrops. Plants can be maintained in these areas in fallow fields after harvesting and persist until the next crop matures.

4.1.2 *Melinis minutiflora*

An indigenous grass, *Melinis minutiflora,* produces many volatile chemicals even when intact (Gohole *et al*., 2003). *Melinis minutiflora* planted in alternate rows with maize reduced stemborer infestations of the main crop from 39.2% to 4.6% (Khan *et al*., 1997a). Kasl (2004) also showed that the chemical volatiles from *M. minutiflora* are attractive to a pupal parasitoid, X*anthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae). Of the combination of chemicals that are produced by *M. minutiflora*, the active chemical, (E)-4, 8-dimethyl-1, 3, 7-nonatriene (Nonatriene) is a chemical also produced by maize (Kfir *et al*., 2002; Kasl 2004). Nonatriene compounds were shown by Khan *et al*., (1997a) to be responsible for the repellency of *M. minutiflora* to *C. partellus*.

4.1.2.1 Physical properties of *M. minutiflora*

Melinis minutiflora is grown in South Africa and has been found in other countries in Africa (Gohole *et al*., 2003). It is a valuable hay and pasture grass, with well known anti-tick properties especially when still green (Khan *et al*., 1998) and has drought resistance (Gohole *et al*., 2003). This chapter presents the agricultural and economic benefit of *M. minutiflora* used as a push crop in the field, in a stimulo-deterrent diversion strategy to manage *E. saccharina* in sugarcane.

Kasl (2004) showed in field trials at Pongola; in the northern region of the KwaZulu-Natal (Figure 4.1), that there was a significant difference in *E. saccharina* damage between treatment and control plots, with higher *E. saccharina* populations in control plots. At her Gingindlovu site, on the north coast of KwaZulu Natal, (Coastal 2 in Figure 4.1) there was no significant difference between treatment and control plots, and no significant difference in *E. saccharina* infestation as distance from the *M. minutiflora* increased. The watering regime (irrigated or not irrigated) and environmental conditions were used to explain this result (Kasl, 2004). Field trials in the current study with this grass tested across both areas of the sugarcane industry, and tested the intercrop setup in the field rather than just the influence of distance from the grass affecting pest populations, as tested in the previous study by Kasl (2004).

4.1.3 Control methods used for managing *E. saccharina*

The main objective in controlling the damage by larvae of *E. saccharina* is to stop the moths from getting onto sugarcane stalks. Oviposition by adult moths ideally should not take place on sugarcane. According to Vandermeer (1989) there are three possible mechanisms for reducing pest infestation in an intercropped system. The first is that an introduced non-host plant disrupts the ability of the pest to attack the proper host plant by making the insect pest perceive the field to be undesirable, i.e. a repellent plant field. The second being that an intercropped non-host plant attracts the pest away from the host plant by being more attractive than the host crop, or thirdly the intercropping setup attracts natural enemies of the pest which will reduce the pest population on the host plants by giving the crop field a volatile odour that is attractive to parasitoids. These are not mutually exclusive and in the best situation, all three mechanisms should be employed to reduce the pest infestation. In the present study, attracting the pest away from sugarcane is not possible because the plants tested in laboratory trials were not shown to be more attractive to *E. saccharina* than the current host plant, sugarcane.

Different planting arrangements of *M. minutiflora* intercropped between rows of sugarcane could give different levels of success as a repellent crop. Planting a row of *M. minutiflora* in the place of a row of sugarcane is expected to spread the volatile given off by this repellent grass through the adjoining rows, but it may not make up for the loss of a row with the benefit it provides in reduction of pest infestation. Border planting of *M. minutiflora* on the edge of the drainage or irrigation lines around a field plot can repel pest species, but the volatile effects may not reach far into the field depending on how large the field area is. Planting repellent grasses in the space between rows of sugarcane could be as efficient as replacing a row of sugarcane with non-host plants, if the sugarcane canopy does not shade out the push plant and stop it growing; the volatile effect will then be lost.

4.1.4. Sugarcane agriculture

Sugarcane is an annual upright grass planted as seed cane. Seed cane as cut sections of sugarcane stalk are placed in the soil in rows approximately 0.9-1.5m apart. Stalks and roots grow from the nodes of these cuttings. One season of sugarcane growth, generally a year to 18 months, is cut and sent to the mill, and the field is allowed to grow back from the roots, called a ratoon. This leaves a space between rows wide enough to walk down and enough space between sugarcane rows for stalks not to compete for sunlight and moisture. Irrigation or drainage lines or roads often run through the field of sugarcane on a farm, subdividing it into blocks. Each block can be treated separately and harvested at different times and have different varieties planted in it. Sugarcane is generally planted towards the end of the year or at the beginning of the new year, and on the South African coast is grown for a year and then harvested. This is done to stop infestation damage from stemborers which feed on mature plants. Sugarcane grown in the KwaZulu-Natal Midlands can be ‘carried over’ to the following season and harvested at 18 months or even 24 months as problems with pest damage are not as severe in the Midlands. A second ‘non-crop’ can be planted just after harvest, or at the same time as the seed cane. Roads and irrigation lines are good areas to plant intercrops because of the space in and around the sugarcane fields. This intercrop can establish quickly into thick undergrowth, spreading out to fill up the available space and stop other undesirable, weedy plants moving into these areas.

Because insecticides are expensive (Khan *et al.*, 2000) and labour intensive to apply, farmers are willing to try alternative methods of pest control, if it is easier and sustainable. Intercropping can be applied to small scale and larger scale sugarcane agriculture and

there are benefits of a second crop from the introduced plants being taken from the same land.

What is lacking for use in the sugarcane industry is a plant that can effectively repel moths, and that will grow in the wetland areas around sugarcane fields. The reason for doing the tests as shown in chapters 2 and 3, is the end result, if any plants are suitable; to plant them into a field setup as was done and reported in this chapter.

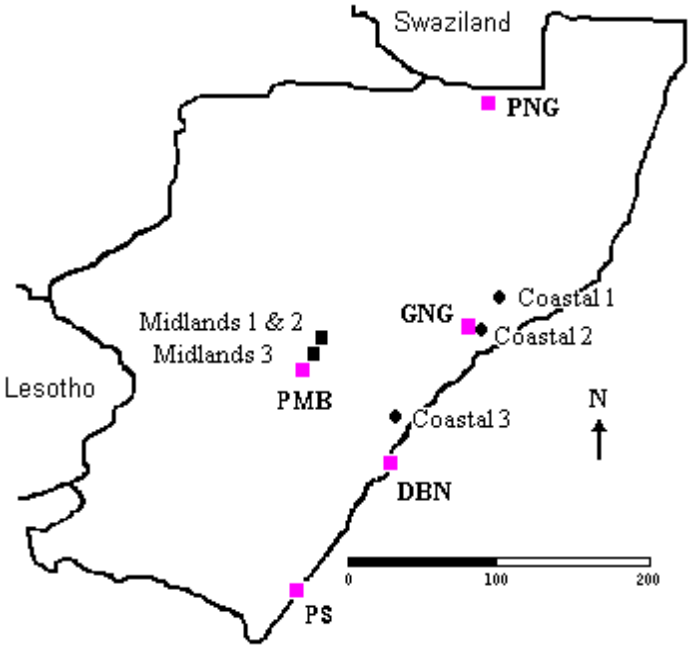


Figure 4.1: Distribution of field sites around KwaZulu-Natal province. Black blocks indicate numbered field sites in the Midlands (low population pressure) and black circles are numbered field sites on the coast (high population pressure). Red blocks indicate cities and towns, DBN – Durban, PMB – Pietermaritzburg, PS – Port Shepstone, PNG – Pongola, GNG - Gingindlovu

4.2. Methods and Materials

4.2.1. Fields sites locations

Seedlings of *Melinis minutiflora* were planted in sugarcane fields at a commercial farm in Emoyeni (28°57′ S; 31°39′ E) (Figure 4.1, Coastal 1),at the SASRI research farm in Gingindlovu (29°01`46.4“ S; 31°36`42.5“ E) (Figure 4.1, Coastal 2), on the north coast of KwaZulu-Natal, and at the SASRI field sites in Mount Edgecombe (29°42.5’ S; 31°20’ E) (Figure 4.1, Coastal 3). Two commercial farms, in the Midlands North area (29°35′ S; 30°30′ E) (Figure 4.1, Midlands 1 and 2) and (Figure 4.1, Midlands 3), 100km inland of the coast of KwaZulu-Natal were also used. Each farmer had setup his fields differently from the others, so each site had fields of different sizes; therefore each experimental setup incorporated *M. minutiflora* into different row spacings and placements of the repellent grass.

These different arrangements were advantageous in testing the best planting density for this grass, despite being confined by the dimensions of the fields. *Melinis minutiflora* was planted along lines of sugarcane that allowed sunlight to the grass and was not shaded out as the sugarcane increased in height. The disadvantage of using commercial farms was that replicates between farms were not identical.

On each farm, control and treatment plots of equal size were planted with sugarcane monocultures of the same sugarcane variety at each individual field site. The control plots within farms were at least a field length of 50 m away from the treatment plots. Kasl (2004) found that *E. saccharina* populations were unaffected by *M. minutiflora*, planted 50 rows away in field trials. However, at Coastal 3 field site (Figure 4.1) the control and treatment fields were only 10 metres apart (Figure 4.4) as this was the size of the trial field available.

4.2.2. General field site descriptions

Field trials for this study were conducted in two different areas of the KwaZulu Natal sugarcane growing region, with different levels of population pressure from *E. saccharina*, comprising three field sites in the Coastal regions, and three inland field sites, in the Midlands area (Figure 4.1). Population pressure from *E. saccharina* is high along the coast, and low in the Midlands which is largely due to the varieties planted in these two different areas of the sugarcane industry. Varieties of sugarcane that give higher yields in the coastal agroclimatic zone, on the specific soil types, which are mostly rainfed, are often varieties that are more susceptible to *E. saccharina* (Redshaw and Donaldson, 2002). In the Midlands region, sugarcane fields are often irrigated and because *E. saccharina* attacks water stressed sugarcane, these fields are not as susceptible to stemborer damage (Redshaw and Donaldson, 2002).

Fields of sugarcane were planted as seed cane in some field sites, before the intercrop treatments were planted. In other field sites, a second ratoon crop was used and the treatment was planted just after harvesting the preceding crop. The sugarcane fields used incorporated both irrigated and rain fed fields. Each field site was divided into blocks; numbers were assigned to the blocks and random numbers chosen to determine where three treatment plots would be situated within the larger field sites. Three control blocks were also chosen randomly around the field.

The natural host plants, *Cyperus papyrus* (L.) and *Cyperus dives* (Delile.) Cyperaceae (Atkinson, 1980) were found by Kasl (2004) in cage trials to be attractive to this moth. All

of the field sites had *Cyperus dives*, the natural host plants of *E. saccharina*, growing along the banks of a river or wetland close to each experimental plot. Sugarcane rows were planted along the contours across the whole field at all sites.

4.2.3 Experimental Procedure

Melinis minutiflora was planted in between the rows of the sugarcane and next to rows in the treatment plots in various positions and densities in the field sites as shown in Figures 4.2-4.6. No additional grass other than sugarcane was planted in the respective control plots of each field site. One seedling of *M. minutiflora* was planted every 500 mm next to the row of sugarcane in the treatment by pushing it into a hole in the row, then the hole was covered with soil and watered. Seedlings were left to grow in the field until the sugarcane was ready to be harvested. Field trials were monitored during the trial by sampling the sugarcane for number of internodes in the sugarcane, sugarcane height, and density, *E. saccharina* presence and damage to sugarcane, at the end of the trial before the sugarcane was harvested.

Melinis minutiflora was planted in one of three different setups across the three fields sites in both the Midlands and the Coastal areas (Table 4.1). In Coastal 1 and Midlands 1, sugarcane was planted in rows with demarcated treatment and control plots. Along the edge of the irrigation line and water drainage lines that ran along the top, middle and bottom row of the treatment plots, a row of *M. minutiflora* was planted. In Coastal 2 and Midlands 3 where molasses grass was planted in place of a row of sugarcane, one row every 10 rows of sugarcane was killed using the glyphosphate herbicide, Roundup and was ploughed out. *Melinis minutiflora* was then planted in the place of each removed row. In the Coastal 3 and Midlands 2 field sites, *M. minutiflora* was planted between the rows of sugarcane in the interrows on the edge and middle line of each treatment plot.

Table 4.1. Field sites characteristics where *M. minutiflora* was planted to test its effect as an intercrop on *E. saccharina* infestation.

Site #	Region/ *E. saccharina* Population	Irrigated / Rain-fed	Cane planting method	Treatment planting time	Cane Variety/ Susceptibility	Treatment Spacing	Planting Date of sugarcane	Placement of treatment
1	Coastal 1 High	Rain fed	Seed Cane	Before sugarcane	N29 Intermediate	20 rows	February 2004	Drainage line
2	Coastal 2 High	Rain fed	Ratoon	After sugarcane	NCo376 Resistant	10 rows	April 2004	In place of sugarcane
3	Coastal 3 High	Rain fed	Seed Cane	After sugarcane	N11 Susceptible	5 rows	June 2005	Inter row
4	Midlands 1 Low	Irrigated	Seed cane	Before sugarcane	N39 Resistant	20 rows	December 2003	Irrigation line
5	Midlands 2 Low	Irrigated	Seed Cane	Before sugarcane	N36 Intermediate	20 rows	December 2003	Inter row
6	Midlands 3 Low	Rain fed	Ratoon	After sugarcane	N16 Susceptible	10 rows	December 2003	In place of sugarcane

4.2.4. General sampling procedure

At each site, a sample was taken between when the sugarcane was a year old and at sixteen months in the Coastal area or at 18 months old in the Midlands area. Ten sugarcane stalks, collected at intervals of one stalk every five metres, were removed and destructively sampled in treatment plots at the first row of the first treatment plot, designated as the sugarcane row nearest to the *M. minutiflora*. Stalks were then taken from the row in the middle of the plot between *M. minutiflora* rows, and the next row after this that had *M. minutiflora*. This pattern was repeated for the remainder of the field plots, and the same respective rows in the control plots.

Sugarcane stalks were split along their length; the total number of internodes, and the numbers of internodes damaged were counted for each stalk. Any *E. saccharina* larvae still in the stalks were collected and placed in a 30 ml plastic vial, with a gauze lid, filled with artificial rearing diet (Conlong *et al.*, 1988). Collected larvae were reared at the SASRI Insect Unit, at 28^{o}C, 75% relative humidity to determine whether any parasitoids had infested the collected larvae or pupae.

4.2.5. Statistical analysis

Normality tests were done on combined survey data of each field site followed by Kruskal-Wallis analysis to test for significant differences between the treatment plots and

control plots in the percentage of internodes damaged within a field site. Mann-Whitney rank sum tests were conducted to assess differences in the percentage of internode damage between control and treatment plots within a site when the assumptions of normality and homogeneity of data were violated. For data found to be normal, a two sample T-test was performed to test the differences in *E. saccharina* numbers between control and treatment plots. This was conducted on combined data from all treatment plots and all control plots of each field site, except for Coastal 3 field site. A Wald test for fixed effects was done to determine the significance of difference in mean *E. saccharina* found between the control plot and treatment plot for Coastal 3.

4.2.6. Specific field details

4.2.6.1 Coastal 1

A field of sugarcane was planted with *M. minutiflora*, on the parallel edges of 50m square blocks. Field plots were arranged within the field site so that drainage lines ran along the top and bottom edges of the plots and one drainage line ran through the middle row of each plot (Figure 4.2). The drainage lines were wider than the sugarcane interrows, allowing *M. minutiflora* planted along these lines to grow strongly in the direct sunlight. Control plots were not planted with *M. minutiflora*. *Eldana saccharina* damage surveys were done before harvesting the sugarcane at sixteen months.

Coastal 1 field site

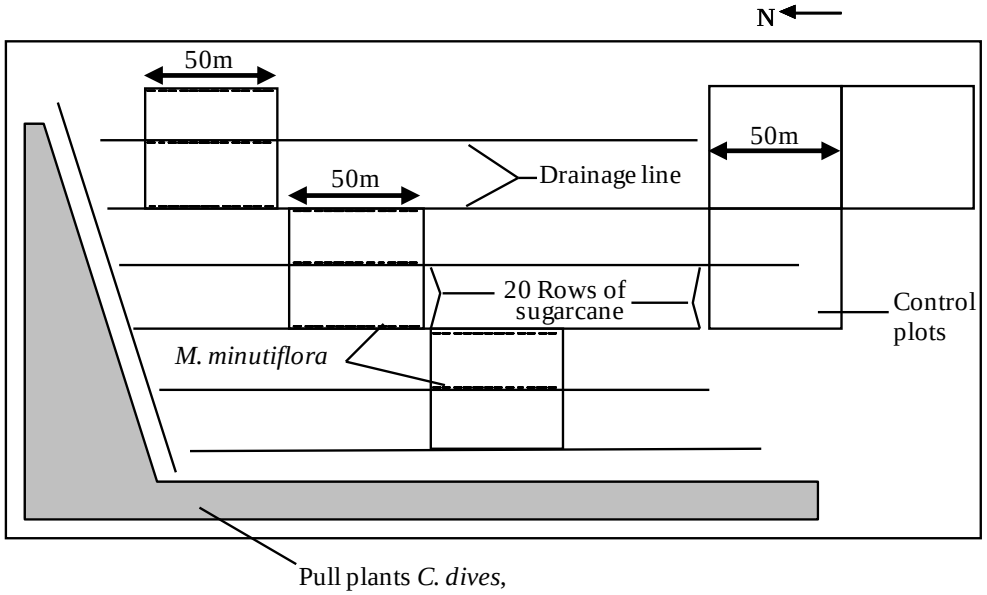


Figure 4.2. Field setup at Coastal 1 field site with *M. minutiflora* planted on the banks of drainage lines in 50 m lines with 20 rows of sugarcane between each row of *M. minutiflora* (dashed line). *Cyperus dives* grew along the bottom and one side of the field site. (Not to scale)

Coastal 2 field site

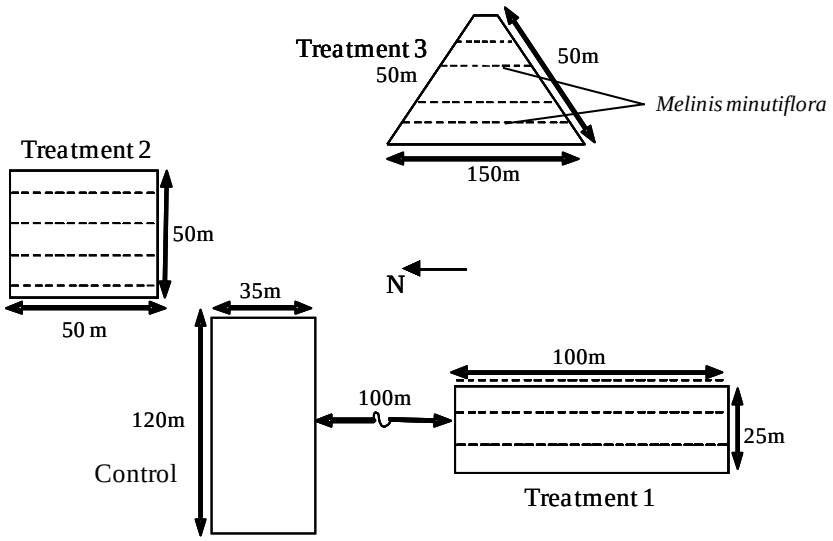


Figure 4.3. Sugarcane field setup at Coastal 2 field site with three treatment plots of different sizes with *M. minutiflora* planted every ten rows (dashed lines) and one control plot with no *M. minutiflora*. Test fields were surrounded by other sugarcane plots not shown here. (Not to scale)

4.2.6.2 Coastal 2

The field trial was conducted at the SASRI farm at Coastal 2, Northern KwaZulu-Natal. Every tenth row of sugarcane was ploughed out from the previous year's ratoon crop. This empty row in the treatment plots of the field sites was planted with one-month old seedlings of *M. minutiflora*. The single control plot did not have *M. minutiflora* (Figure 4.3) and the 'treatment row' was not ploughed out of it. There were three treatment plots and only one control plot available at this field site.

Coastal 3 field site

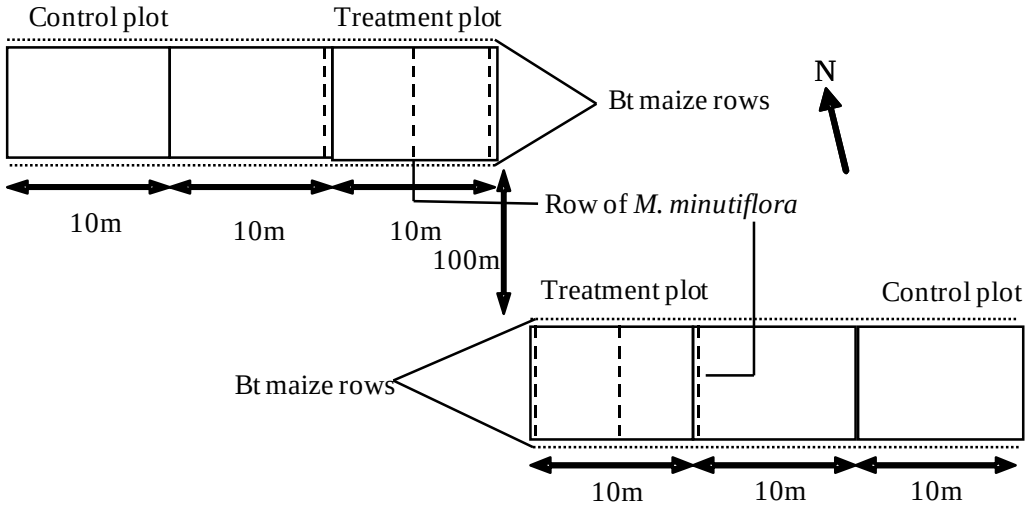


Figure 4.4. Field setup of the two fields at the Coastal 3 field site with control plots and treatment plots. *Melinis minutiflora* was planted in three rows every fifth row on the two outer edges and in the middle row of the treatment plots (dashed line). Plots were only 10 metres long. (Not to scale)

4.2.6.3 Coastal 3

Two fields of sugarcane were used for this trial on the Coastal 3 field site in Mount Edgecombe (Figure 4.4). Fields were demarcated into three sections, the control plot having only sugarcane growing, a plot of the same width as the control with Bt maize planted along the edges perpendicular to the sugarcane row direction and the treatment plot having three rows of *M. minutiflora* planted in the inter row, every fifth row, and Bt maize planted along the perpendicular edges. The second plot was designed to separate the treatment and control plots to remove the effect of the *M. minutiflora* from the treatment so that there was no drift effect by volatiles from *M. minutiflora* into the control plot. Six months into the trial all Bt maize was accidentally removed and was not included into the analysis of sugarcane in the field trial. Surveys were done on rows of sugarcane next to *M. minutiflora* as well as every second or third row between *M. minutiflora* lines, and in the same numbered rows in the rest of the plots.

Midlands 1 and Midlands 2 field sites

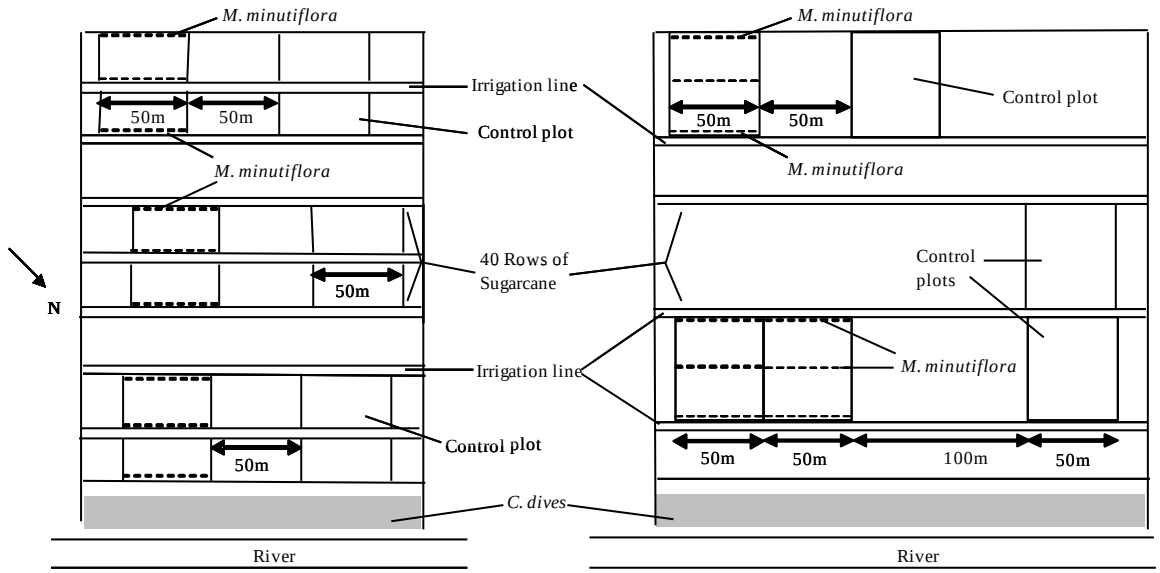


Figure 4.5. Field setup of the Midlands 1 (left) and Midlands 2 (right) field sites with *M. minutiflora* (dashed line) intercropped in treatment plots along the irrigation lines and control plots without it. *Cyperus dives* grows along the river at the bottom of the field sites. (Not to scale)

4.2.6.4 Midlands 1

The treatment plots of Midlands 1 had three irrigation lines planted with 50m of *M. minutiflora* on two sides and one row of *M. minutiflora* intercropped in the middle row on a soil ridge of the field plots (Figure 4.5). Three treatment plots were planted in the field,

with three corresponding control plots planted further along the same irrigation lines, without *M. minutiflora*.

4.2.6.5 Midlands 2

In the second field trial, 300m away, on the same farm *M. minutiflora* seedlings were planted in 50m lengths, 20 rows apart, across the treatment blocks in an inter row. The sugarcane canopy covered the inter rows as the sugarcane grew, but the grass continued to grow well during the trial. Three rows of *M. minutiflora*, two rows on the top and bottom of the treatment plots and one row in the middle of the treatment plot between the rows of sugarcane, were planted (Figure 4.5). Three corresponding control plots were marked out in the same field without *M. minutiflora* between the rows of sugarcane.

4.2.6.6 Midlands 3

Two fields of sugarcane on a commercial farm were planted with *M. minutiflora*, in two 25m long parallel rows at each treatment plot, one on the outside edge, and one in the middle row of each plot in place of a row of sugarcane, which was killed with herbicide before planting the grass. These plots were smaller than the other two Midlands sites so there were seven rows of sugarcane between *M. minutiflora* rows and not twenty as in the other Midlands sites. Three respective control plots were marked out in randomly selected blocks in the field site, covering the same surface area as the treatment plots (Figure 4.6).

Midlands 3 field site

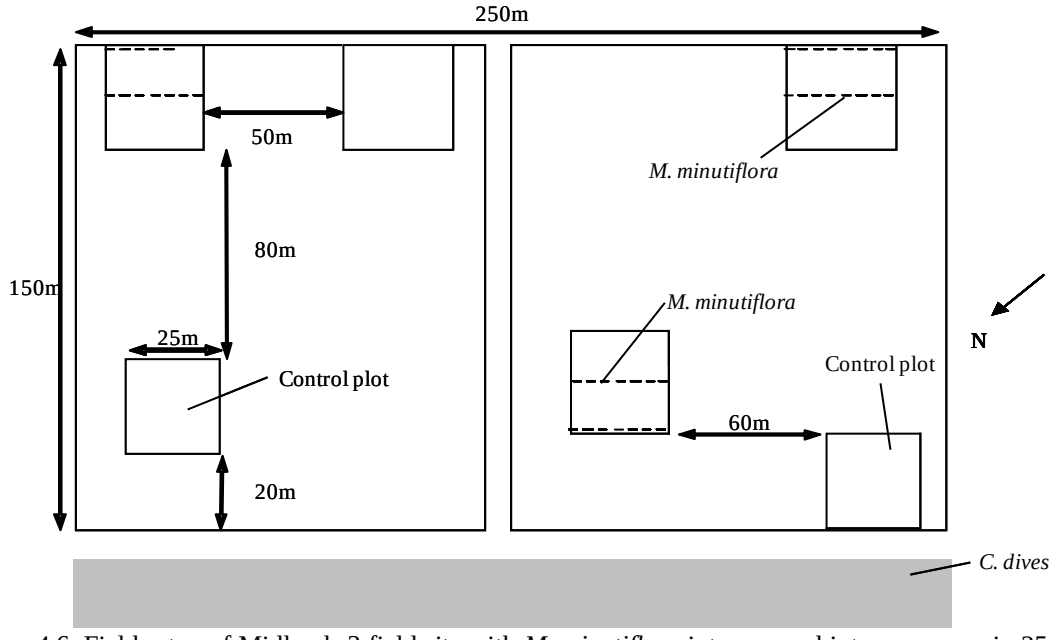


Figure 4.6. Field setup of Midlands 3 field site with *M. minutiflora* intercropped into sugarcane in 25m long rows in the treatment plots (dashed line). *Melinis minutiflora* was planted 7 rows apart, where a row of sugarcane was removed from the treatment plots. Control plots have no *M. minutiflora* planted (Not to scale)

4.2.6 Economic benefit

The economic benefit of planting *M. minutiflora* as a push crop in sugarcane was calculated from the cost of planting the grass within a hectare of a sugarcane field, the average cost of planting a hectare of sugarcane, and a reduction in loss of usable sugar due to pest infestation. Expected income per hectare was calculated from an industry average sugarcane yield of 60 tons per hectare (Anonymous, 2006), percentage sucrose at 13.7% (Anil Haripasad, personal communication[1]) and the current average Recoverable Value (RV) taken as R1670.00 per ton. Recoverable Value is the sugar price paid at the mill depending on the quality and quantity of the sucrose. Income loss from infestation was calculated at 1% internodes damaged (taken from field results) was equal to 1% loss in usable sugar (King, 1989) and expressed in Rands per hectare (Table 4.2). Net income is the expected income minus income lost from infestation. Revenue in Rands per hectare was calculated by subtracting the cultivation cost of sugarcane and the cultivation cost of *M. minutiflora* per hectare from the net income. The economic benefit is the difference in revenue between treatment and control plots (Table 4.2).

[1] Anil Haripasad, Pers comm. Management Accounting Officer, SASA, email: Anil_Haripasad@sasa.org.za

4.3. Results

Mixed results were obtained from field trials, showing a good effect of the *M. minutiflora* at two sites, and apparent negative results at another two sites and no visible effect from the grass presence at the remaining two field sites. Economic benefits of planting *M. minutiflora* were either positive or negative. Data was collected just before harvest and the resultant data are shown.

4.3.1.1. Coastal 1

Results from Coastal 1 show a significant reduction in damage from *E. saccharina* between treatment and control plots (U=7751, Mann Whitney U test, P=0.001), caused by the presence of *M. minutiflora* in the treatment plots (Figure 4.7). The average number of damaged internodes in the control plots is more than twice that recorded in the treatment plots (Figure 4.7). Similarly the mean number of *E. saccharina* found in the control plots is greater than that found in the treatment plots (Figure 4.7) although not a statistically significant difference (t=1,88, T-test, P=0.065, df=63).

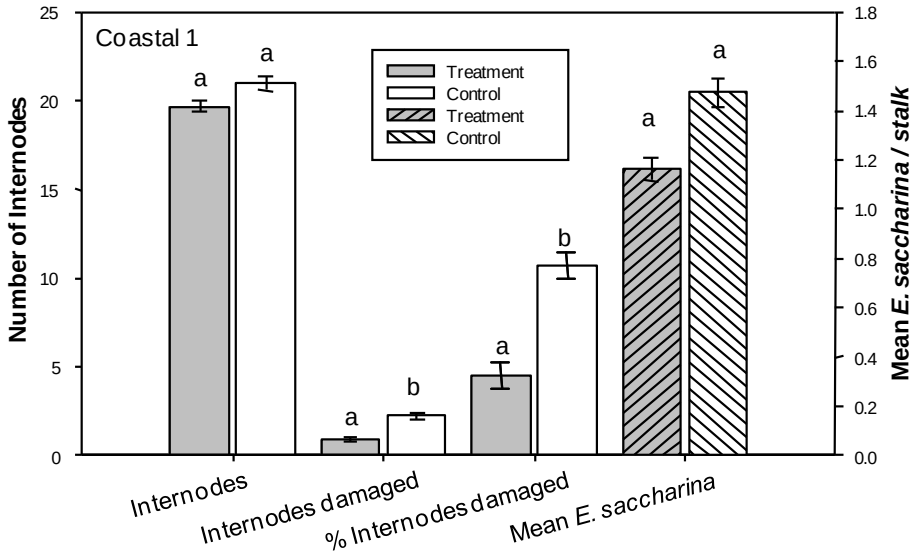


Figure 4.7 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Coastal 1 after 16 months. (Mean per plot ±SE) Pairs of columns with the same letters above are not significantly different from each other. (Mann-Whitney U test. P< 0.05, n = 150)

4.3.1.2. Coastal 2

Figure 4.8 shows that there is equal growth in the treatment and control plots at Coastal 2 and the damage by *E. saccharina* is low, but significantly greater in the treatment plots than in the control plots (Figure 4.8). This is shown in the percentage internodes damaged in the treatment plots when compared with the control plots which is significant (U=11857, Mann Whitney U test, P= 0.001). There were more *E. saccharina* larvae found in the treatment plots than in the control plots but this is not significant, and the numbers of larvae were very low compared to the Coastal 1 site.

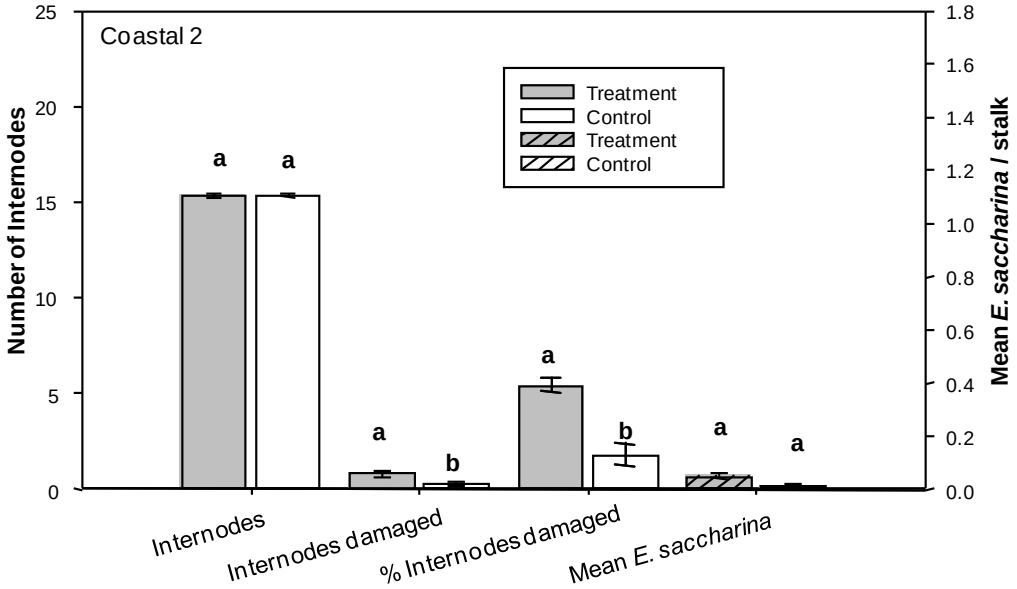


Figure 4.8 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Coastal 2 after one year. (Mean per plot ±SE) Pairs of columns with the same letters above are not significantly different from each other. (Mann-Whitney U test. P< 0.05, n = 100)

4.3.1.3. Coastal 3

A significant difference in the percentage of internodes damaged was found between treatment and control plots (U=4202, Mann Whitney U test, P=0.001) at Coastal 3 site (Figure 4.9). There is no significant difference in the mean number of *E. saccharina* collected from the treatment plots when compared with the control plots (χ^2= 2.4, Wald test, P= 0.3, df =2).

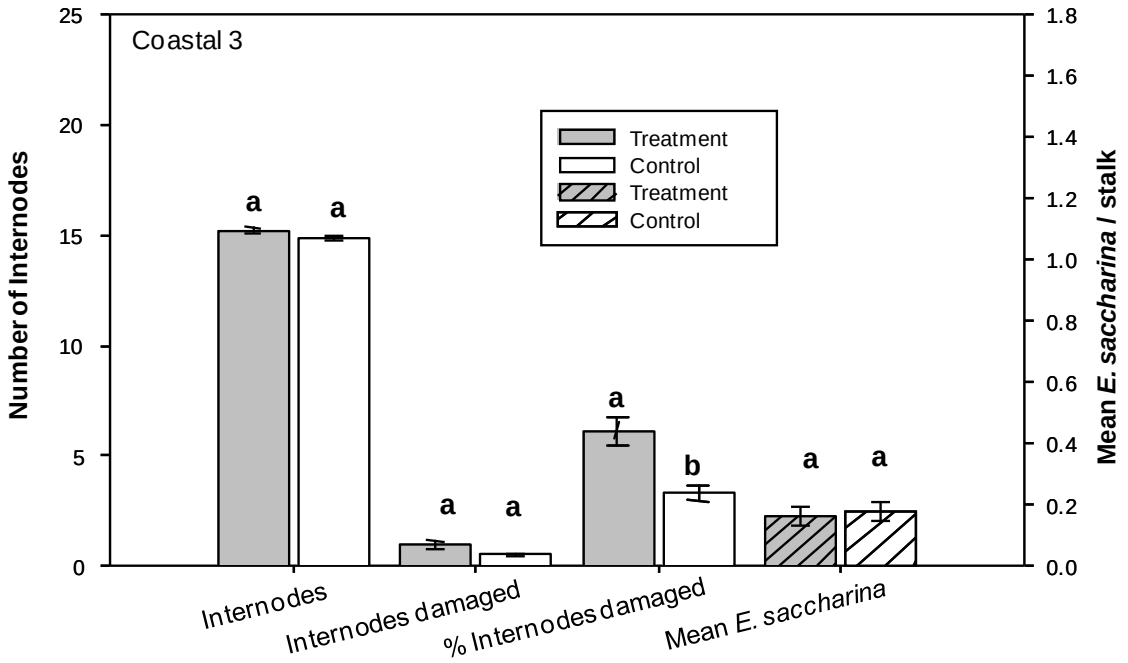


Figure 4.9 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Coastal 3 after one year. (Mean per plot ±SE) Pairs of columns with the same letters above are not significantly different from each other. (Wald test. χ^2 <0.05, n = 100)

4.3.1.4. Midlands 1

A significant difference in the average number of internodes per stalk between treatment and control was found at the Midlands 1 site (Figure 4.10). The number of internodes in a stalk is directly related to the stalk height, which therefore indicates there was a difference in growth between the treatment and control plots. Portions of the treatment fields were cut at seven months by the farm owner. The data from these parts of the field that were cut were removed from the analysis; however the difference in height was still present.

The results of comparison of damage by *E. saccharina* between the treatment and the control plots is statistically significant (H=2.744, *P*=0.002, χ^2 test, df=1) (Figure 4.10) because no *E. saccharina* were found in the treatment plots.

No significant difference was seen between the number of *E. saccharina* collected from control and treatment blocks. There was only one *E. saccharina* found in the control plot out of the total 150 stalks sampled from the control plots (Figure 4.10). No *E. saccharina* were collected from the treatment blocks so no statistical analysis could be done.

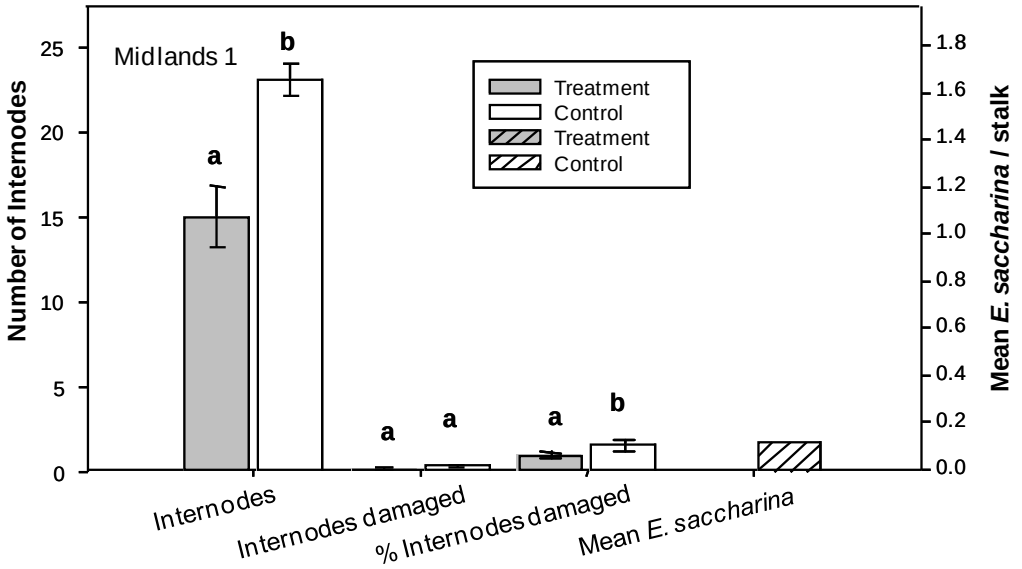


Figure 4.10 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Midlands 1 after 18 months. Columns with the same letters above are not significantly different from each other. (Mann-Whitney U test. $P < 0.05$, n = 90)

4.3.1.5. Midlands 2

Figure 4.11 shows a non significant difference in the average height and number of internodes of sugarcane stalks between the treatment and the control plots at the Midlands 2 site. There was no significant difference in the percentage of internodes damaged between treatment and control plots (H=0.043, P=0.571, χ^2 test, df=1), and there were no *E. saccharina* recovered from this field site (Figure 4.11) because *E. saccharina* populations were so low.

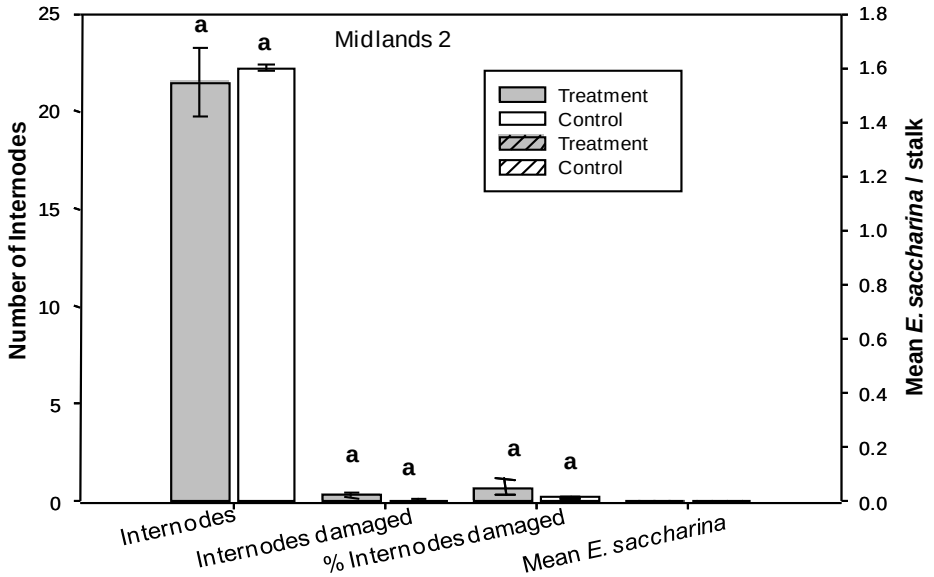


Figure 4.11 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Midlands 2 after 18 months. Pairs of columns with the same letters above are not significantly different from each other. (χ^2 test. $P < 0.05$, n = 90)

4.3.1.6. Midlands 3

There was a significant difference in the mean number of internodes per stalk between the treatment and control plots (Mann Whitney U test, P= 0.015) at the Midlands 3 site. The stalks in the control plots grew significantly taller than the stalks from the treatment plots (Figure 4.12). There was no significant difference in percentage internodes damaged between treatment and control plots (U=1024.5, Mann Whitney U test, P= 0.922). There were more *E. saccharina* larvae found in the treatment plots than in the control plots, but populations were again less than 0.2 individuals per stalk.

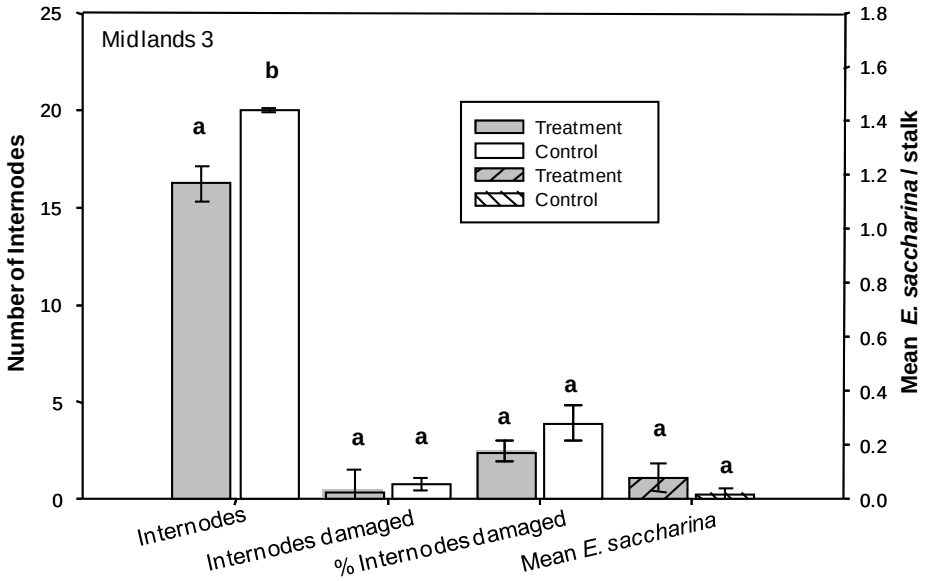


Figure 4.12 The effect of intercropping *M. minutiflora* grass on; Internodes per stalk of sugarcane, internodes damaged per stalk of sugarcane and percentage of total internodes damaged. (Mean per plot ±SE) Mean number of *E. saccharina* collected per treatment at Midlands 3 after 18 months. Pairs of columns with the same letters above are not significantly different from each other. (Mann-Whitney U test. P< 0.05, n = 45)

4.3.2. Cost benefit analysis

Planting of *M. minutiflora* as a push crop at the Coastal 1 field site was found to reduce the percentage internodes damaged from 10.7% to 4.5% (Table 4.2). This reduction in damage resulted in an increase in net income from R6390 per hectare in the control, to R7151 per hectare in the treatment where *M. minutiflora* was planted; a net economic benefit of R761 per hectare with the cost of planting *M. minutiflora* subtracted. Similarly, planting *M. minutiflora* in the Midlands 1 field site reduced the damaged internodes percentage from 1.5% to 0.9%, which was calculated as a net economic benefit of R9 per hectare (Table 4.2).

Planting *M. minutiflora* in the Midlands 3 field site reduced the percentage internodes damaged from 4.3% to 2.8%, which was calculated as R121 profit from planting this grass (Table 4.2). In the other three field sites, Coastal 2, Coastal 3 and Midlands 2, the cost

benefit to the farmer came out as negative amounts. At these sites it costs more to plant molasses grass in these fields than the damage *E. saccharina* caused (Table 4.2).

At the Coastal 2 field site, the percentage internodes damaged in the treatment plot was 5.34% and 1.8% in the control plots. The cost endured from planting *M. minutiflora* in this field is a loss of R578. A similar loss of R583 was shown from planting *M. minutiflora* in the treatment plots of the Coastal 3 field site, giving a percentage internodes damaged of 7.44%. The control plots of Coastal 3 had 3.87% internodes damaged. The percentage internodes bored in the treatment and control plots of the Midlands 2 field site were 0.48% and 0.36% respectively. The increase in damage in the treatment plot as well as the cost of planting the grass was calculated as a loss of R108 for this field.

Table 4.2: Cost benefit analysis of planting *Melinis minutiflora* as a push plant against *E. saccharina* in sugarcane

	Cost of planting *M. minutiflora* (R/Ha)	Infestation (% Internodes bored)	Yield reduction from infestation (R/Ha)	Net Yield (R/Ha)	Revenue (R/Ha)	Benefit (R/Ha)
Coastal 1 Control	0	10.7	1473	12 294	6390.	
Treatment	91.94	4.5	620	13 147	7152	**762**
Coastal 2 Control	0	1.81	249	13 518	7614	
Treatment	91.94	5.34	735	13 032	7036	**-578**
Coastal 3 Control	0	3.87	533	13 234	7330	
Treatment	91.94	7.44	1024	12 743	6747	**-583**
Midlands 1 Control	0	1.05	145	13 622	7718	
Treatment	91.94	0.31	43	13 724	7728	**10**
Midlands 2 Control	0	0.36	50	13 717	7813	
Treatment	91.94	0.48	66	13 701	7705	**-108**
Midlands 3 Control	0	4.36	600	13 167	7263	
Treatment	91.94	2.81	387	13 380	7384	**121**

NB: the average sugarcane yield of 80 tonnes per hectare, 13.74 % sucrose yield and the average RV price of R1670 per tonne was used in these calculations. The cultivation cost of a hectare of sugarcane is calculated at R5904. The gross expected yield in R/Ha is R13 767.

4.4. Discussion

4.4.1. Field trials

Melinis minutiflora was planted at six different sites in two different areas of the South African sugarcane region, inland in the KwaZulu Natal midlands and along the coast. In these areas the influence of this grass on *E. saccharina* was not the same. Different field arrangements were tested with different planting times, row spacing and placement of *M. minutiflora*. The field setup in Coastal 1 where *M. minutiflora* was planted in the drainage lines and in Midlands 1 where the *M. minutiflora* was planted in the irrigation lines of sugarcane fields showed that there was a significant positive effect of this non-crop grass. Coastal 1 and Midlands 1 field sites had a similar planting setup with the grass planted on the borders of the irrigation and drainage lines, and the same density of 20 rows of sugarcane apart. This field setup showed a positive result and a significant reduction in *E. saccharina* populations in sugarcane fields (Figure 4.7 and Figure 4.10). There were many *E. saccharina* present in the Coastal field plots in the high population pressure area, but the Midlands field trials also showed a positive significant difference in number of internodes damaged between treatment and control plots despite the levels of *E. saccharina* in Midlands 1 being too low for a reduction in pest populations to be shown. This particular field setup has potential to yield beneficial results in sugarcane in both high and low population pressure areas. The difference between these fields is that the Midlands field site was irrigated while the more significant positive result was seen in a rainfed field of Coastal 1 on the coast (Figure 4.7).

Coastal 2 and Midlands 3, with *M. minutiflora* arranged in place of a row of sugarcane, were not as successful in showing the repellent effect of this grass on *E. saccharina* populations. Field trials at Coastal 2 showed a significant, negative response, and were more infested with *E. saccharina* where the *M. minutiflora* was planted between the rows than in the control plot where the grass was not planted (Figure 4.8). Although more damage was shown in the control plots Midlands 3 field site showed no significant difference in damage (Figure 4.12).

The mean number of *E. saccharina* larvae collected from this particular field site were also not significantly different but were higher in the treatment plot than in the control plots (Figure 4.12). This field site can neither support nor disprove the effect of this grass on *E. saccharina* infestation.

Coastal 3 and Midlands 2 field sites had the same setup of *M. minutiflora*, planted in-between the rows of sugarcane in the interrows (Table 4.3). The results from Midlands 2 field site, showed no result to confirm or refute the effectiveness of this non-crop grass as a repellent crop against *E. saccharina* infestations. One reason for this was that the numbers of *E. saccharina* in this particular field were very low and no *E. saccharina* were collected at the surveys (Table 4.3). Additionally little damage was seen in the stalks of either control or treatment plots (Figure 4.11). Fields at Coastal 3 had significantly more damage shown in treatment plots than in control plots (Figure 4.9). At the Coastal 3 field sites, the rows of *M. minutiflora* were planted closer together, every fifth row, and only 10 metres away from the control plot rows. The reason for the higher level of *E. saccharina* damage in the treatment plots may be that the treatment plot was small and close to the other plots and could not move *E. saccharina* away from the field into the neighboring natural host plants, *Cyperus dives*.

Table 4.3. Field sites characteristics where *M. minutiflora* was planted to test its effect as an intercrop on *E. saccharina* infestation

Region/*E. saccharina* Population	Irrigated/ Rain fed	Sugarcane planting method	Treatment planting time	Highest *E. saccharina* populations	Effect of treatment	Placement of treatment
Coastal 1 High	Rain fed	Seed Cane	Before sugarcane	1st	push	Drainage line
Coastal 2 High	Rain fed	Ratoon	After sugarcane	4th	pull	In place of sugarcane
Coastal 3 High	Rain fed	Seed Cane	After sugarcane	2nd	pull	Inter row
Midlands 1 Low	Irrigated	Seed cane	Before sugarcane	5th	push	Irrigation line
Midlands 2 Low	Irrigated	Seed Cane	Before Sugarcane	6th	No effect	Inter row
Midlands 3 Low	Rain fed	Ratoon	After sugarcane	3rd	No effect	In place of sugarcane

The repellent action of this grass was shown to be more effective over a big field with space for *M. minutiflora* to establish thick undergrowth alongside a field plot, as will be shown in the next chapter. Field sites with the grass planted in closely spaced sugarcane rows did not show the same benefit as was seen in field sites where rows of *M. minutiflora* were planted between higher numbers of sugarcane rows. The reason for this is possibly the number of rows of sugarcane between rows of *M. minutiflora*, but rather with the width of the field and the biomass of the intercropped *M. minutiflora*.

Our results are inconsistent with the findings of field trials by Kasl (2004). In the Pongola trial done by Kasl (2004) where *M. minutiflora* was intercropped, treatments were shown to have less damaged internodes and fewer *E. saccharina* larvae collected (Kasl, 2004). Field trials in this current study have only given this positive result for two field sites (Coastal 1, Figure 4.7; Midlands 1, Figure 4.10). The other field sites showed no significant effect from *M. minutiflora* intercropped into treatment plots, or a negative effect. These field sites (Coastal 2 and Coastal 3, Figures 4.8, Figure 4.9) showed significantly more damage to internodes in the treatment plots than in the control plots. On these field sites a negative effect from *M. minutiflora* can be concluded. The positive results and previous study by Kasl (2004) suggest potentially beneficial effects of *M. minutiflora*, repelling *E. saccharina* away from sugarcane. In a different crop, Khan *et al*., (1997a) found that *M. minutiflora* planted in alternate rows with maize was able to significantly reduce stemborer infestation of the main crop. These results require further research.

Table 4.1 shows the different sugarcane planting times in relation to the grass planting time. When cross checked with the results in Figure 4.7 - 4.12, they can be separated into three groups. Fields that had a positive result from the introduction of the grass, Coastal 1 and Midlands 1, fields that had no significant effect from the grass, Midlands 2 and Midlands 3 and the fields that showed a negative response to the grass in the field, Coastal 2 and Coastal 3. The two field sites where a positive result was shown are also field sites where *M. minutiflora* was planted a few weeks before the sugarcane, which was from seed cane (Table 4.3). Therefore the grass had a chance to grow and establish itself before the sugarcane competed with the grass for sunlight. Grass volume will affect the amount of volatile released into the sugarcane to act as a repellent of *E. saccharina*. The sugarcane in the field sites where a negative result was recorded was planted before the grass and, as in the case of Coastal 2, was from a ratoon crop. The sugarcane in these two field sites had a

chance to establish in the rows with a closed canopy soon after the grass was planted. The grass could not grow to the same biomass densities as were seen in Coastal 1, (Chapter 5). Therefore presumably less volatile was released into the field sites, and the grass struggled to compete with the sugarcane. The last two field sites are the exception to this idea. The third Coastal field site was in a smaller field than the other field sites and the lines of *M. minutiflora* were closer together (five metres of sugarcane between the rows of grass). The *E. saccharina* populations in this area were high; and the ability of a few lines of grass to repel the *E. saccharina* from the surrounding sugarcane rows was not possible. *Melinis minutiflora* was tested in this field site and obtained a negative result. The third field site in the Midlands did not yield a significant result. The infestation levels, though higher in the control plots were in a field site that had a ratoon crop and was planted after the sugarcane was cut and had started to regrow (Table 4.3). Therefore, the grass was unable to achieve widespread growth and compete as the sugarcane grew faster and reduced the spread of this grass between the rows of sugarcane.

Intercropping this grass with sugarcane has advantages more than just being able to reduce pest numbers. The insect diversity in a monocropped field of sugarcane is very low (Haddad *et al*., 2001) and monocropped fields are prone to losses in yield due to species of pests being able to build up numbers in areas of reduced diversity (Talekar *et al*., 1984). The introduction of this grass into the field and having other plants present along the borders and in the irrigation and roadways of fields increases diversity which promotes natural predators which in turn decreases pest populations (Haddad *et al*., 2001). Natural predators are attracted to fields with higher plant diversity by the greater diversity of resources. Another habitat management aspect of *M. minutiflora* that was not tested in these field trials is the effect that volatile emissions from this grass have on stemborer parasitoids. *Melinis minutiflora* was shown by Gohole *et al*., (2005) to increase parasitism of stemborers by *C. sesamiae* due to the attractive volatiles produced by this grass. Kasl (2004) worked on another stemborer parasitoid, *X. stemmator* which was shown to be attracted to *M. minutiflora* in olfactometer trials and increased foraging in sugarcane when placed with this grass in cage trials. *Melinis minutiflora* has benefits additional to these such as weed suppression. The potential positive effects this grass has with pest control, and parasitoid attraction suggests that *M. minutiflora* should be re-tested in high *E. saccharina* population regions but planted early and in spaces where it will not be shaded out.

4.4.2. Cost benefit analysis

The cost benefit analysis of planting a hectare of sugarcane with *M. minutiflora* shows an economic benefit linked to reduced *E. saccharina* infestation. This benefit was shown in a field from the Midlands where the population pressure was low, and in the Coastal region where population pressure from *E. saccharina* was high in the field. Both field sites show there is an increase in available profit, despite using industry averages. Field sites where there was low population pressure from *E. saccharina* would not yield the economic benefit of planting this grass. The money that is spent on planting this grass in the field may not return a profit from the increase in yield of sugarcane, achieved from a reduction in pest infestation. If the levels of pest species are too low to be significantly affected by this grass there will be no significant increase in yield to increase the profit. The other field sites, where the economic benefit analysis was done, showed a negative response to planting this grass in the field. These fields are influenced by the planting date that made the presence of the grass a cost rather than a benefit in the field. This grass has the potential to be an economically beneficial crop adding to the yield from a crop; however it needs to have a positive effect in order to cover the cost of additional planting in the field. This positive effect could potentially come from weed suppression, which is discussed in the next chapter.

Chapter 5:
Competition of *Melinis minutiflora* and weedy plants in sugarcane

5.1. Introduction

Weed management extends beyond just removing undesirable vegetation from a crop field. Though weeds may cause yield loss, they can also have beneficial effects (Altieri and Liebman, 1988). Grassy field margins or areas of encouraged natural plant growth where any plant is allowed to grow and spread near to a crop field, will most likely have weedy plant species occupying available spaces. Areas of natural plant growth were tested to assess the impact of herbaceous field borders on populations of European corn borer, *Ostrinia nubilalis* Hubner (Lepidoptera: Crambidae) (Stamps *et al.*, 2007). It was found that natural plant growth borders reduced the populations of this pest in corn fields, and also reduced weed presence. There is potential benefit in allowing non-crop plants to grow on the borders of crop fields.

5.1.1. Weedy plants

An agricultural weed is a plant, often introduced from a distant geographical location, which is undesirable in a particular agricultural setting because of its crop reducing properties (Altieri and Liebman, 1988). Weeds can also serve as reservoirs for pest insects and diseases, and interfere with harvesting and milling of cereal crops, or be poisonous to humans and livestock (Hill, 1977). Weeds compete directly with crops in the field for sunlight, moisture and nutrients and can thus reduce crop yield (Altieri and Nicholls, 2004). Worldwide a total yield loss of 10% can be attributed to competitive effects of weeds on crops (Altieri and Liebman, 1988). Crop fields that have weeds growing around the edges and along roads and irrigation lines, have higher biodiversity than monocropped fields (Bajwa and Kogan, 2004). The use of weedy plant species as cover crops in an agricultural system can add organic matter to the soil, suppress or prevent other annual weed growth, reduce the cost of other weed control, reduce soil erosion, and minimize surface water run-off (Anderson, 1996).

5.1.2. Weed Control

Weed control is concerned with reducing the negative effects of weeds in the field where cash crops are grown. Herbicides are the principal control method for unwanted weeds in crop

fields, sprayed across fields in large scale agriculture (Coll, 2006). Agricultural herbicides are generally broad spectrum, meaning they kill a range of plant types, as there is seldom just one weedy plant in a field but a complex of plant species all competing with each other and with the cash crop. Herbicides are affected by several factors: The soil type and preparation, rainfall and weather, sunlight, temperature, micro-organisms, the kind of weeds, varietal response of the crop and crop tolerance. Herbicides are not, however, always the best option for weed control and not always available, especially for small-scale farmers, because they are expensive and are labour-intensive to apply. Herbicides are dangerous if not handled correctly and often the lack of proper protective clothing and faulty spraying equipment is the leading cause of chemical intoxication on small scale farms in developing countries (Forget, 1991).

Methods used by small scale farmers for weed control are crop interference, crop rotation and hand weeding. Crop interference is a fundamental method of non-chemical weed control (Jordan, 1993), and involves weed suppression by the crop plant itself. Living mulches are also introduced crops that can have weed suppressing properties.

Living mulches are cover crops planted and grown with the main crop because of their weed control and soil quality benefits (Prasifka *et al.*, 2006). Legumes planted as living mulches may reduce fertilizer requirements by providing fixed nitrogen to the main crop (Prasifka *et al.*, 2006). An intercrop of *Desmodium uncinatum* reduced damage to maize by striga weed (Anonymous, 1999). Experiments revealed that *D. uncinatum* roots release chemicals that undermine the growth of weeds with an allelopathic effect.

Living mulches can be used as pest control in two ways. Firstly the presence of living mulch in a field can impair the pest insects from finding a host plant and secondly, living mulches can attract natural enemies which reduce pest populations indirectly (Prasifka *et al.*, 2006). These are two properties common with intercrops, but living mulches are different from intercrops in that their primary purpose in the field is the nutritional benefit of the main crop.

5.1.3. Green manuring

Green manures are plants within a main crop for the benefits they give to the crop. Green manuring was found to reduce both weed populations and dry matter content of weeds as compared to the addition of nitrogen fertilizer alone. Green manuring significantly increased the amount of nitrogen in the soil of sugarcane crops in India (Buragohain and Medhi, 1999). Various green manures are found to grow without fertilizers as well as having weed

suppressing properties (by smothering or allelopathy), pest and disease suppression and adding to overall soil health (Rhodes, 2006). Organic agriculture is involved with these types of biological inputs instead of synthetic chemicals; inputs that contribute to soil conservation, crop rotation and using green manures.

5.1.4. Damage by weeds in sugarcane

Sugarcane is a perennial crop, and three to five ratoons - a crop growing from the roots of the previous harvest - can be made from a single planting, before it needs to be replaced and planted again from seed cane. Problem weeds such as the perennial, Bermuda grass, *Cynodon dactylon*, (L.) Pers. often increase in each successive year, reducing the sugarcane plant populations to a point where replanting of the sugarcane is needed (Etheredge *et al.*, 2004). Weeds of sugarcane are both broadleaf and grasses, and at present there is no biological control against weeds of sugarcane. Transgenic sugarcane with resistance to glyphosate has been developed and evaluated in the field (Gianessi *et al.*, 2002). Glyphosate could replace current herbicides used in sugarcane for weed control because it is more effective when used with a resistant sugarcane variety rather than both pre-emergence and post emergence herbicides (Gianessi *et al.*, 2002). Weeds cause a loss of tonnage, reduce sugar recovery in the mill, and limit the number of ratoon crops (Gianessi *et al.*, 2002). Weeds, therefore, influence the sugar yield that can be extracted from stalks. Depending on the amount of weeds present in a field, the yield of a crop can range from 100 % full yield down to 5% yield (Singh and Tomar, 2003).

Cultural control for weeds includes the use of fast growing varieties of sugarcane that shorten the critical period of competition. The use of trash mulch, such as leaving the dead leaves from sugarcane in the field which suppresses weed growth, conserves moisture and reduces soil erosion. Legumes planted in other cash crops to take advantage of the available space in crop fields, can also stop weed populations and generate additional income by being used as a second concurrent crop.

5.1.5. Benefits of weeds

Weeds cannot always be assumed to be completely damaging and in need of immediate control. Some weeds may be useful in the field, despite competing with the main crop (Altieri and Liebman, 1988). Useful crops and shorter lived plants are usually used as intercrops; however certain weedy plants may be good as intercrops. Monocropped fields assist insect

pest infestations by providing concentrated resources and uniform physical conditions which promote pest invasions. Pest insects are more likely to stay in monocropped host crops because they can complete their whole life cycle in that simple environment (Altieri and Whitcomb, 1980). Wild grasses are possible reservoirs for pest stemborers and thought to be responsible for some pest outbreaks on crops (Overholt *et al.*, 2003). Despite this, researchers have seen that the natural increase in biodiversity of multiple cropping systems increases the quality and quantity of natural enemy fauna (Bajwa and Kogan, 2004), which in turn decreases the pest populations. Additionally, outbreaks of certain types of crop pests are more likely to occur in weed free fields rather than weed diversified crop systems (Altieri and Whitcomb, 1980) because pests have only one crop to focus on and are not distracted to move to another less viable plant. Additional plants in the field that have predator-attractive properties will reduce pest populations by introducing predatory species into the field which will feed on the pest organisms. Koji *et al.*, (2007) found that Guinea grass used in habitat management served as a good agent to selectively enhance arthropod predators of stemborers and additionally acted as a sink for the pest.

5.1.6. *Melinis minutiflora* revisited

Chapter Four examined *M. minutiflora*, molasses grass, an indigenous grass that has pest repellent properties in certain field setups. Observations during these studies in sugarcane fields, suggested that other weedy plants were suppressed where molasses grass was planted, which spread to all available spaces between the sugarcane rows. However, it did not spread further into the crop than the first row of sugarcane. Areas that are not influenced by *M. minutiflora* should have significantly more weeds than areas with *M. minutiflora* present. This grass may be viewed by farmers as a possible weed because it spreads widely and grows quickly. It is possible that farmers will be unlikely to intercrop this grass into their sugarcane fields if they believe it will compete with the sugarcane and reduce growth and sucrose yield. Therefore, an experiment was performed to determine if molasses grass had any competitive effect on the growth and yield of sugarcane and whether this grass was able to compete with weeds already in the field sites.

5.2. Methods and Materials

The effect of *M. minutiflora* on sugarcane yield and weed biomass was measured to determine if the grass could be considered as a living mulch for weed control in addition to *E. saccharina* control.

5.2.1. Impact of *Melinis minutiflora* on adjacent sugarcane growth

Melinis minutiflora was planted in the fields of sugarcane as described in Chapter 4. In the three Midlands field sites and Coastal 1 field site, sugarcane height and density of stalks per metre was recorded at three points along a sugarcane row on the edge of each treatment plot where *M. minutiflora* was planted; and the row exactly in the middle of the treatment plot; a row not adjacent to a *M. minutiflora* row (Figure 5.1). A two metre long measuring pole was held up directly next to the sugarcane stalk and the height measurement to the top leaf tip taken for that stalk. The number of sugarcane stalks along one metre of the row were counted and recorded along with the height measurement. This was done three times at equally spaced points in the treatment and control rows, for five rows in each plot (Figure 5.1). Midlands 3 field site had only two rows of *M. minutiflora* growing in the treatment block, and 7 rows of sugarcane between the rows of *M. minutiflora*. Only two rows of sugarcane adjacent to *M. minutiflora* on the edge of the treatment plot and in the middle and two rows on the edge and middle row of control plots, had measurements taken. Coastal 1 field site had sugarcane height and density measurements taken. Measurements for height and density were collected from one row in each control plot while data was collected from the sugarcane directly next to the *M. minutiflora* in a treatment plot and from sugarcane in rows in the middle of a treatment plot, not directly adjacent to *M. minutiflora*.

5.2.2. Impact of *Melinis minutiflora* on weed biomass

Reduction in weed biomass, in treatment plots, was measured by collecting and weighing weedy plant material, including any *M. minutiflora* on the edges of treatment plots, and comparing these weights to the weight of weedy plant material collected from the edges of the control plots. A quadrat, 0.5 X 1m, with the short axis of the quadrat against the sugarcane row, and the long axis jutting into the irrigation or drainage line, was placed over all weedy plant material next to the edge row of sugarcane stalks at three equally spaced points along each collection row (Figure 5.1). The quadrat was then pushed down to the base of the plants and all plant material in this area was cut at ground level using shears, collected in paper bags, and returned to the laboratory where it was dried at 70º C and then weighed for 7 days until mass remained constant. The *M. minutiflora* weedy material from each quadrat in the treatment plots was separated into another bag before being dried and weighed as above. Biomass data for *M. minutiflora* and other weedy plant material on the irrigation lines of treatment plots and weedy plant material in control plots, were collected to correlate with sugarcane yield and/or quality of sugarcane in the rows adjacent to where *M. minutiflora* was

planted. In order to test the quality of the sugarcane, especially of the sugarcane growing next to *M. minutiflora*, a sucrose yield analysis was done.

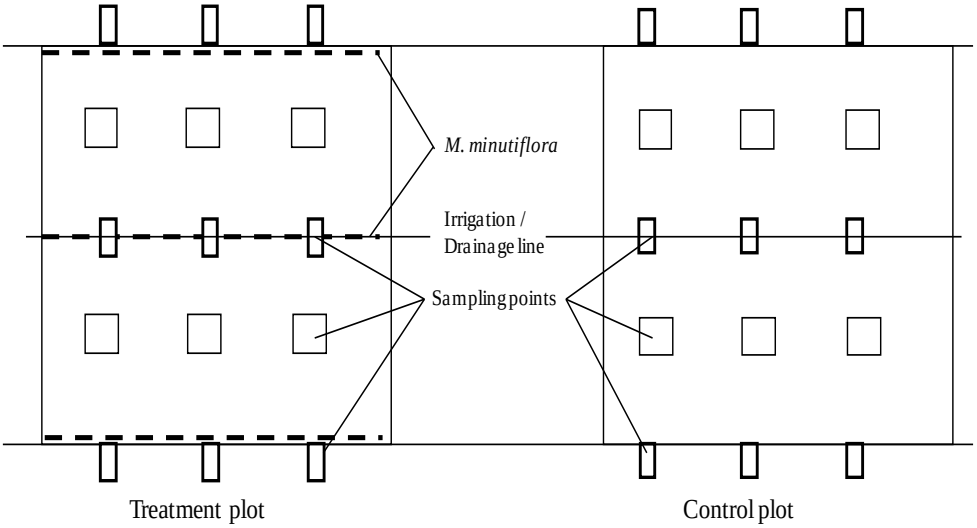


Figure 5.1: Sampling points for height and density measurements of sugarcane and weed biomass collection within representative treatment and control plots. Height and density measurements were taken from areas marked as boxes, (bold and non-bold). Weed biomass was collected at points where boxes are in bold. Stalks for ERC% cane (see below) were taken from the same rows as indicated boxes.

5.2.3. Sucrose yield analysis

Sucrose yield analysis was done on stalks from all three field sites in the Midlands and only the Coastal 1 field site. Twelve stalks, at intervals of one stalk every four metres, were taken from the edge rows of treatment and control plots, i.e. the rows either adjacent to the *M. minutiflora* rows in the treatment plots, or the rows adjacent to the demarcated drainage/irrigation lines in the control plot. Twelve stalks (one stalk every four metres) were also taken from the tenth row of each treatment plots midway between the *M. minutiflora* lines (Figure 5.1) except for Midlands 3, which had only the edge rows sampled. Sucrose yield analysis was done in the SASRI millroom using standard procedures developed at SASRI to give an indication of the percentage sucrose from the sugarcane. The values of Estimated Recoverable Crystal as a percentage of total sugarcane (ERC% cane) were used in the statistical analysis to measure the differences between treatment and control plots, and between collection points within plots.

5.2.4. Statistical Analysis

Differences in sugarcane height and density between treatment and control plots were tested with an ANOVA at P=0.05. Weed biomass results were also tested with an ANOVA for significant differences between collections points in the treatment plots and between treatment and control plots. Mann-Whitney U-test (Wilcoxon rank-sum) test was used where

the assumptions of normality and homogeneity of variance were violated. Significant differences in ERC% cane between each level of the main effects (i.e. Plot, Position, Treatments) and their interactions were established using the Wald's test in Restricted Maximum Likelihood analysis (REML; Genstat Ver. 8.0). A Wald's test was performed because three effects of each field (plot number, position in plot i.e. edge or middle, and treatment or control) were compared simultaneously for significant differences. The Least Significant Difference (LSD) pairwise multiple comparison test was used to quantify significant differences after positive interactions were shown by Wald's test.

5.3. Results

5.3.1. The impact of *M. minutiflora* on sugarcane height and density

The height and density taken from the control plots and at two points in the treatment plots in the Coastal 1 field site and at Midlands 1, 2 and 3 field sites (Figure 5.2) showed no difference in sugarcane height or density between treatment and control plots except Coastal 1 (Figure 5.2 A).

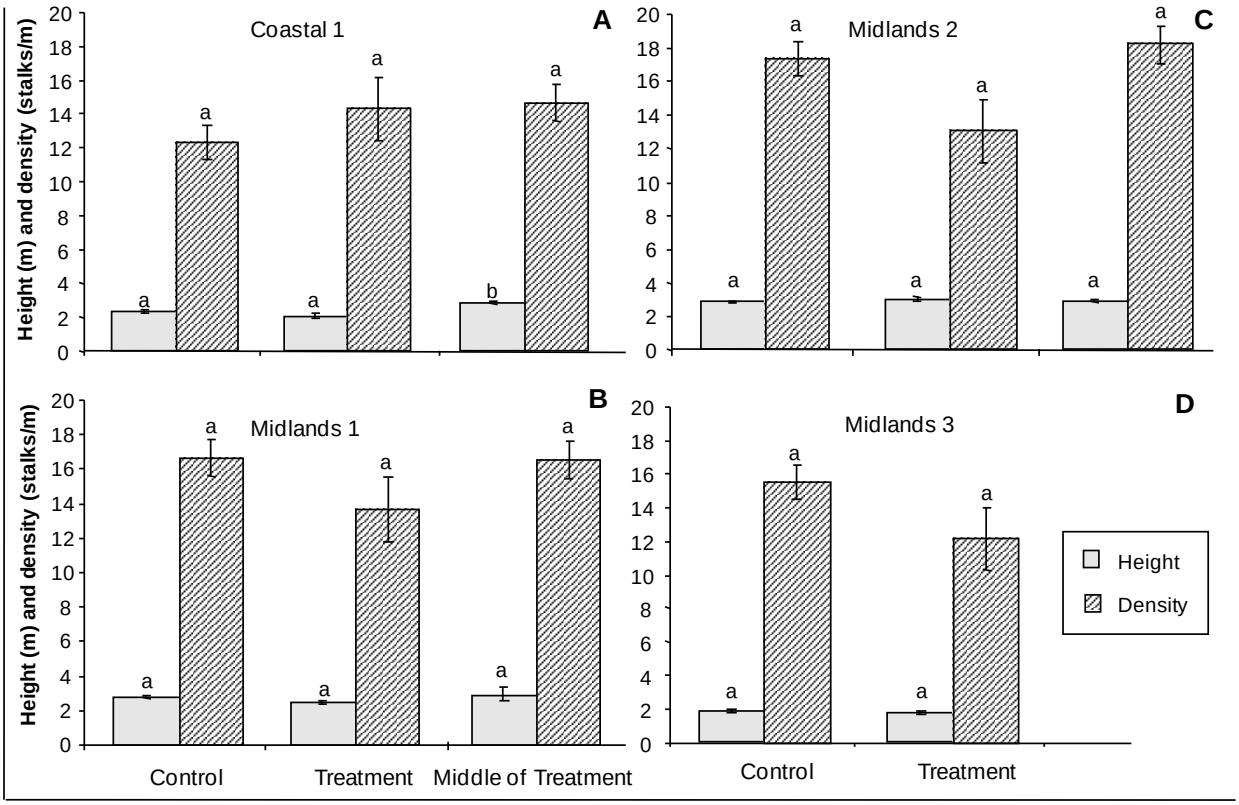


Figure 5.2: The effect of *M. minutiflora* on sugarcane height and density (±SE) in control plots, along edge of treatment plots and middle of treatment plots at Coastal 1 (A), Midlands 1 (B), Midlands 2 (C) and Midlands 3 field site (D). Same patterned means within each graph, with different letters above are significantly different from each other (Mann Whitney U-test, $P<0.05$).

5.3.2. Impact of *M. minutiflora* on weedy plant species

The biomass of weeds growing in the irrigation lines and along the edges of sugarcane field sites was generally reduced by the presence of *M. minutiflora* intercropped with sugarcane (Figure 5.3). The differences between the control plots and treatment plots showed significant reductions in weed biomass when comparing the amount of other weeds in the control plots to the amount of weed biomass collected from the treatment plots, except the Midlands 3 field site. At the Midlands 3 field site the amount of weed biomass that is not *M. minutiflora*, was similar in the treatment plots and the control plots. There were no weeds in Coastal 1 field site due to a herbicide programme at this field, so no comparison between treatment and control plots was made. The biomass of *M. minutiflora* in this field was compared with other field sites to give an idea of the amount of biomass this grass can attain if the weedy species between rows are removed before this grass is planted.

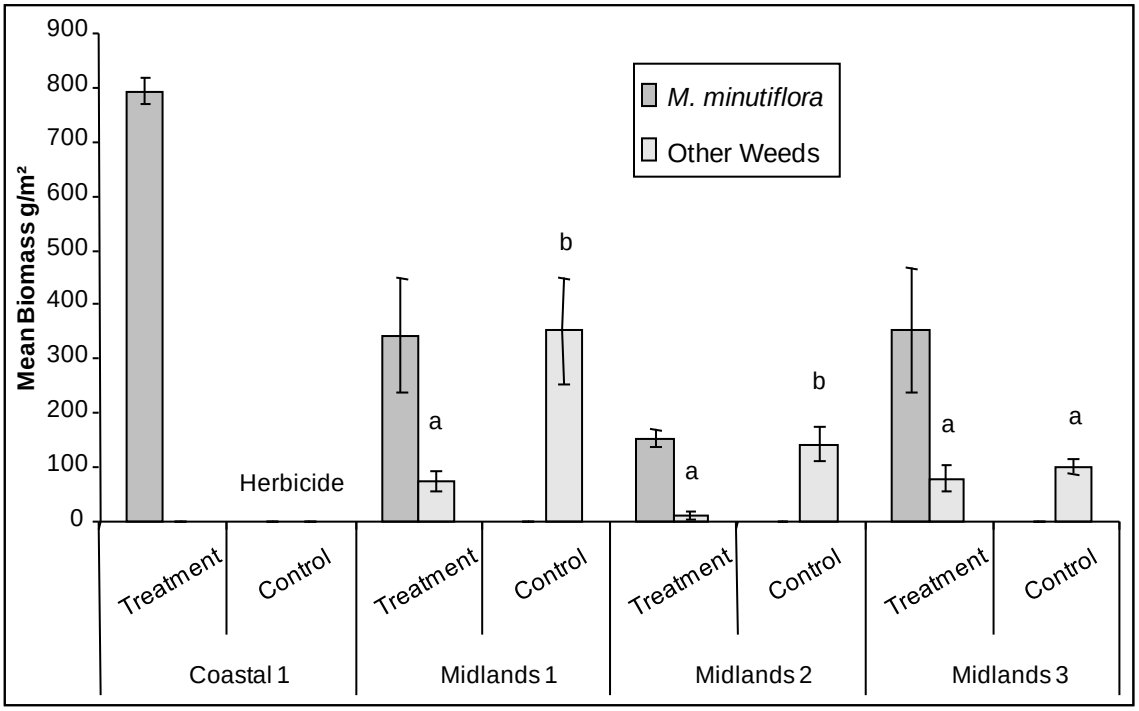


Figure 5.3: Mean weed biomass (±SE) from treatment and control plots of Coastal 1 and Midlands field sites. The biomass of *M. minutiflora* from treatment plots is separated from other weedy biomass to allow comparison. Means within each field site of other weeds, followed by the same letter, indicate no significant difference (t-test, $P<0.05$).

5.3.3. Sucrose yield analysis

An analysis of sugarcane yield using Estimated Recoverable Crystal as a percentage of total stalk (ERC%) in comparison between treatment and control plots, between data collection points within treatment plots, edge and middle of plot, and between four field sites, showed that the only significant difference was between the field sites (Figure 5.4) (χ^2=0.001, Wald

test, P= 0.05, df=3). This is an expected result as the field sites are in different areas with different varieties of sugarcane grown in each field.

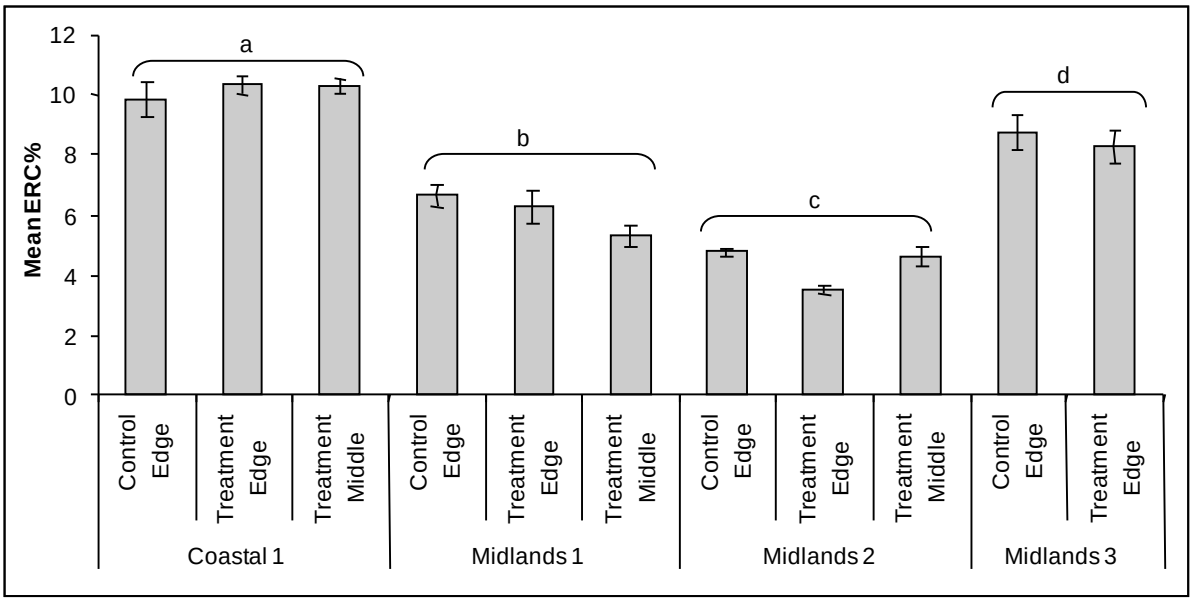


Figure 5.4 Mean ERC% cane measured from stalks taken from treatment and control plots at Coastal 1 and Midlands field sites. Grouped means followed by the same letter are not significantly different (Wald test, P<0.05).

5.4. Discussion

5.4.1. Impact of *M. minutiflora* on sugarcane height and density

In the Coastal 1 field site, the height and density of sugarcane at the edge of the treatment plots was not significantly different from the control plots. However, the middle rows of the treatment plots were shown to be significantly taller compared to the edge rows of the treatment plots and the control plots (Figure 5.2, A). A reduction in the number of nodes per stalk and mean internodes length in sugarcane influences the height of the stalks (Lingle *et al*., 2000). The height and density of sugarcane in the middle and on the edge rows of the treatment plots of Midlands 1 field site are not significantly different from each other or from the control plots (Figure 5.2, B). There was also no significant difference in height or stalk density at the Midlands 2 and Midlands 3 field sites (Figure 5.2, C, D) which shows that the presence of *M. minutiflora* in these field sites does not affect the growth of sugarcane in these fields. The planting of *M. minutiflora* in irrigation/drainage ditches next to sugarcane did not impede the growth of sugarcane, as stalk density and height in the Midlands field sites were not significantly different between rows next to *M. minutiflora* and rows in the middle of the treatment and control fields. However the difference in height in the middle rows of the treatment plot, in the Coastal 1 field site, may be due to the stalks being in a closed canopy and growing upward to reach sunlight. The stalks on the edges of the treatment plots are able

to extend laterally into the more open row where *M. minutiflora* was planted and therefore do not compete as much in height for sunlight.

5.4.2. Impact of *Melinis minutiflora* on weedy plant species

The comparison of weed biomasses between treatment and control plots (Figure 5.3) shows a significant reduction in weed biomass of the treatment plot in the Midlands 1 field site, where *M. minutiflora* out competes the weeds already present. *Melinis minutiflora* being planted in the irrigation lines had a positive effect on the field by reducing the amount of other weeds present, and thereby reducing the possible competition with sugarcane and possible invasion of the crop by common weeds of sugarcane observed in these field sites, such as *Amaranthus hybridus* (pigweed) and *Conyza bonariensis* (flaxleaf fleabane).

In the field site in Coastal 1 no other weed biomass was present and all the plant material collected was *M. minutiflora* because of the herbicide programme. In comparison with the other field sites, the biomass of *M. minutiflora* from Coastal 1 was greater than at any other field site. *Melinis minutiflora* was able to grow unhindered in between the rows of sugarcane on the drainage lines of Coastal 1. *Melinis minutiflora* was shown to reduce other weeds within the field sites where this grass was planted (Figure 5.3). A herbicide trial should be performed to determine if planting *M. minutiflora* is beneficial to crop yield or whether it is equal to having no weeds in terms of any decrease in yield from the field. Additionally, this grass should be more cost effective than using herbicides.

5.4.3. Sucrose yield analysis

There were no significant differences in the sucrose yield (ERC% cane) between respective pairs of control and treatment plots or between sampling points in the field plots (Figure 5.4). The presence of *M. minutiflora* in and around treatment fields had no adverse effects on the amount of sucrose yield from the sugarcane in the field.

The previous chapter, showed *Melinis minutiflora* in a large field site, to have potential pest population reducing properties in sugarcane. This chapter shows that the grass is advantageous in the sugarcane field as it does not significantly compete with sugarcane and has weed suppressing potential. The mean number of *E. saccharina* larvae collected from the Coastal 1 field site was the highest of all the field sites, but it also had the most reduction in populations between treatment and control plots. The herbicide programme at this field site may indicate the benefit of not having weeds between the rows of sugarcane. Predators that

would normally stay or be attracted by the weedy species between the crop rows and find larvae while in the field would not be attracted by weed species to search in the field. The introduction of this grass, *M. minutiflora* may attract these predatory insects to the field (Kasl, 2004) in the same way other weeds can harbour natural enemies and prevent stemborers from reaching damaging levels on crops (Schulthess *et al.*, 1997; Sengonca *et al.*, 2002).

Weeds in sugarcane are problematic due to properties of sugarcane agriculture. Sugarcane is planted in rows roughly 0.9-1.5m wide. Sugarcane is generally divided into plots with roads and water ways running between the plots. These open areas along the rows of sugarcane are the ideal for weeds to establish. Maize is generally planted at 1.2m spacing to allow access between the rows during harvesting. This spacing allows more weeds to develop in between the rows as sunlight and water are more available and weeds grow faster when not competing in the shade of the main crop (Seavoy, 1973). Weeds are fast growing plants and can germinate, become established and produce seeds for future establishment in the field before the sugarcane canopy closes. Shade from the main crop kills perennial weeds that compete with the crop (Seavoy, 1973). The critical period of weed competition in sugarcane is the first 90 days of crop growth (Rainbolt and Dusky, 2007). Sugarcane is irrigated in some regions which gives the weeds, already in the soil, a chance to germinate and grow rapidly. Weeds in sugarcane may remove water via transpiration and further reduce the growth of the sugarcane. Soils are usually highly fertilized which also helps weeds to establish and spread seeds rapidly. In a ratoon crop year of sugarcane, the soil is less prepared and weeds are not dug out as vigorously at the beginning of the growing season as when planting a seed cane crop. All these factors can help promote weed growth in sugarcane.

Weed competition is a mutual, negative interaction between organisms by means of interference or indirect exploitation of shared resources (Ghersa and Roush, 1993). Plant breeding that would allow a crop to compete with weedy species and maintain the yield is not yet possible. However introducing plants into the crop field, which interfere with the weeds instead of the crop but do no damage to the crops in the field, is a possible alternative. Crop interference is more effective than weed tolerance. Weed tolerant crops only have a small drop in yield, but do not stop the weed seed populations that can cause future yield loss (Jordan, 1993). Intercropped plants can have weed control advantages in two ways. Increased crop yield and decreased weed growth, is possible if the intercropped plant is better at taking resources than weedy plants or reduces weed growth through allelopathy. Alternatively, if intercrops can use resources not exploited by weeds or can convert nutrients for uptake by the

main crop, such as a nitrogen fixing legumes, it will give these plants advantages for better growth than the weeds (Liebman and Dyck, 1993). Increasing the soil cover and intercepting light by the crops canopy would shorten the time available for weeds to grow and spread (Baumann *et al*., 2001)

The spreading of *M. minutiflora* shows the potential rapid space-filling character this grass has. Grass spreading reduced the available space for other weed species to get into the open space of the drainage line. It was evident at the trial sites that *M. minutiflora* is not shade tolerant, as it did not encroach into the adjacent sugarcane row. Secondly at Midlands 1, *M. minutiflora* grew rapidly soon after it was planted as a seedling. It competed with other weeds growing along the edges of sugarcane rows and out-competed them, showing a difference in weed biomass of 350 g/m^2 in the control to 70g/m^2 in the treatment plot (Figure 5.3). This grass did not compete with the sugarcane in these rows as shown by no significant difference in height and density in most field sites. The one field site where a significant difference in height is seen is Coastal 1 (Figure 5.2). Sunlight could influence the height of the sugarcane because leaves on the stalks on the more open edges of the field do not extend as far upward to get sunlight as stalks in the middle of the field do. Competition causes morphological changes in vertical shift of leaf area distribution (Baumann *et al*., 2001).

Fields used for only one crop no longer appear to be suitable in crop management as there are long term advantages related with mixed cropping (Altieri and Whitcomb, 1980). Benefits of mixed cropping include pest control by repelling stemborers which was shown as a possibility with *M. minutiflora* in Chapter 4, and weed competing properties also associated with *M. minutiflora* in this chapter. Intercropping a plant that can intercept sunlight to immature, weakly competitive weed seedlings and stop their growth can contribute to long term weed suppression (Baumann *et al*., 2001)

Cover crops and living mulches such as hairy vetch and winter rye, have benefit in crop production particularly for ground cover and soil erosion control but can also conserve soil nitrogen and increase soil organic matter suppressing weeds (Hartwig and Ammon, 2002). The weed suppression characteristics of winter cereal crops, barley, wheat and oats cultivars were studied using the weed *Galium aparine* L. (Seavers and Wright, 1999). Significant differences were found in the suppressive abilities of these crop species and Oats was the most effective species (Seavers and Wright, 1999). Intercropping into a field with these three

cereal crops could have beneficial effects for farmers by increasing crop production and suppressing weeds already in the crop fields.

Strip cropping haricot bean (*Phaseolus vulgaris* L.) with maize in weedy and weed free conditions tested the abundance of a tachinid parasitoid and predatory wasps associated with African bollworm. The tachinids were found to be more abundant in the strip crops and weedy fields than the monocrops. Predatory wasps were unaffected by intercrops, but were two to eight times more abundant in weedy planted plots than weed free plots, due to the increase in the habitat diversity (Abate, 1991). Haymes and Lee (1999) found that in a wheat and field bean intercrop field experiment, weed competition resulted in better performance of the wheat cultivar. *Orobanche crenata* is a weedy root parasite causing damage to legume crops. Infestation by this parasite on faba bean and pea was reduced by intercropping with oats. The inhibition of *O. crenata* seed germination was via allelochemicals from the roots of the cereal crop (Fernandes-Aparicioa *et al*., 2006). Damage to maize by pests was significantly greater in monocrop fields than in the maize and leucaerna intercrop fields. This was linked to a reduced loss in yield per plant (Ogol *et al*., 1999). The negative effects of plant competition tend to be balanced out by the positive effects of protection from pests in this experiment (Ogol *et al*., 1999).

Farmers get reduced weeds, and in exchange they have to tolerate weedy intercrops or non crop plants that have benefits in the field, but are still essentially weedy plants.
Farmers may still think of *M. minutiflora* as an indigenous weed. This study has shown that there is no competition effect from this grass and additionally there is a weed suppression benefits that this grass gives. Therefore, there is a good reason to use this grass in sugarcane agriculture.

Chapter 6: Conclusions

Habitat management is a discipline within the confines of cultural and biological control methods used to reduce pest species in crop fields. Push-pull plants (Pyke, 1978) in stimulo-deterrent diversion strategies, as first explained by Miller and Cowles (1990), gave rise to searches for potential host plants that could be used to repel pest species away from crops, or pull them towards alternate crops.

This current study was started in 2004 following on from a PhD study in sugarcane in a similar vein. The laboratory results of that study were taken into the field in this study. Additionally, further laboratory experiments were done to determine the suitability of certain non crop plants in a push and pull strategy to reduce the infestation population of *E. saccharina* in sugarcane. There have been stemborer studies done on plants used as "pull" plants and "push" plants and many have been successfully used in agriculture. Khan *et al.*, (1997a) showed *M. minutiflora* was effective in pushing *E. saccharina* away from maize plants. Van den Berg (2006) used *P. purpureum* as a push plant to reduce infestation of maize by *C. partellus*, while Khan *et al*., (2007) used it to reduce *B. fusca* infestations.

The first part of this study (Chapters two and three) involved further testing in the laboratory of potential pull plants, which had previously been found to have stemborers feeding on them. Olfactometer and oviposition trials were used to determine whether there was any attraction by these plants to *E. saccharina.*

Oviposition trials in cages did not yield significant results to indicate that *E. saccharina* could make a specific oviposition choice for one of the tested plants or sugarcane. *Eldana saccharina* females are not particular about where to lay their eggs, as oviposition does not seem to be influenced by the presence of potential host plants for their offspring. In previous studies on *E. saccharina* it has been shown that the dead leaf material on a plant is chosen for oviposition (Atkinson, 1980; Leslie, 1990). The presence of cryptic oviposition sites on the test plants was a possible explanation by which plants were chosen by female moths to lay their eggs, and the biomass of dead leaf material did show a possible relationship with the number of eggs laid.

The stimulo-deterrent diversion strategy does not show potential in this study if the chemicals from test plants do not affect the pest insects to attract them or repel them away from crop fields. If the physical characteristics of a plant do not affect the pest then finding appropriate plants for 'push-pull' will be a challenge. *Eldana saccharina* is an intractable insect and did not behave as was expected in this experiment. Kasl (2004) found that *E. saccharina* larvae were attracted to its natural host plant, *C. papyrus* more than sugarcane. A different group of test plants may give a more significant result in oviposition and olfactometer trials.

The results from olfactometer experiments (Chapter three), testing the effect of chemical volatiles at a different range on *E. saccharina* host selection were not able to show that the volatiles of potential pull plants attracted *E. saccharina* more than sugarcane. The effect of males in the olfactometer boxes added another dimension to the experiments about which sex makes the choice for a host plant. If one sex does choose the host plant, the question should be asked, does the other sex search for a mate on that plant? The results show that females moved out of the boxes more than male moths but did not make a significant choice for any test plants. Female moths were not attracted to males in the experiments, possibly because male moths were not able to call for females effectively. When male moths are tested in future studies, it may help to understand more of the courtship behaviour of *E. saccharina*, but certainly seems at this stage that this insect does not respond to plant volatiles.

From the results of the second and third chapters a conclusion is made that these particular plants do not appear to support further exploration of their use in habitat management programmes against *E. saccharina* in sugarcane fields. However, *E. saccharina* populations have been reduced by planting *P. purpureum*, around maize fields (Kfir *et al*., 2002). Van den Berg *et al.* (2003), showed that *V. zizanioides* was attractive to another stemborer, *C. partellus* for oviposition and could be used in the field to attract this pest species away from the main crop. Testing *V. zizanioides* with sugarcane against *E. saccharina* in this study, did not support this result. These experiments were setup to test plants for their potential as push or pull plants. Different plants with supposed different volatile profiles need to be tested in future studies to find a plant suitable for planting into sugarcane to attract *E. saccharina* away from the crop field.

The study nevertheless continued with field trials across the Coastal and Midlands regions of the sugarcane industry using *M. minutiflora,* which although not producing significant results in the laboratory has been shown to reduce stemborers in the field. Positive results were found in two field sites where the populations of *E. saccharina* were reduced in treatment plots where *M. minutiflora* was planted, compared to control plots, which confirmed the potential of this grass proposed by Kasl (2004). In the other field sites, negative results or non significant results were achieved. The potential of this grass was apparent when planting the grass as seedlings, just after harvesting sugarcane so that the grass was able to establish before the sugarcane increases in height in the next season and shades out the grass. The correct spacing and placement of intercrops between rows of sugarcane and size of field in which to use this grass needs further studies.

The fifth chapter followed on from the field results of chapter four, and the characteristics of *M. minutiflora* were further tested and shown to have weed suppressing properties, but did not compete with sugarcane when planted next to rows of sugarcane. The biomass of planted *M. minutiflora* in the field sites was more than that of other weeds in those fields. This non-crop grass can be planted in new sugarcane fields before weeds are able to establish. It would suppress weeds and fill up available space between rows of sugarcane to prevent future weed growth. Farmers should therefore be encouraged to use this non-crop grass in their fields to decrease other weeds that could possibly reduce the yield. The results in the fifth chapter do not show an increase in yield due to the presence of *M. minutiflora*, but they also do not show a significant reduction in ERC% cane because of the intercrop. Other measured impacts of *M. minutiflora* could be reduced erosion, reduced nematodes, and general increase in field biodiversity.

Green farming is an option that should be considered for future sugarcane agriculture. The amount of chemicals and insecticides that have been used in the sugarcane industry has caused controversy, especially because of the negative aspects such as expense and broad spectrum effect that these chemicals have on the environment. Green farming, using natural methods and biological control to overcome pest and weed problems, is worth consideration with a plant such as *M. minutiflora.* Other new plants in the crop field environment will increase the habitat diversity in the area, which has many other benefits such as shelter and

alternative food sources, such as nectar, for natural enemies (Abate, 1991). At their worst these results show that there is nothing to lose by trying these new "greener" methods of pest control and improving the environment, and at their best will reduce pest numbers and other negative effects often attributed to sugarcane farming.
The use of *M. minutiflora* in fields of sugarcane can be recommended for its potential pest repellent and weed suppressing properties.

There are two possible ways to move forward from this point, find new plants to influence *E. saccharina* or find a new target in the sugarcane crop system for plants to have an effect on. Natural parasitoids and predators of stemborer pests do occur in close proximity to sugarcane fields. The stemborer parasitoid of *E. saccharina*, *Goniozus indicus* Ashmead (Hymenoptera: Bethylidae), was found to use chemical cues from *Cyperus papyrus* to locate its host (Smith *et al*., 2006). This offers the opportunity to draw this agent into the sugarcane crop. Stemborer infested sugarcane may be missing cues that attract parasitoids into the field (Conlong and Kasl, 2000). *Cotesia sesamiae* was attracted to a field of sorghum planted with *M. minutiflora*, and attacked lepidoptera cereal stemborers (Gohole *et al*., 2005). Altieri *et al*., (1981) showed that extracts from *Amaranthus* sp. increased egg parasitism of corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) in soybean, cowpea, tomato and cotton (Verkerk *et al*., 1998). Future habitat management with non-crop plants and sugarcane may concentrate on attracting natural predators and parasitoids into the field to reduce *E. saccharina* infestations.

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