# Propositions

1. Controlling the elusive banana weevil is more difficult than many researchers think (*this thesis*)

2. There are quite some applications of pheromones which have not been well exploited and yet are the best contributions to environmentally benign pest control options that can bring hope to both people and the planet (*this thesis*).

3. Without use of infochemicals, foraging by arthropod carnivores would be impossible regardless of dietary specialization (Steidle, J.LM. & van Loon, J.J.A. 2003. *Entomologia Experimentalis et Applicata*, 108:133-148).

4. There are no things known in a way of emotion or appetite, ambition or achievement, that could surpass in richness and strength the excitement of entomological exploration (Price, P.W. American Entomologist, 49 (3) 2003).

5. Insects as a group receive minimal legislative protection because of small size and distant evolutionary relationship to humans (Metrick, A & Weitzman, M.L.1996. Patterns of behaviour in endangered species preservation. *Land Economics*, 72:1-16).

6. Plants which can listen to talking neighbours that are attacked by herbivores have a selective advantage over those that are deaf (Dicke, M., Agrawal, A.A. & Bruin, J. 2003. Plants talk, but are they deaf? *Trends in Plant Sciences*, 8:403-405)

7. The low impact of agricultural research in Africa is due to the lack of countervailing power of farmers to set the research agenda (Röling, N., Houkonnou, D., Offei, S.K., Tossou, R. & Van Huis, A. 2004. Linking science and farmers' innovative capacity, (in press)).

8. In central Uganda a feast without bananas is a feast without food.

William Tinzaara

**Chemical ecology and integrated management of the banana weevil *Cosmopolites sordidus* in Uganda.**

Wageningen, 25 February 2005

# Chemical ecology and integrated management of the banana weevil *Cosmopolites sordidus* in Uganda

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**William Tinzaara**

# Chemical ecology and integrated management of the banana weevil *Cosmopolites sordidus* in Uganda

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ter verkrijging van de graad van doctor
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## Abstract

Infochemicals (pheromones and kairomones) may potentially be used for control of the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Cosmopolites sordidus* is a major pest of East African highland banana and plantains in most banana growing regions of the world. The weevil produces an aggregation pheromone that attracts both males and females. The attractive isomer sordidin has been identified and synthesized, and is commercially available. The objective of the research project described in this thesis was to investigate whether an infochemical-based trapping system can be used to control *C. sordidus* under Ugandan conditions. In laboratory and field experiments, *C. sordidus* responded in an additive way to the combination of the fermented plant tissue and the aggregation pheromone. The effect was, however, more pronounced in laboratory than field experiments. Several factors such as the pest biology, pheromone efficacy, trap parameters, cropping system and environmental factors were found to variously influence the effectiveness of the pheromone-baited traps. The effects of doubling pheromone trap densities from 4 to 8 per ha on *C. sordidus* population density and plant damage were negligible in an on-farm experiment. The pheromone-trapping system on farmers' fields was therefore not effective at the trap density recommended by the supplier (4 traps per ha). Olfactory responses of the banana weevil predators *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) and *Pheidole megacephala* (Hymenoptera: Formicidae) towards volatiles from banana pseudostem tissue (kairomones) and the synthetic banana weevil pheromone were evaluated in a two-choice olfactometer. Both predators discriminated between fermenting banana pseudostem tissue and clean air. There was no evidence that the pheromone influences predator distribution around the trap in the field. In experiments to investigate whether pheromone trapping can be integrated with use of entomopathogenic fungi, *Beauveria bassiana* to control *C. sordidus*, we observed that weevils can be aggregated on banana mats on which pheromone-baited traps are placed and on adjacent mats. Infected weevils were also observed to transmit the fungal pathogen to healthy individuals in the field. Weevil mortality due to pathogen infection was significantly higher in plots where aggregation pheromone was used in combination with *B. bassiana* compared to when the pathogen was applied without the pheromone. The data demonstrate that the banana weevil aggregation pheromone could be used to enhance the dissemination of *B. bassiana* for the control of *C. sordidus*. This project provides experimental evidence to further develop the application of the synthetic aggregation pheromone to control *C. sordidus* in Ugandan banana production by small scale farmers. The aggregation pheromone should be considered to be a good component of an IPM system in which it may not be effective by itself but stimulate several mortality factors for the control of the banana weevil. The next major strategy for use of pheromones is therefore to further exploit the potential to integrate entomopathogenic fungi and nematodes in the trapping system.

Chapter **1**

# General introduction


## Abstract

Infochemicals play an important role in the biology of many insect species. An understanding of their role in plant-herbivore-carnivore interactions can be used in the development of tools for the enhancement of environmentally benign alternatives to synthetic pesticides. This review discusses how chemical information mediates ecological interactions between organisms and the role of infochemicals in integrated pest management programmes. Infochemicals can be used in pest monitoring and as a control measure through mating disruption, mass trapping and as a means of aggregating herbivores at delivery sites for biological control agents. Particular emphasis is placed on the potential of using pheromones and kairomones in the management of the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), a pest in banana plantations of East African highland banana and plantains in most banana growing regions of the world. *Cosmopolites sordidus* produces an aggregation pheromone that attracts both males and females. This pheromone has been identified and synthesized and is being recommended as an effective method of trapping and controlling the weevil. The synergism between banana plant extracts (kairomones) and the synthetic pheromone in attracting *C. sordidus* should be better exploited. Research areas that can provide information for the development of an infochemical-based trapping system for the management of *C. sordidus* are discussed. Finally, research objectives and a thesis outline are presented.



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# 1 INTRODUCTION

All organisms utilise information they perceive from their environment to maximise fitness, e.g. by improving food location, reproduction and predator avoidance. Information on environmental conditions is often available through chemical cues (Bell & Carde, 1984; Carde & Bell, 1995; Dicke, 1999a). Chemicals involved in conveying information in intra- and inter-specific interactions between organisms are termed infochemicals (Table 1) and constitute a subcategory of semiochemicals (Dicke & Sabelis, 1988). The study of chemicals mediating interactions between organisms, either within the same species (pheromones) or from different species (allelochemicals), forms the research field of chemical ecology (Metcalf & Metcalf, 1992; Roitberg & Isman, 1992). Chemical information is regarded as a key factor mediating behavioural and ecological interactions between insects and plants. In recent years, increased research attention has been directed towards the role of chemical information in arthropod biology (Whittaker & Feeny, 1971; Bell & Carde, 1984; Metcalf & Metcalf, 1992; Roitberg & Isman, 1992; Carde & Bell, 1995; Dicke, 1999a; Dicke & Vet, 1999).

In this review, chemically mediated interactions between organisms and the role of infochemicals in pest management are discussed. Our objectives are to summarise how infochemicals have been used in pest control, indirectly through monitoring and directly through mating disruption, mass trapping and by integration with biological control methods. Limited information is available on infochemicals and the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). We therefore review the existing literature on coleopterans so as to gain an insight into the possible role of infochemicals in the development of an integrated pest management programme for *C. sordidus* and in particular the development of an infochemical based trapping system for the control of this weevil.

# 2 CHEMICAL INFORMATION IN ECOLOGICAL INTERACTIONS

Chemicals produced by plants and insects play a major role in the behavioural responses that determine the performance, survival and development of the insects (Vet & Dicke 1992; Steidle & van Loon, 2003; Cournoyer & Boivin, 2004; McGregor & Gillespie, 2004). Chemicals involved in transmission of information between individuals of the same species are termed pheromones (Table 1). These include sex pheromones, aggregation pheromones and trail pheromones (David & Birch, 1986; Ridgway *et al.,* 1990; Agelopoulos *et al.,* 1999; Bartelt, 1999). Sex pheromones are produced by one sex (usually the female) and attract members of the opposite sex for mating. By comparison,

aggregation pheromones lead to aggregation of members of both sexes resulting in mating and aggregation at a food source (Foster and Harris, 1997). These are often produced by males. Male produced aggregation pheromones have been demonstrated for a number of weevil species such as *Rhynchophorus palmarum* (L) (Rochat *et al.,* 1991), *R. cruentatus* (Weissling *et al.,* 1993), *R. phoenicis* (Fabricus) (Gries *et al.,* 1993), *Metamasius hemipterus* (L) (Giblin-Davis *et al.,* 1994b), *C. sordidus* (Budenberg *et al.,* 1993b), and *Sitophilus* spp. (Walgenbach *et al.,* 1983). Social insects, such as ants and termites, often produce trail pheromones by which individuals may guide other members of the colony to food sources (Blum, 1974; Bell & Carde, 1984). Other pheromones produced by phytophagous insects include anti-aggregation pheromones that ensure adequate spacing when resources are limited (Prokopy, 1981), oviposition-deterring pheromones that females use to mark the hosts on which they have laid eggs (Heard, 1995), and alarm pheromones that serve to warn conspecific insects of impending danger (Bowers *et al.,* 1972). Knowledge of pheromones for carnivorous insects is still rudimentary, with a few pheromones indicated for parasitoids (e.g egg marking pheromones) and predators (Aldrich, 1999).

Herbivores are known to use plant volatiles (kairomones) to locate a food plant (Visser, 1986). When herbivores feed on a plant, cell damage results and there is release of volatiles from the wound site (Dicke *et al.,* 1990). The amount of volatiles released by herbivores or by herbivore-infested plants may affect herbivore response to the plant. The response to an information source by the herbivore may depend on physiological state, previous experiences and abiotic conditions (Dicke *et al.,* 1998). Host plant selection by herbivores may be affected by infochemicals from competitors (Schoonhoven, 1990) and natural enemies (Grostal & Dicke, 1999).

Chemical information on herbivores presence and identity may be essential for successful location of herbivores by carnivore. Plants may respond to herbivore attack by producing chemical cues that attract carnivores to herbivore-infested plants (Dicke, 1999a, b). The herbivore-damaged plant may emit relatively large amounts of plant volatiles that are not emitted or only emitted in trace amounts by mechanically damaged plants (Dicke *et al.,* 1990).The amount of plant volatiles released after herbivore attack may be different among species, plant genotypes, and plant parts and can be affected by abiotic and biotic factors such as species, instars and densities of the herbivores (Vet & Dicke, 1992). Corn seedlings release large amounts of volatile terpenoids after damage inflicted by *Spodoptera* caterpillars, which attract the larval parasitoid *Cotesia marginiventris* (Turlings *et al.,* 1990). Similarly, the predatory mite *Phytoseiulus persimilis* is attracted by spider mite induced volatiles produced by bean or cucumber plants (Dicke *et al.,* 1990). The anthocorid predators (*Anthocoris nemorum* and *Orius* spp) (Heteroptera: Anthocoridae) were attracted to pear

trees infested by pear psylla under field conditions (Drukker *et al.*, 1995). Prey searching and location is not the only process triggered by herbivore-induced plant volatiles. Some predators such as *Metasyrphus corollae* (Syrphidae), *Chrysopa carnea* (Chrysopidae) and *Coccinella septempunctata* (Coccinellidae) use these chemical cues to select oviposition sites (Lewis, 1977; Shonouda *et al.*, 1998; Steidle & van Loon, 2002).

Table 1. Infochemical terminology according to Dicke and Sabelis, 1988

**Infochemical**: A chemical that, in the natural context conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response

***Pheromone*:** An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism [(+, -) pheromone], to the receiver [(-, +) pheromone], or to both [(+, +) pheromone]

***Allelochemical*:** An infochemical that mediates an interaction between two individuals that belong to different species.

*Allomone*: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 1 but not to organism 2.

*Kairomone*: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 2, but not to organism 1.

*Synomone*: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when contacts an individual of another species (organism 2), evokes in the recover a behavioural or physiological response that is adaptively favourable to both organism 1 and 2.

# 3 INFOCHEMICALS AND INSECT PEST CONTROL

Infochemicals, and pheromones in particular, have been used widely in pest management and have been reported to have the advantage of being non-toxic to humans and arthoropods (David & Birch, 1986; Ridgway *et al.*, 1990; Agelopoulos *et al.*, 1999). Pheromones perform most effectively when employed as part of the integrated control programme, where they can be used either indirectly for monitoring (Wall, 1989), or directly to manipulate pest populations (Griffiths *et al.*, 1991). They offer the prospect of establishing contact with microbial control agents by attracting pests into an

environment where these agents might be best preserved. This can apply to fungal pathogens, mobile insect predators and parasitoids (Pickett, 1988). The knowledge on infochemical use by plants, herbivores and carnivores can be utilised in the development of new integrated pest control strategies.

### 3.1 Pheromones

Successful chemical identification of insect pheromones (Butenandt *et al.*, 1959) led to an interest in pheromone research. Pheromones were considered a new generation of pest control agents and rapid progress was made in the identification and isolation of pheromones from a wide range of insects (Table 2). Sex and aggregation pheromones are common among weevils. However, the state of knowledge of weevil pheromones varies broadly among species. For some, there is only an indication that a pheromone exists but for others, there have been detailed behavioural and physiological studies, chemical isolation, identification, pheromone synthesis, and commercial development (Table 2).

Pheromones and other behaviour modifying chemicals hold a great potential as tools for pest management (Silverstein, 1981; Phillips, 1997; Agelopoulos *et al.*, 1999; Suckling, 2000). Pheromones have been used in both monitoring insect populations and in direct control (Phillips, 1997; Agelopoulos *et al.*, 1999). Pheromone traps provide an easy and efficient way of detecting insect populations in the field and in storage facilities (Phillips, 1997). Control of insect pests can be achieved either by mating disruption (Burkholder & Ma, 1985) or by mass trapping using pheromone baited traps that lure insects to their death (Giblin-Davis *et al.*, 1994b) (Table 2). Pheromones also have the potential to lure pests into traps containing entomopathogens that the visiting pests would then spread throughout their population (Vega *et al*., 1995; Klein & Lacey, 1999).

Although there have been limited successes, the potential exists for using pheromones to control coleopteran populations by mass trapping. The advantage is that, with few exceptions, coleopterans use aggregation pheromones, attracting both sexes which negatively affect the reproductive capacity of the population (Trematerra, 1997; Bartelt, 1999). This makes mass trapping using aggregation pheromones theoretically possible, in contrast to the systems with female produced sex pheromones where more than 90% of males must be captured to effect any depletion of the following generation (Bartelt, 1999). Mass trapping is most promising for insect pests with low fecundity, slow population build up, limited dispersal and long life span (Giblin-Davis *et al.*, 1996a). Pheromone traps are species specific and the negative impact on non-targets is limited. Other benefits of using pheromones in pest management include low costs and low potential for development of pest resistance.

### *3.1.1 Monitoring*

Pheromones have been used for monitoring pest populations of crop pests and orchard pests (Wall, 1989), stored products pests (Burkholder, 1990) and forestry pests (Borden, 1993). Monitoring can assist in detecting the arrival of dispersing and migrating insect pests, timing of control measures, risk assessment and population density estimates (McVeigh *et al.,* 1990; Howse *et al.,* 1998). Monitoring involves establishing a quantitative relationship between pheromone trap catches of a particular pest and the plant damage caused by it. The relationship is then used to define trap catch values that could be used to identify the economic threshold level of a pest for which control measures are necessary (Suckling, 2000). The monitoring systems have enabled more effective targeting of all major pest control tactics including pesticides and biopesticides (Suckling, 2000).

Although commercial advances in pheromone-related pest monitoring and control technology have tremendous potential (Howse *et al.,* 1998; Smit *et al.,* 1997), there remain challenging technological issues in the development and use of monitoring and control systems (Suckling, 2000). These include the design of attractant release systems, trap design and deployment systems, data analysis and interpretation and the development of expert decision systems. Obtaining quantitative information about pest populations from trap catch data has proved difficult in many cases, especially for strongly flying, highly mobile species which may be able to escape from sticky substances commonly used in insect traps (Srivastava *et al.,* 1992). In some cases pest species have been trapped successfully in the field but their presence did not correlate well with pest density and damage levels (David & Birch, 1986). There have also been examples of insect species not being caught by ineffective traps and causing crop damage (Tadas *et al.,* 1994).

### *3.1.2 Mating disruption*

Mating disruption involves permeating the pest environment with sex pheromones so that the probability of a female being found by a male, mating and laying viable eggs is reduced below a point where economically significant damage occurs (Carde & Minks, 1995; Evenden *et al.,* 2000). Pest control by use of synthetic pheromones to disrupt normal mating behaviour is now operational for a number of lepidopteran species such as the pink boll worm, *Pectinophora gossypiella,* the cotton boll worm, *Helicoverpa armigera* and the rice stem borer, *Chilo suppressalis* (Campion *et al.,* 1989; Hall *et al.,* 1994; Carde & Minks, 1995; Cork *et al.,* 1996; Tamhankar *et al.,* 2000). Use of pheromones in mating disruption of coleopteran pests has been reported for *Cylas formicarius* (F) (Mason and Jansson, 1991) and for the African sweet potato weevils, *C. brunneus* and *C. puncticollis* (Downham *et al.,* 2001).

Table 2. Examples of weevils and beetle species of which pheromones have been reported and chemically identified

| Species | Subfamily | Principle damage | Source | Identified? | Chemical/commercial name | Type of use | Reference |
|---|---|---|---|---|---|---|---|
| *Anthonomus grandis* Boheman (boll weevil) | Curculioninae | Cotton buds, bolls | Male | Yes | Granlure, grandisol | Monitoring | Tumlinson *et al.*, 1969; Hardee *et al.*, 1972 |
| *Conotrachelus nenuphar* (Herbist) (plum curculio) | Cryptorhynchinae | Unripe fruit of apple, plum | Male | Yes | Grandisoic acid (8) | - | Eller and Bartelt 1996 |
| *Cosmopolites sordidus* (Germar) banana weevil | Rhynchophorinae | Banana plant corms | Male | Yes | Cosmolure | Mass trapping | Budenberg *et al.*, 1993b<br>Beauhaire *et al.*,1995<br>Jayaraman *et al.*,1997 |
| *Cylas formicarius* (Fabricius) (sweet potato weevil) | Apioninae | Sweet potato vines , roots, tubers | Female | Yes | (Z)-3-dodecen-1-ol (E)-butenoaete | Monitoring, Mass trapping | Jansson *et al.*, 1989, 1993 |
| *Dynamis borassi* (Fabricius) (palm weevil) | Rhynchophorinae | Inflorensce and crown of palms | Male<br>kairomone | Yes<br>No | Ferrugineol | -<br>- | Giblin-Davis *et al.*, 1997 |
| *Diaprepes abbreviatus* (L) (West Indian sugarcane root stalk borer) | Otiorrhynchinae | Citrus foliage | Male and female | No | - | - | Harari & Landolt, 1997 |
| *Popillia japonica* (Newman) (Japanese beetle) | Rutelinae | Plant flowers, fresh leaves, grasses | Female | Yes | Japonilure | Monitoring | Tumlinson *et al.*, 1977 |
| *Prostephanus trunctus* (Horn) Larger grain borer | Bostrichidae (family) | Storage grains | Male | Yes | Trun-call | Monitoring | Cork *et al.*, 1991 |
| *Metamasius hemipterus* (L.) (West Indian sugarcane borer) | Rhynchophorinae | Sugarcane, banana, palm stems | Male | Yes | Ferrugineol | Mass trapping | Giblin-Davis *et al.*, 1994b |
| *Rhynchophorus cruentatus* (Fabricius) (palmetto weevil) | Rhynchophorinae | Palm stems | Male | Yes | Rhynchophorol | Mass trapping | Weissling *et al.*, 1993 |
| *Rhynchophorus ferrugineus* (Asian palm weevil) | Rhynchophorinae | Palm stems | Male | Yes | Ferrrugeneol | Mass trapping | Hallett *et al.*, 1993 |
| *Rhynchophorus palmarum* (L.) (American palm weevil | Rhynchophorinae | Palm stems | Male | Yes | Rhynchophorol | Mass trapping | Rochat *et al.*, 1991 |
| *Rhynchophorus pheonicis* (F.) (African palm weevil) | Rhynchophorinae | Palm stems | Male | Yes | Phoenicol | Mass trapping | Gries *et al.*, 1993 |
| *Sitophilus granarius* (L.) (granary weevil) | Rhynchophorinae | Stored grains | Male | Yes | Sitophilate | Mass trapping, monitoring | Walgenbach *et al.*, 1983 |
| *Prostephanus trunctus* (Horn) Larger grain borer | Bostrichidae (family) | Storage grains | Male | Yes | Trun-call | Monitoring | Cork *et al.*, 1991 |

One potential weakness of mating disruption is that this technique provides no safeguards against immigration of mated females from outside the area treated with a disruptant (Carde & Minks, 1995). Thus, mating disruption can work best as an area-wide management tool (Staten *et al.,* 1997) rather than being used by an individual farmer. A high population density of the target insect can also limit the control efforts especially if there is competition between synthetic and female produced plumes (Carde & Minks, 1995). Lures involved in mating disruption should be formulated to release at sufficiently high rates to prevent mating (Suckling, 2000). Effective mating disruption requires a high proportion of males failing to mate females and therefore, long-lived species that can mate on multiple occasions are more difficult to control (Suckling, 2000).

### *3.1.3 Mass trapping*

The aim of mass trapping is to control insect species by capturing a very large proportion of the pest population by deploying pheromone traps in sufficient numbers. Such an approach assumes that a reduction in the adult population will lead to a further population decrease in the next generation (Birch & Haynes, 1982). In mass trapping strategies it is important to know the proportion of the population that is captured and the relationship between this proportion and the reduction in the population of the next generation.

In lepidopterans, mass trapping using sex pheromones has varying degrees of area-wide and prolonged success (Kehat *et al.*, 1980; Mafra-Neto & Habib, 1996; Tamhankar *et al.,* 2000). This may have two causes: 1. the sex pheromone (produced mostly by females) attracts only males (and then not all of them) and 2. the males are polygamous and a small proportion of untrapped males can continue to fertilise a substantial number of females with high egg laying potential (Tamhankar *et al.,* 2000).

Several studies have tested the potential of the mass trapping strategy for the control of coleopteran insects. This strategy was tested against the spruce bark beetles *Ips typographus* (L)(Bakke, 1981), the Japanese beetle, *Popillia japonica* Newman (Ladd & Klein, 1982), the boll weevil *Anthonomus grandis* grandis Boheman (Hardee, 1982), the European bark beetle, *Scolytus multistriatus* (Marsham) and the ambrosia beetle *Gnathotrichus sulcatus* (LeConte) (Birch and Haynes, 1982). Some authors (Oehlschlager *et al.,* 1995; Alvarez, 1996) have reported successful suppression of the pest populations by use of mass trapping. Use of pheromone traps significantly reduced the damage from *Cylas* infestation on potato plants in the Dominican Republic (Alvarez, 1996). Oehlschlager *et al.* (1995) described a 17 months mass trapping trial in Costa Rica during which the population density of the weevil *Rhynchophorus palmarum* in a 30 ha commercial palm plantation was substantially reduced

using 6 traps/ha. Cost-benefit analyses of using pheromones in mass trapping for successful studies have however rarely been carried out.

Mass trapping using pheromones should be considered as one component of a set of pest management practices. Deployment of traps alone may not result in suppression of a pest population, especially when the target insect is abundant and has a high multiplication rate (Birch & Haynes, 1982; Weislein, 1992; James *et al.*, 1996). Cuthbert *et al.* (1977) indicated that large numbers of naturally attractive sites (e.g crop residues) competed with traps and hence reduced the trapping effect of scolytid beetles, *S. multistriatus*. Therefore, to suppress populations of the elm bark beetle, *S. multistriatus* (Birch & Haynes, 1982) and the spruce bark beetles, *Ips typographus* (Weislein, 1992), mass trapping should be conducted in conjunction with rigorous sanitation programs. The same is true for the mass trapping of *Carpophilus spp*. in stone fruit orchards (James *et al.*, 1996) and *R. palmarum* in plantations of African oil palm (Oehlschlager *et al.*, 1995).

High trap densities and high trapping frequency are often needed to obtain satisfactory levels of population suppression (Lloyd *et al.*, 1981). Continuous trapping of *A. grandis* at high population levels indicated that as many as 92% of the males emerging from a population were captured with a trap density of 14 traps/ha (Lloyd *et al.*, 1981). Such high trap densities are not practical as they render the technique too costly. In contrast, with low population levels, a density of 2.5 traps/ha resulted in a high rate of elimination of *A. grandis* (Legget *et al.*, 1989). Mass trapping tends to be more effective at low population densities as was reported for *A. grandis* (Lloyd *et al.*, 1981; Legget *et al.*, 1989; Ridgway *et al.*, 1990).

Two major questions have always been raised regarding trap density (David & Birch, 1986; Howse *et al.*, 1998). First, what trap density is required to adequately sample the pest population? Second, at what density do traps interact thereby influencing the size of the catch in the individual traps? Answers to these questions will help in optimising trap placement that is critical to the success of mass trapping efforts.

### *3.1.4 Trap effectiveness*

A number of factors influence the rate of captures of insects in pheromone traps. Pheromone dose and release rate influence how traps perform relative to natural pheromone sources and thus determine how well they attract insects (Muirhead-Thompson, 1991; De Groot & De Barr, 1998). Insects may respond to the colour of a trap and certain trap designs capture insects more efficiently (Giblin-Davis *et al.*, 1996b; Smit *et al.*, 1997; De Groot & De Barr, 1998). In field studies using aggregation pheromones as trap baits, all trap colours tested were equally efficient in attracting *M. hemipterus* to ground mounted

bucket traps (Giblin-Davis *et al.*, 1996a) and *R. palmarum* to tree mounted traps (Oehlschlager *et al.*, 1993). *Anthonomus grandis* responded positively to yellow from a distance of approximately 7 m and at 2 m the majority of weevils orient to this colour rather than the pheromone (Legget & Cross, 1978). Placement of the traps near, within, or away from the host plants may affect the trap capture depending on the link between the host and mate finding (McNeil 1991; Muirhead-Thompson, 1991). Catches may also correspond to the timing of placement of the traps in populations with distinct generations (Jansson *et al.*, 1991).

Trap catches in most cases correlate to environmental factors such as wind direction and speed, rainfall and temperature (Jansson *et al.*, 1989; Sappington & Spurgeon, 2000). Wind speed and direction may alter pheromone plume shape and size, and create pheromone concentration gradients within the plume. Upwind orientation by insects to pheromones is well known. Information on the influence of wind is not available for ground dwelling insects where wind speed is negligible. Little research has been carried out to examine the influence of relative humidity on pheromone-mediated communication in Coleoptera. For Lepidoptera, Miller and McDougall (1973) found a negative relationship between trap catch and relative humidity in a 12-year trapping study on the spruce budworm. Responsiveness of pests to pheromones has been demonstrated to increase with temperature (Burkholder & Bousch, 1974; Burkholder & Ma, 1985). Variation in pheromone trap catches with temperature may be attributed to increased response in the receiver and better distribution of the chemicals.

The trap efficiency is further affected by the pest biology. Mated females of *A. grandis* were less responsive to male produced sex pheromone (grandlure) than virgin females, both in laboratory bioassays and in field tests (Hardee *et al.*, 1969). In insect species in which virgin females are trapped, the reduction in the subsequent generation may be directly related to the proportion removed from the population. If females are trapped after mating and after having laid some eggs, the efficiency of the method decreases. If males were trapped, a very large proportion probably would have to be captured before there is a noticeable impact on the next generation. This is true for many insect species in which both males and females are capable of mating many times (Borden, 1977).

The pheromone trap efficacy is also influenced by interaction of the cropping system and the biology of an insect pest (Hebblethwaite, 1989). For polyphagous pests that have non-crops as host plants, uncultivated areas and weed populations are important elements of the farming system relevant to pest incidence. For pests having only one crop as host, the pattern of cultivation and proportion of land cultivated are some of the factors that determine their importance. The pheromone trapping efficiency is influenced by the cropping patterns (monocropping or mixed cropping), but it is also possible that within a cropping pattern there are still zones where the pest may be more important than

elsewhere which may affect the trap catches. To develop an effective pheromone trapping system, biological information, such as the number of generations per year, range of host plants, dispersal rate, distribution pattern and migratory potential of the pest is generally needed (Borden, 1977).

### 3.2 Kairomones

Many insects use kairomones to find their host plants (Visser, 1986). Among the coleopterans, the boll weevil, *A. grandis*, orients to volatile chemicals emanating from its host plant cotton (Hardee *et al.*, 1966) and also responds to crushed cotton squares (Dickens, 1989). Colorado potato beetles are attracted to volatiles from undamaged potato plants (Visser, 1986) but volatiles from mechanically damaged or herbivore-damaged potato plants elicit a stronger attraction in the beetles (Bolter *et al.*, 1997). Female and male sugar cane stalk borer weevil, *Diaprepes* abbreviatus (L) were reported to be attracted to volatiles from damaged food (Harari & Landolt, 1997). Male and females of the plum curculio, *Conotrachelus nenuphar* (Herbst) are similarly attracted to host odor (Prokopy *et al.*, 1995). Several studies have demonstrated the attractiveness of pseudostem and sugar cane stalk odours to palm weevils in the field (Giblin-Davis *et al.*, 1994a). Mixtures of odorants synergistically attracted the Japanese beetle, *Popillia japonica* (Tumlinson *et al.*, 1977) and bark beetles (Byers, 1992). There are hardly any data about the maximum distance from which kairomones can attract insects.

The efficiency of the kairomones in attracting insects depends on the odour quality and/or the amount released. Bark beetles prefer to attack and colonise dying trees that have odours that are distinctively different from those of healthy trees (Metcalf & Metcalf, 1992). Fermented plant tissues produce a spectrum of odorants that are significantly different from those released by healthy plants (Metcalf & Metcalf, 1992; Giblin-Davis *et al.*, 1994a; Braimah & van Emden, 1999; Rochat *et al.*, 2000). Fermented sap exuding from dead or wounded palms was highly attractive to *R. cruentatus* (Giblin-Davis *et al.*, 1996a). Moist fermenting tissue from various palm species, fruits, sugarcane, pineapple and molasses are similarly attractive to palm weevils (Giblin-Davis *et al.*, 1994a). In olfactometer bioassays, kairomones triggered the primary attraction of African palm weevils, *R. palmarum*, to oil palm. Fermentation processes were important to elicit this behaviour (Rochat *et al.*, 2000).

In addition to being used as attractants of insects to traps, kairomones can be used to enhance attractiveness of insects to pheromone-baited traps (Phillips *et al.*, 1984; Giblin-Davis *et al.*, 1994a; Cerda *et al.*, 1999). They may also be used in the dissemination of entomopathogens to target insects (Vega *et al.*, 2000). Plant-produced kairomones that mediate host selection by phytophagous insects could also be exploited to enhance breeding for host plant resistance.

### 3.3 Synergism between pheromones and kairomones

Host plant volatiles (kairomones) are known to enhance the effectiveness of pheromone traps in attracting weevils (Dickens, 1984; Phillips *et al.*, 1984; Giblin-Davis *et al.*, 1994a; Cerda *et al.*, 1999). In the pine weevil *Pissodes nemorensis*, the male-produced aggregation pheromones grandisol and grandisal were attractive in the field only when deployed with odours from the host plant (Phillips *et al.*, 1984). The maize weevil, *Sitophilus zeamais*, responded significantly more to male produced pheromones deployed with grain than to either pheromone or grain separately (Walgenbach *et al.*, 1987). Ambrosia beetles in the genus *Gnathotrichus* utilise male produced pheromones that act synergistically with the host-derived compounds α-pinene and ethanol (Borden *et al.*, 1980). Green leaf volatiles have a synergistic effect on the attraction of the boll weevil to its pheromone (Dickens, 1984). The combination of *R. palmarum* weevils and palm stem was attractive to both sexes of this species, and males without host material were not attractive to either sex in field trials (Rochat *et al.*, 1991). Attraction of palm weevils by synthetic pheromone rhynchophorol is synergised by the addition of host material, such as palm stem or sugar cane (Oehlschlager *et al.*, 1993), or by host odour compounds, such as ethyl acetate (Jaffe *et al.*, 1993). Attraction of male and female *R. cruentatus* to male pheromone 5-methyl-4-octanol (cruentol) is synergised by volatiles from host plant material, such as Sabal palm stem tissue (Giblin-Davis *et al.*, 1994a). The response in the field of the West Indian sugar cane borer, *M. hemipterus*, to the aggregation pheromone was enhanced by fermented and fresh plant volatiles (Giblin-Davis *et al.*, 1994b). Increasing quantities of sugar cane or host tissue generally increase attractiveness of pheromone-baited traps to *M. hemipterus* (Oehlschlager *et al.*, 1993; Giblin-Davis *et al.*, 1994a).

### 3.4 Infochemicals and biological control

#### *3.4.1 Predators and parasitoids*

There has been increasing interest in the implementation of biological control programs using parasitoids and predators and the efficacy of these natural enemies may be enhanced by infochemicals (Vet & Dicke, 1992; Dicke, 1999a). Application of infochemicals in pest control is, however, impossible without knowledge of the behaviour induced by these chemicals (Gross, 1981). Infochemical use, both within and between species can be utilised in pest management by either exploiting the way the natural enemy responds or by manipulating the source of the infochemical (Dicke *et al.*, 1990; Bottrell *et al.*, 1998). For example, infochemicals can be used to enhance the searching efficiency, host utilisation and reproductive capacity of natural enemies (Lewis & Nordlund, 1985; Noldus, 1989; Renwick, 1992; Bottrell *et al.*, 1998).

There are few cases of the applied uses of infochemicals to manipulate the behaviour of predators in the field by infochemicals. However, data on the role of infochemicals in predator foraging have become available for some groups such as predatory mites (Sabelis and Dicke 1985; Dicke & Sabelis, 1988), bark beetle predators (Aukema *et al.,* 2000), heteropteran predators (Van Loon *et al.,* 2000) and coccinellids (Liu and Sengonca, 1994; Steidle and van Loon, 2002). The predator *Rhizophagus grandis* (Gyll.) (Coleoptera: Rhizophagidae) is attracted to traps baited with a kairomone produced by the bark beetle *Dendroctonus micans* Kug (Coleoptera: Scotylidae) (Aukema *et al.,* 2000) and in this way the predator distribution in the field can be monitored. The search time of predators may be reduced through increased aggregation at infested sites or kairomone sources (Vet & Dicke, 1992).

The performance of natural enemies can be enhanced by changing their environment by using infochemicals. Predators and parasitoids may be retained in the target area or their search and attack behaviour may be improved (Noldus, 1989; Lewis & Martin, 1990). Hagen *et al.* (1971) used artificial honeydew as stimulus to increase predation of *Heliothis zea* by *Chrysoperla carnea* (Stephens). The chemical provided both a kairomone and food supplement that increased predator density around cotton plants. Detailed behavioural studies in patches of nectaried or nectariless cotton plants containing host larvae revealed that when nectar was present in the patch, the wasp *Microplitis croceipes* (Cresson), a parasitoid of *H. zea* larvae stayed longer and parasitised more hosts than when nectar was absent (Stapel *et al.,* 1997). Similarly the release of a synthetic blend of the sex pheromone of *H. zea* in cotton increased parasitism of eggs from 21% in control to 36% in treated plots (Lewis *et al.,* 1982).

A thorough behavioural analysis is needed for drawing conclusions on the role of infochemicals in predator and parasitoid foraging strategies and thus to employ such chemicals in pest control (Noldus, 1989; Dicke *et al.,* 1990; Renwick, 1992; Dicke, 1999a). To apply infochemicals in pest control we need to: (i) know desirable traits of natural enemies and the mechanism(s) by which infochemicals influence these traits, (ii) have the technological ability to manipulate these traits prior to and/or after release, and (iii) potential negative effects such as the attraction of pest insects (Bolter *et al.,* 1997).

### *3.4.2 Fungal pathogen dissemination*

Although certain fungal pathogens have been recognised as potential biological control agents of insect pests (Kaya *et al.,* 1993; Nankinga, 1994), their commercial use has been limited by problems to technically deliver them to the target pests (Kaya *et al.,* 1993; Lacey *et al.,* 1994; Nankinga *et al.,* 1996; Nankinga and Ogenga-Latigo, 1996). Practical delivery of biocontrol agents to the damaged area of the plant remains a challenge because conventional application techniques are typically not cost effective and the target organisms are often difficult to reach. One possible mechanism of targeted delivery is by

contaminating insects with pathogens and letting them spread the infective material (McLaughlin *et al.*, 1969; Shapas *et al.*, 1977; Pell *et al.*, 1993; Lacey *et al.*, 1994). Pheromone traps have been identified as possible lures for inoculation devices and subsequent pathogen dissemination (Vega *et al.*, 1995; Klein & Lacey, 1999; Vega *et al.*, 2000).

The movement of infested hosts is considered to be one of most important ways in which a pathogen can be transmitted and dispersed to new habitats (Shapas *et al.*, 1977; Vega *et al.*, 2000). With an effective pheromone and luring device, it may be relatively easy to expose the attracted insects to a pathogen. These insects would then leave the trap on habituation to the pheromone and return to the crop disseminating the entomopathogen among their own population (auto-dissemination). The hoped for advantage of such inoculation rather than immediate killing with the insecticide would be the potential for the transmission of the pathogen to other insects in the immediate area magnifying the effect of the treatment. It would be relatively easy in this inoculation technique to contaminate insect stages hidden inside the plant tissues with pathogens especially when the egg laying female is infested (Zimmermann *et al.*, 1992). This technique allows for specific and targeted delivery of the bioactive agent that would not be possible using conventional methods (Vega *et al.*, 2000). The effectiveness of the auto-dissemination delivery system of fungal pathogens may however be influenced by the biology (the population dynamics and rate of dispersal) of the target insects.

Various reports are available where pheromones have been used effectively in the dissemination of fungal pathogens (McLaughlin, 1969; Schwalbe *et al.*, 1974; Shapas *et al.*, 1977; Vega *et al.*, 1995; Klein & Lacey, 1999). A bait containing a feeding stimulant and the protozoan, *Mattesia grandis* was effectively used in spreading the pathogen throughout cotton fields for the control of *A.* grandis (McLaughlin, 1969). In laboratory tests, 96% of the test insects placed in the inoculation devices treated with pheromone and pathogen got contaminated compared to 56% that picked pathogens from inoculation devices containing only *Mattesia* pathogens (Schwalbe *et al.*, 1974). In the field, over 95% of the Japanese beetle (*Popillia japonica*) that passed through the auto-dissemination traps during a one-week period died within the 10-day observation period while the control mortality during the same period was 8% (Klein and Lacey, 1999). The inoculative device becomes practical especially when the trap is baited with a pheromone lure that aggregates insects of both sexes (Vega *et al.*, 1995).

# 4 INFOCHEMICALS AND BANANA WEEVIL CONTROL

*Cosmopolites sordidus* is considered to be one of the most serious pests of bananas (Stover and Simmonds, 1987). The insect can cause yield loss up to 100% (Sengooba, 1986; Koppenhofer *et al.*,

1994) through sucker death, toppling and snapping (Sikora *et al.,* 1989; Rukazambuga *et al.,* 1998; McIntyre *et al.,* 2001), and shortens plantation life spans if not controlled (Rukazambuga *et al.,* 1998; Gold *et al.,* 2004). For information on biology and population dynamics of the banana weevil, see extensive reviews by Gold (1998), Gold *et al.* (1999b; 2001) and Masanza (2003).

Control of this pest has depended on the use of synthetic insecticides, which though feasible for larger commercial growers, is beyond the economic capacity of most banana producers in developing countries such as Uganda. *Cosmopolites sordidus* is resistant to a wide range of chemicals (Collins *et al.,* 1991; Gold *et al.,* 1999a). There are hardly any bananas and plantain varieties identified with useful tolerance or resistance to *C. sordidus* (Ortiz *et al.,* 1995). The control of the weevil by cultural methods such as mulching or the use of split pseudostem traps to catch and kill resident and invading weevil populations has been only partially successful (Okech *et al.,* 1999; Gold *et al.,* 2002). Trapping as a control method is influenced by environmental factors, weevil biology, trapping intensity and frequency (Bakyalire, 1992; Gold, 1998). Efficacy of trapping is also related to trap density, trap placement, quality of traps, size of traps and frequency of collection (Bakyalire, 1992; Ogenga-Latigo & Bakyalire, 1993; Koppenhofer *et al.,* 1994). Re-invasion from neighbours' fields may be another problem (Okech *et al.,* 1999; Gold *et al.,* 2001). Enhanced trapping using infochemicals including pheromones and kairomones has been identified as a means that can be used to develop an effective method of trapping and controlling the weevil. Below we discuss the role of pheromones and kairomones, and their integration with biological control methods for the management of *C. sordidus*.

### 4.1 Pheromones

Evidence for a male-produced aggregation pheromone to which both females and males of *C. sordidus* respond was first provided by Budenberg *et al.* (1993b). Beauhaire *et al.* (1995) detected six male specific compounds with electroantennogram (EAG) activity in volatile collections. They identified and synthesised sordidin (2,8-dioxa-1-ethyl-3,5,7-trimethylbicyclo[3.2.1]octane) (Figure 1), which was the most abundant of the volatiles. The absolute stereochemistry of the natural sordidin was later determined (Mori *et al.,* 1996; Fletcher *et al.,* 1997). Ndiege *et al.* (1996) and Jayamaran *et al.* (1997) developed a large-scale synthesis of racemic sordidin that made field-testing possible. It was attractive to both males and females, confirming its function as an aggregation pheromone. This has been synthesized to provide cosmolure pheromones that together with traps are commercially available from ChemTica International in Costa Rica.

The use of pheromone lures (Cosmolure) for trapping *C. sordidus* has been reported in Costa Rica as a promising option (Alpizar *et al.,* 1999; Oehlschlager *et al.,* 2000). Trials of mass trapping in

plantations of banana and plantains with pheromone traps on plots of 1-250 ha in size, and traps at a density of four per ha were effective in reducing weevil damage to banana corm by over 60% and bunch weight was increased by 20% (Alpizar *et al.,* 1999; Oehlschlager *et al.,* 2000). Alpizar *et al.,* (1999) proposed that trap density could be reduced once weevil populations and damage have been reduced to low levels. In Costa Rica, trap interference starts to decrease at a separation of 20 m (C. Oehlschlager, pers. commun.), and the effective radius was estimated to vary from 5 to 15 m. Consequently, a trap density of four traps/ha and moving them every month at regular space intervals was found effective to bring *C. sordidus* populations to low levels in the banana plantation in 4-5 months (Alpizar *et al.,* 1999; Oehlschlager *et al.,* 2000). Preliminary studies conducted in Uganda showed pheromones to be 18 times more attractive to *C. sordidus* than pseudostem traps (Tinzaara *et al.,* 2000).

In laboratory and field studies, male produced aggregation pheromones for some coleopteran insects are ineffective unless combined with host plant volatiles (Oehlschlager *et al.,* 1992; Giblin-Davis *et al.,* 1996a). The weevil appears to show a similar trend of synergistic combinations of species-specific pheromone and host plant volatiles (Ndiege *et al.,* 1996; Jayaraman *et al.,* 1997).

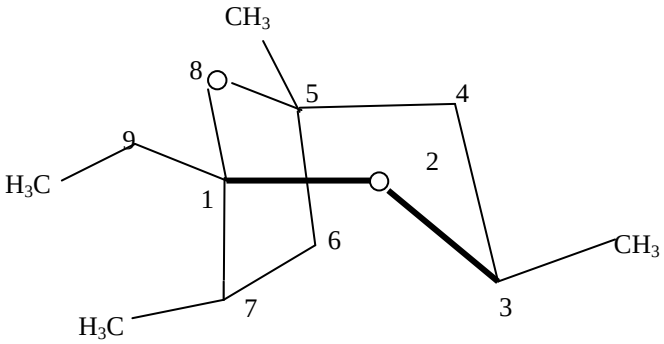
Mass trapping using infochemicals especially pheromones is particularly promising for the control of *C. sordidus* since the weevils crawl and do not disperse, both sexes are attracted, and their reproductive capacity is low (Alpizar *et al.,* 1999). Use of pheromones may provide a weevil control option that is less labour intensive compared to the use of pseudostem traps although costs are likely to increase as pheromones will require importation, distribution and storage. The development of the infochemical-based trapping system could also be used as a means of disseminating entomopathogens with baited traps which allow both entry and exit of weevils. Further research is necessary to develop these ideas

### 4.2 Kairomone

Cuille (1950) first suggested that plant kairomones attract the banana weevil to the host plants. Volatiles from the susceptible AAA cooking banana attracted more weevils than the AB resistant desert types (Budenberg *et al.,* 1993a). Seshu Reddy *et al.* (1993) showed that baits of cooking type bananas are more attractive to the weevil than those made of desert type bananas. Decaying banana material is more attractive than fresh material (Budenberg *et al.,* 1993a) although it is reported that fresh pseudostems are more attractive than fermented material (Hord & Flippin, 1956; Delattre, 1980). *Cosmopolites sordidus* seems to prefer corm odours from plants with fruits to corm odours from young or adult plants, or from adult plants with flowers (Cerda *et al.,* 1996). Preliminary studies conducted at ICIPE in Kenya indicate

that use of kairomone-based trapping systems with processed and buried banana materials attract weevils (S. Lux, pers. commun.). This trapping system might be integrated with entomopathogens.

The potential of using banana kairomones, especially fermented tissues, as synergists to synthetic pheromones has not been well exploited, although there is information indicating that banana plant volatiles could be used to enhance the attractiveness of synthetic pheromones (Ndiege, 1996; Jayaraman *et al.,* 1997). Further research is needed on host plant odours to optimise the use of kairomones in an infochemical-based trapping system for *C. sordidus* control.




(1S,3R,5R,7S)-1

Figure 1. Configuration of sordidin, a major component of the pheromone cosmolure+

### 4.3 Infochemicals and biological control of *C. sordidus*

Studies regarding the effect of infochemicals on the behaviour of predators of *C. sordidus* are currently not available. However, ant and non-ant predators for *C. sordidus* have been reported from Kenya (Koppenhofer *et al.,* 1992), Uganda (Tinzaara *et al.,* 1999, Abera, 2004.), and Cuba (Roche & Abreu, 1983; Bendicho, 1987). These predators were mostly found associated with weevil environments in rotten pseudostem tissues and in larval tunnels (Koppenhofer *et al.,* 1992; Tinzaara *et al.,* 1999). Attraction of predators by the volatiles from the plant-weevil complex has not been investigated yet. It is possibe that these predators are attracted to the weevil environments by volatiles produced by the host plant, the weevil or the weevil-plant complex. Although synthetic pheromones (cosmolure+) are known to be specific to *C. sordidus*, their effect on the behaviour of predators has not been investigated. Information on how infochemicals influence the behaviour of the predators may increase their chance of being used in the field as biological control agents.

The potential of biological control of *C. sordidus* using indigenous entomopathogenic fungi has been recently investigated (Pena *et al.,* 1995; Nankinga & Ogenga-Latigo, 1996; Nankinga *et al.,* 1996;

Nankinga, 1999; Schoeman & Schoeman, 1999; Nankinga & Moore, 2000). *Beauveria bassiana* showed a great potential for effective control of *C. sordidus* (Pena *et al.,* 1995; Nankinga *et al.,* 1996). One of the limiting factors is the lack of an effective delivery technique to the target insects (Nankinga, 1999). The potential of weevils to be used in transferring pathogens from infested to uninfested weevils was demonstrated by Schoeman and Schoeman (1999). Dissemination of *B. bassiana* using pseudostem traps was tested but found ineffective (Nankinga, 1999). The development of a pheromone lure could be used as a means for aggregating weevils to disseminate the entomopathogens. Use of pheromone lures as successful devices for dissemination of *B. bassiana* have been reported effective for other beetle species (Vega *et al.,* 1995; Klein & Lacey, 1999). An above ground ramp trap that allows both entry and exit of weevils (Alpizar *et al.,* 1999, Oehlschlager *et al.,* 2000; Tinzaara *et al.,* 2000) could be used in this regard.

# 5 RESEARCH NEEDS

Studies available indicate that there is a potential for using infochemicals to control *C. sordidus* (Alpizar *et al.,* 1999; Oehlschlager *et al.,* 2000; Tinzaara *et al*., 2000). Further information is needed to develop a cost-effective infochemical-based trapping method to control *C. sordidus*. Information is lacking on how infochemicals relate to weevil behaviour and environmental conditions. Other questions to be addressed are: does adult removal using infochemicals have an impact on weevil population dynamics and damage; and can infochemicals be integrated with other methods such as biological control?

The efficiency of the pheromone traps may be influenced, among other factors, by the physiological status of the pest such as reproductive maturation and the mating status (Borden 1977; Jansson *et al.,* 1991). Although pheromone lures are known to attract female and male weevils equally (Alpizar *et al.,* 1999; Tinzaara *et al.,* 2000), there is lack of information on the effect of the pest mating status and the age of *C. sordidus* on catches in pheromone-baited traps. We hypothesize that if mature and mated individuals do not respond to the pheromone lures, then trapping will cause limited suppression of the weevil population in the field.

Catches per trap are reduced when baited traps with synthetic pheromones are placed close to each other (trap interference) compared to widely spaced single traps (Schlyter, 1992; Byers, 1999). The effective trap radius has direct implications on the trap density used and the costs involved and therefore there is a need to get a more accurate value of the radius before pheromone traps can be used in mass trapping. Trap placement influences the capture rates for many insect pests (McNeil, 1991; Muirhead-Thompson, 1991). It is not clear whether the banana host plant has any influence on pheromone trap

catches although we hypothesise that traps placed close to mats capture more weevils than traps placed between mats. Information is therefore required on the attraction range of the pheromone trap, its effective radius and on trap location relative to the host plant.

High trap densities may be needed to obtain high levels of pest population suppression (Howse *et al.*, 1998). High trap densities may not be practical especially to resource-poor farmers as they render the method too costly. The trap density of four traps per hectare and moving the traps to new locations once a month was reported effective in reducing weevil populations to low levels within 4-5 months in Costa Rica (Alpizar *et al.*, 1999). The assumptions here are: (1) the weevil movements are limited and there is no reinvasion of areas that have already been trapped; (2) the traps remove a high proportion of weevils within the trap area in a month. The percentage population reduction using pheromone traps was however estimated using trap catch numbers and not overall population densities in the banana plantations. The effectiveness of a trap density of four traps per hectare needs to be evaluated under different agro-ecological conditions. Placing traps in the whole plantation without changing trap location monthly could be cost effective if time taken to reduce weevil populations is low. Information is needed on the proportion of weevils trapped per unit area so as to make decisions on when to change the location of the traps (in case of mobile traps) and when to stop trapping when the population has been reduced to low levels. The rate of reduction of weevil population and damage using different trap densities needs to be determined. The question that should be answered here is which trap density is most cost effective. The pheromone trap catches need to be correlated to environmental factors such as wind direction, rainfall and temperature. Such information would assist the farmer in managing the trap.

Deployment of pheromone traps alone may not result in pest population suppression when host material is abundant, providing good conditions for pest multiplication (Birch & Haynes, 1982; Vite & Baader, 1990; James *et al.*, 1996). Many crop residues compete with traps, thereby reducing the effect of the traps. The effect of banana residue management on pheromone trap catches is not known. Mulching influences weevil movements in banana plantations (C. Gold and G. Kagezi, unpubl. data), but how much this would affect pheromone trap catches is not known. Therefore, the influence of farm management practices such as mulching and sanitation on pheromone trap catches needs to be studied.

Herbivore damaged plants are known to produce volatiles that attract carnivores (Dicke, 1999a). Such volatiles may lead to increased carnivore density around the plant that may lead to reduced herbivore damage. Predators of *C. sordidus* are normally found in weevil infested rotten pseudostems and galleries (Koppenhofer *et al.*, 1992; Koppenhofer, 1993; Tinzaara *et al.*, 1999; Abera, 2004.). Are these predators attracted to such environment by volatiles produced by the host plant, weevil or the weevil-plant complex? The role of pheromones in influencing such an interaction has not been

investigated. The potential of using *B. bassiana* in Uganda to control *C. sordidus* control was promising (Nankinga, 1999; Nankinga & Moore, 2000). However, one of the limiting factors is the lack of an effective delivery technique (Nankinga, 1999; Roy & Pell, 2000). Use of pheromone lures to infest beetles with *B. bassiana* and to disseminate it to other beetles is possible (Klein & Lacey, 1999; Vega *et al.*, 2000), but needs to be studied for *C. sordidus.*

In conclusion, there is limited information on use of infochemicals for the control of *C. sordidus*. However, according to the available information on other coleopteran insects, there is a potential for development of an infochemical-based trapping system for the control of *C. sordidus.* There are however research gaps that need to be investigated to generate information for use in developing an infochemical-based trapping system for the control of *C. sordidus*. Research aimed at investigating some of the aspects mentioned in this review has been initiated in Uganda.

# 6 RESEARCH OBJECTIVES AND THESIS OUTLINE

## 6.1 Research objectives

The overall objective of this research project was to investigate whether an infochemical-based trapping system can be used to control the banana weevil, *Cosmopolites sordidus* under Ugandan conditions. The research focus was to elucidate the effects of weevil aggregation pheromone and host-plant kairomone on weevil trapping as related to weevil behaviour and environmental conditions. Secondly, the study investigated the impact of adult removal on weevil population dynamics and damage to the host plant. Finally, the potential was investigated of combining infochemical (pheromone and kairomone) use with biological control using predators and entomopathogens for the management of the banana weevil. Specific objectives of this research were:

1) To evaluate the relative attractivity of host plant volatiles and the aggregation pheromone to the banana weevil.
2) To evaluate factors that influence pheromone trap effectiveness in capturing the banana weevil.
3) To determine the effect of pheromone trap density on banana weevil population density and damage.
4) To evaluate olfactory responses of predators of the banana weevil to host plant volatiles and the aggregation pheromone.
5) To determine the effect of aggregation pheromone on the dissemination of an entomopathogenic fungus, *B. bassiana,* for the control of the banana weevil.

## 6.2 Thesis outline

In **chapter 2**, I evaluated bioassay set-ups for use in investigating orientation responses of the banana weevil to host plant volatiles and the synthetic aggregation pheromone. Results of weevil orientation responses in the different bioassay set-ups indicated that the banana weevil responds in an additive way to the combination of volatiles from fermented banana pseudostem and the synthetic pheromone. Detailed laboratory and field studies were then conducted to investigate whether host plant odours enhance the effect of the aggregation pheromone on attraction of the banana weevil (**chapter 3)**. The effect of the dose and age of the pseudostem tissue in enhancing the aggregation pheromone was evaluated.

The efficiency of the pheromone traps may be influenced by factors related to the physiological status of the insect, trap parameters, environmental factors and farm practices. An understanding of how these factors influence pheromone trap catches would assist in trap deployment for the control of the banana weevil. **Chapter 4** describes studies on the effect of sex, age, the female mating status and density of the banana weevil on response to the aggregation pheromone. The effect of trap parameters (e.g trap placement, trap type and trap radius) on pheromone trap catches are investigated in **chapter 5**. This chapter also describes studies on the relationship between pheromone trap catches and environmental factors such as rainfall, relative humidity, temperature and wind speed.

Mulching is commonly practiced by farmers in Uganda as a means to conserve soil moisture and reduce soil erosion. Studies to test the hypothesis that mulching may impede dissemination of the pheromone through the field leading to reduced trap catches are described in **chapter 6**. My studies determined the effect of mulching levels on numbers and sex of weevils captured in pheromone-baited traps. The distances moved by weevils relative to the pheromone-baited traps in mulched and unmulched areas have been determined.

The success of the pheromone trapping system depends on whether it can suppress the pest population and the subsequent damage to the crop. To assess this, I conducted experiments described in **Chapter 7**. In Costa Rica, a trap density of 4 traps per hectare was recommended to be effective in reducing banana weevil populations to low levels after 4-5 months. The effectiveness of this trap density (4 traps per ha) was questionable in Ugandan conditions. The effect of increasing pheromone trap density on trap efficiency was therefore investigated.

In **Chapter 8**, I investigate olfactory responses of the predators of the banana weevil to host plant volatiles and the aggregation pheromone. The predators such as *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) and *Pheidole megacephala* (Hymenoptera: Formicidae) are mostly found

in environments harbouring weevils, such as pseudostem traps and rotten pseudostem tissue. I investigated whether these two predator species are attracted to such environments by volatiles produced by the host plant, weevil or the weevil-plant complex.

In **chapter 9**, I investigate the potential of using the aggregation pheromone to enhance dissemination of the fungal pathogen *Beauveria bassiana* to control *C. sordidus*. Candidate fungal pathogens have been identified for use in the pest management strategy of the banana weevil but their use has been limited by the lack of an effective delivery system. The pheromone might be used to aggregate weevils at field delivery sites of the pathogen. The successful use of pheromone lures to enhance the dissemination of *B. bassiana* would require that weevils can enter a trap, be exposed to the fungus and leave the trap. I studied whether dissemination would be greater when the pathogen is placed inside the pheromone trap, around the trap or around the trap and on a few adjacent mats.

Finally, the data of this research project are integrated in **chapter 10** to present an overview on infochemicals and the management of the banana weevil. I discuss the role of the aggregation pheromone in mass trapping, synergistic effects of host plant odours to the aggregation pheromone, integration of the pheromone with biological control for the management of the banana weevil. This project provides ample experimental evidence to further develop the application of the synthetic aggregation pheromone to control the banana weevil in Ugandan banana production by small scale farmers.

**Acknowledgement**

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Chapter **2**

# Different bioassays for investigating orientation responses of the banana weevil, *Cosmopolites sordidus*, show additive effects of host plant volatiles and the synthetic pheromone


## Abstract

Three different bioassay methods to investigate orientation behaviour of the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) to host plant volatiles and the synthetic pheromone (cosmolure+) were compared. A locomotion compensator was used to record walking tracks in response to three odour sources separately. The data show that *C. sordidus* uses odour-conditioned anemotaxis in its orientation to the odour sources tested. Of the two olfactometers tested, a dual port olfactometer using a continuous airflow, showed stronger discrimination by *C. sordidus* to the different odours compared with a double pitfall olfactometer. The results of all three bioassays indicate that *C. sordidus* respond in an additive way to the combination of fermentation plant volatiles and the synthetic pheromone.



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# 1 INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is considered a major pest in most banana growing regions of the world (Stover & Simmonds, 1987), particularly for cooking bananas and plantains (Sikora *et al*., 1989; Kiggundu, 2000). The weevil causes yield reductions through impeding sucker establishment in newly planted crops (McIntyre *et al*., 2001), plant loss and bunch size reduction (Rukazambuga *et al*., 1998) and mat die-out (Gold *et al*., unpubl. data). This pest can cause yield losses up to 100% (Sengooba, 1986; Koppenhofer *et al*., 1994) if not controlled.

*Cosmopolites sordidus* populations may be monitored in the field (Mitchell, 1978) and controlled (Gold *et al*., 2002) using split pseudostem or rhizome traps. Cuille (1950) and Budenberg *et al*. (1993a) reported attraction of the weevil by volatiles from banana pseudostem and rhizome (kairomones). Fresh and rotting materials have been reported to be equally attractive (Budenberg *et al*., 1993a) although other reports indicate that fresh stems are more attractive than fermenting material (Hord & Flippin, 1956). *Cosmopolites sordidus* prefers corm odours from plants with fruits to corm odours from young adult plants with or without flowers (Cerda *et al*., 1996). Kairomones can also be used to enhance the attractiveness of the pheromone (Budenberg *et al*., 1993b; Oehlschlager *et al*., 2000) but this has not been investigated in any detail.

Evidence of male-produced aggregation pheromone to which both females and males of *C. sordidus* respond was first provided by Budenberg *et al*. (1993b). Beauhaire *et al*. (1995) detected six male-specific compounds eliciting electroantennogram (EAG) activity in volatile collections. They identified and synthesised sordidin that was the most abundant of the volatiles. The absolute stereochemistry of the natural sordidin (1S,3R,5R,7S)-(+)-1-ethyl-3,5,7-trimethyl-2,8-dioxabicyclo[3.2.1]octane) was later determined (Mori *et al*., 1996; Fletcher *et al*., 1997). Ndiege *et al*. (1996) and Jayamaran *et al*. (1997) developed a large-scale synthesis of racemic sordidin that made field-testing possible. It was attractive to both males and females, confirming its function as an aggregation pheromone. This has been formulated at ChemTica International in Costa Rica to provide so-called Cosmolure+ pheromone. Use of Cosmolure+ for trapping weevils has been reported in Costa Rica as a promising option (Alpizar *et al*., 1999; Oehlschlager *et al*., 2000). Preliminary studies conducted in Uganda showed the pheromone to be up to 18 times more attractive to *C. sordidus* than pseudostem traps (Tinzaara *et al*., 2000).

*Cosmopolites sordidus* orientation response to kairomones and aggregation pheromone has been investigated using a variety of bioassay set-ups, such as olfactometers (Budenberg *et al*.,

1993a, b; Treverrow, 1994; Cerda *et al*., 1999; Ndiege *et al*., 1996), choice chambers (Bakyalire & Ogenga-Latigo, 1994) and a locomotion compensator (Mgenzi, 1999). As no systematic comparison of bioassay set-ups has been made, it remains unclear how bioassay set-up affects weevil responses to infochemicals.

We compared different bioassay set-ups to investigate orientation responses of *C. sordidus* to host plant volatiles and the synthetic pheromone used either singly or in combination. We addressed the following questions: 1. Do fermenting banana odours significantly increase attraction of *C. sordidus* to pheromone? 2. How attractive are fermenting banana odours to *C. sordidus* compared to pheromone?

# 2 MATERIALS AND METHODS

## 2.1 Site

The work was conducted at the Laboratory of Entomology, Wageningen University, in a dark room at 21-25 $^{o}$C and 60-70% relative humidity. During the locomotion compensator experiments, the red light source (red fluorescent tube, Philips "36W/15 red", Philips, Eindhoven, The Netherlands) was only switched on after the weevil had walked for 900 seconds on the sphere, to assist in changing treatments and to place another weevil on the sphere. The red light source was always switched on during the olfactometer experiments.

## 2.2 Insects

Experiments were conducted using weevils collected from Uganda three weeks prior to the first experiments. The insects were maintained in an incubator at 22 $^{o}$C and 70-80% relative humidity. They were fed on banana corm and pseudostem pieces that were secured from Uganda and kept in a refrigerator at 4 $^{o}$C until use. The photoperiod cycle (L12: D12) was shifted such that the scotophase was from 09.00 am to 21.00 pm. Weevils (without distinction by sex) were used in bioassays not earlier than 3 weeks after reversing the photoperiod cycle. Previous observations indicated that the sex of the weevil had no effect on response to host plant volatiles (Budenberg *et al*., 1993a) and to the synthetic pheromone (Alpizar *et al*., 1999; Tinzaara *et al*., 2000) and therefore weevils were not sexed in these experiments. Weevils used for experiments were placed individually in clean plastic boxes (15 x 10 x 8 cm) without food for 12 hours before the bioassay. For the locomotion compensator tests, a piece of glass-pearl coated reflective material was glued onto the elytra of the weevil at the beginning of the starvation period.

## 2.2 Treatments

Three different odour sources were used in the study: (i) fermented pseudostem tissue, (ii) pheromone, (iii) pheromone + fermented pseudostem tissue. Clean air (iv) was offered as control.

## 2.3 Fermented pseudostem tissue.

The experiments were conducted using banana pseudostem material of East African highland banana plants (*Musa* spp., AAA-EA group), cultivar Mbwazirume. Banana pseudostems were collected from freshly harvested plants in Uganda, packed in a cardboard box and shipped to the Netherlands, Wageningen University. Shipment took five days and some of the material had already begun to ferment upon arrival in Netherlands. The material was chopped into pieces. To get fermenting materials, chopped pseudostem pieces were kept at 21-25 $^{o}$C in plastic containers, producing full fermentation within 7 days. The material was then stored in a freezer and thawed when needed in bioassays. The plant odour source for both the locomotion compensator and olfactometer bioassays was 50g of fermented pseudostem pieces, used singly or in combination with the synthetic pheromone.

## 2.4 Pheromone

The pheromone lure obtained from ChemTica International in Costa Rica consisted of a polyethylene pack containing 90 mg of pheromone (cosmulure+) released at a rate of 3 mg/day (A.C. Oehlschlager, pers. comm.). The cosmolure+ in the pack was pure sordidin with no other additives other than the dye for visualization (A.C. Oehlschlager, pers. comm.). For storage, packs were tightly sealed and kept in a dark cupboard at 21-25 $^{o}$C. After a container with pheromone had been opened, the pheromone packs were stored in the freezer at –5 $^{o}$C. The lures were used within 30 minutes after removal from the freezer.

## 2.5 Bioassay set-ups

Comparing weevil responses to different odour sources was done using a locomotion compensator, a double port olfactometer and a double pitfall olfactometer.

### *2.5.1 Locomotion compensator*

The response of *C. sordidus* to plant volatiles and synthetic pheromone singly or in combination was studied using a combination of a locomotion compensator and a wind tunnel (for detailed

description see Thiery & Visser, 1986). The method involved attaching a piece of reflective material (functioning as a mirror) on the elytra of an insect. Each insect was placed on top of a 50 cm diameter sphere and observed by a detector. The detector used the reflection of a beam of infrared light that was projected onto the insect from a light source above, whereby changes in the position of the insect were detected. As the insect started walking, the change in its position was detected, which initiates two motors to rotate the sphere in the opposite direction with the same walking speed as the insect. Thus, the insect stays on the same position on top of the sphere while walking. Two pulse generators in contact with the sphere detect the rotations of the sphere, and pulses were recorded and analysed by a microprocessor. The recordings of the insect's movements were stored in a computer for calculation of track characteristics.

Locomotory responses of *C. sordidus* were evaluated using the odour sources as indicated above. The fermented pseudostem tissue (50g) was placed in a 10 cm diameter petri dish before placing them in a wind tunnel. The pheromone lure was hung on a tripod stand and whenever being used in combination with pseudostem tissue, it would be hung in such a way that it touched the surface of the pseudostem tissue. An individual weevil was placed on the sphere (2 m from the odour source) over which an air stream with a speed of 5 cm/s carrying the respective odours was blown from the wind tunnel. Wind speed was measured using an anemometer (Therm anemometer 642, Lamprecht, Germany). Each weevil was exposed to an air stream for 900 seconds. Each weevil was exposed to each of the odour sources, randomising the sequence of odour sources over the experimental days. This testing procedure allowed each weevil to have at least one hour of rest between each stimulus. Forty-eight weevils were tested over a period of 10 days. An exhaust fan continuously removed air after it had streamed over the sphere.

The following four track parameters were used to quantify the weevil's behaviour: (i) walking speed (mm/s); (ii) straightness of walking, the quotient of vector length and total track length (range from 0 to 1); (iii) upwind length (mm), the net distance from origin towards the odour source along a straight line after 900 seconds; and (iv) upwind fixation, the quotient of upwind length and total track length (range from –1 to +1).

*2.5.2 Double port olfactometer*

A double port olfactometer as described by Cerda *et al*. (1996) was used for evaluating weevil response to fermented banana plant volatiles and the synthetic pheromone. The apparatus consisted of a plastic tray of 45 x 30 x 14 cm with a lid.

The same odour sources as those used in the locomotion compensator bioassay were used except that both port and pitfall olfactometers allowed exposing a weevil to two odour sources at the same time. The odours compared in this set-up were (i) fermented pseudostem tissue versus clean air, (ii) pheromone versus clean air, (iii) pheromone versus fermented pseudostem tissue, (iv) pheromone versus pheromone + fermented pseudostem tissue, and (v) fermented pseudostem tissue versus pheromone + fermented pseudostem tissue.

Charcoal-filtered air was pumped through jars containing odour sources and into a choice arena. The arena had two inlet ports for the two different odour sources on one end (0.5 cm diameter tubes) and a single exhaust port on the other end. Air containing each odour was led separately to either of the inlet ports. The airflow rate (measured with an airflow-meter) from each odour source into the choice arena was 1 l/min and air was exhausted at the opposite side at a rate of 2 l/min. Weevils were released individually at the down-wind end facing the upwind direction. A maximum of ten minutes was allowed for each weevil to choose between the two odour sources. A weevil was considered to have made a choice on reaching one of the odour ports or if after ten minutes the weevil was within 2 cm from the odour source port. Arms containing odour sources were alternated after testing five individuals to correct for any unforeseen asymmetry in the set-up. Each weevil was used only once and after testing ten individuals, the apparatus was washed with ethanol and air-dried before subsequent use.

*2.5.3 Double pitfall olfactometer*

A double pitfall olfactometer, modified from the set-up used by Budenberg *et al*. (1993b) and Treverrow (1994) was used for evaluation of weevil response to different odour sources. It consisted of a plastic tray (45 x 30 x 14 cm) in which two 200 ml flasks containing different odour sources were placed. A plastic plate sealed the container, with two 4-cm-diameter holes in the middle and 10 cm from each end of the centre line of the long axis. The two flasks protruded through these holes but the rims of the flasks were aligned with the cut surface of the plastic plate. A sheet of paper covering the plastic plate was replaced after each comparison.

The odour sets compared in this set-up were the same as those used in the double port olfactometer except that the fermented pseudostem or pheromone versus clean air were not used because odour sources were limiting.

One weevil was placed in the centre of the arena at a time. After 10 minutes, the position of the weevil (i. e inside jar, within 2 cm from the jar rim and more than 2 cm from the jar rim) was recorded. The positions of the odour sources were alternated after every five tests to compensate for

any unforeseen asymmetry of the set-up. Fresh odour sources were used for every comparison set. The apparatus was washed with alcohol and air-dried before any subsequent use. Each weevil was used only once. Ten individuals were tested for response per day per odour set. At least three odour source comparisons were made per day and each comparison was repeated on four days. Weevils were considered to have made no choice if found beyond 2 cm from the rim of the flask.

### 2.6 Statistical analysis

The walking track parameters of the weevil on the locomotion compensator were analysed using the Wilcoxon's matched pair signed rank test of SPSS for windows release 10 (SPSS Inc., Chicago, 1999), followed by Bonferroni correction for multiple comparisons. Results of the olfactometric bioassays were analysed using the binomial test. Non-responding weevils were excluded from the analysis of the olfactometer tests.

# 3 RESULTS

### *3.1 Locomotion compensator bioassay*

The results of the track parameters recorded with the locomotion compensator for *C. sordidus* indicate that the weevils responded significantly ($P < 0.05$) stronger to the combination of pheromone and fermented pseudostem than to pheromone or pseudostems alone (Table 1). Upwind fixation was found to be the most sensitive parameter to compare the response of *C. sordidus* to different odours. The data on all walking track parameters showed that weevil responses to fermented plant odours or the pheromone were not significantly different (Table 1). Mean straightness was equal ($P > 0.05$) for all treatments tested. Compared to the clean air control, the locomotory response of *C. sordidus* to the pheromone or pseudostem either singly or in combination was generally characterised by an increased degree of upwind fixation (Figure 1), longer upwind length and higher walking speed (Table 1).

### *3.2 Double port olfactometer*

*Cosmopolites sordidus* moved more frequently to the port releasing the synthetic pheromone than to that releasing the odour of fermented pseudostem in a double port olfactometer (Table 2). The weevil responded significantly ($P< 0.05$) more to the combination of pheromone and pseudostem than to the pseudostem alone. The comparison of *C. sordidus* response to the combination of the

pheromone and pseudostem with the pheromone alone did not show a significant difference. The mean number of non-responding weevils was 33% using this bioassay set-up (Table 2).

Table 1: Parameters (mean ± S.E) of walking tracks on a locomotion compensator of *C. sordidus* in response to different odour sources.

| Treatment | Upwind length (mm) | Total length (mm) | Walking speed (mm/s) | Straightness | Upwind fixation |
|---|---|---|---|---|---|
| Clean air | 652 ± 308a | 5814 ± 352ab | 6.5 ± 0.4a | 0.83 ± 0.01a | 0.08± 0.05a |
| Fermented pseudostem | 2169 ± 337b | 5813 ± 325a | 6.5 ± 0.4a | 0.82 ± 0.02a | 0.31± 0.05b |
| Pheromone | 1865 ± 322b | 5805 ± 243a | 6.4 ± 0.3a | 0.82 ± 0.02a | 0.34± 0.05b |
| Pheromone + Fermented pseudostem | 3420 ± 273c | 6608 ± 263b | 7.4 ± 0.3b | 0.82 ± 0.02a | 0.51± 0.04c |

Means followed by a different letter in a column are significantly different (P< 0.05), Wilcoxon's matched pair signed rank test followed with Bonferroni correction of $\alpha = 0.05/6 = 0.008$. Total number of individual weevils exposed to odour sources (n) =48

Table 2: Response of *C. sordidus* to odour sources from fermented banana pseudostem tissue and synthetic pheromone in a double port olfactometer

| Comparison of volatile source A versus B | Source A | Source B | Non-responders | % non-responders |
|---|---|---|---|---|
| Pseudostem versus clean air | 17* | 8 | 15 | 38 |
| Pheromone versus clean air | 18** | 6 | 16 | 40 |
| Pheromone versus pseudostem | 20* | 10 | 10 | 25 |
| Pheromone + pseudostem versus pseudostem | 18* | 9 | 13 | 33 |
| Pheromone + pseudostem versus pheromone | 17 | 12 | 11 | 27 |

*P<0.05 **P<0.01 for a binomial test between two odour sources, n =40 (individuals tested per odour set).

### *3.4 Double pitfall olfactometer*

*Cosmopolites sordidus* did not show a significant difference between the synthetic pheromone and fermented pseudostem odour (Table 3). There was a significantly (P<0.05) higher response of *C. sordidus* to the combination of pheromone and pseudostem than to either the pheromone or volatiles from the pseudostem alone. The mean number of non-responding weevils was 45% using this bioassay set-up (Table 3).

Table 3: Response of *C. sordidus* to odour sources from fermented banana pseudostem tissue and synthetic pheromone in a double pitfall olfactometer.

| Comparison of volatile source A versus B | Source A | Source B | Non-responders | % non-responders |
|---|---|---|---|---|
| Pheromone versus pseudostem | 12 | 9 | 19 | 48 |
| Pheromone + pseudostem versus pseudostem | 16* | 7 | 17 | 43 |
| Pheromone + pseudostem versus pheromone | 17* | 6 | 17 | 43 |

* $P < 0.05$ for a binomial test between two odour sources, n = 40 (individuals tested per odour set)

# 4 DISCUSSION

Kairomones from fermenting host plant tissue that enhance attractiveness of male-produced aggregation pheromone have been reported for various weevils in the subfamily Rhynchophorinae (Budenberg *et al*., 1993b; Weissling *et al*., 1994; Giblin-Davis *et al*., 1996a). The results of our study using a locomotion compensator demonstrated that *C. sordidus* have a significantly stronger response to the combination of the synthetic pheromone and fermented pseudostem odours than to pheromone alone or fermented pseudostem alone. It was unexpected that on the locomotion compensator the synthetic pheromone and fermented pseudostem odours were equally attractive to *C. sordidus*. Previous studies conducted in the laboratory and in the field (Jayaraman *et al*., 1997; Alpizar *et al*., 1999; Tinzaara *et al*., 2000) showed the pheromone to be more attractive than the fresh pseudostem tissue. This discrepancy might be explained by the no-choice situation employed during the locomotion compensator experiments. Secondly, some reports indicate that fermentation of host plant materials generates a strong increase in the amount and variety of the volatiles emitted (Rochat *et al*., 2000). Thirdly, the results could have been affected by the concentrations of fermented pseudostem tissue and the synthetic pheromone that might have been different between our study and those reported previously. Odour release rate, distance to the odour source and air speed all differ between studies. However, one could argue that separated odour fields offered simultaneously as in dual port olfactometers represent an artificial situation and that the no-choice situation is more representative for normal foraging conditions. We plan to test several pheromone-to-kairomone ratios in subsequent tests.

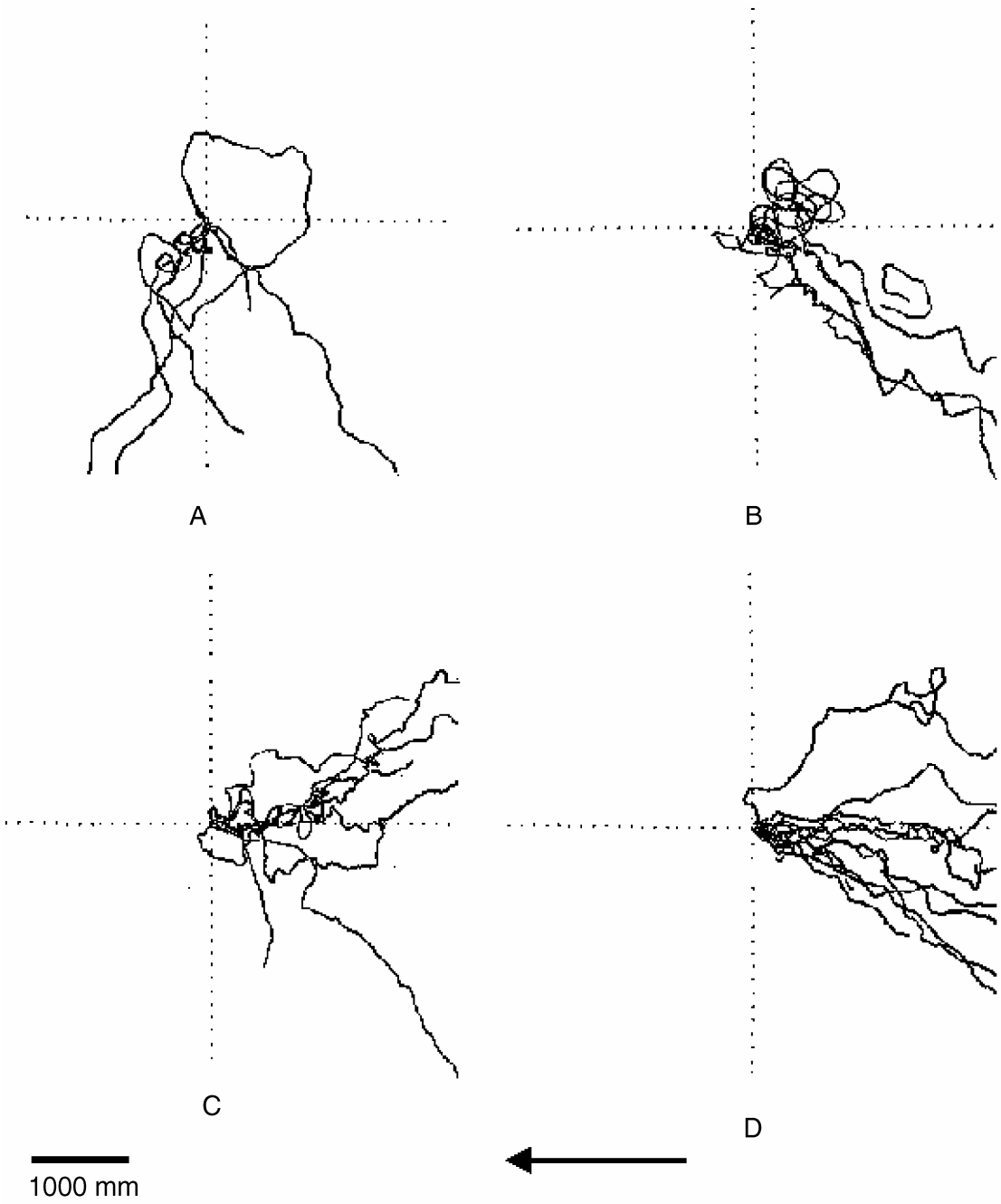


Figure 1. Representative walking tracks of *C. sordidus* on a locomotion compensator in response to: (A) Clean air, (B) fermented pseudostem tissue, (C) pheromone, and (D) pheromone plus fermented pseudostem tissue. Tracks of the same four individuals exposed to each of the odour sources mentioned were randomly selected from the collection of walking tracks recorded during the study. An arrow indicates direction of the air stream over the servosphere.

Our results of the walking track parameters of the banana weevil demonstrate that the response of this weevil to different odours can be studied using a locomotion compensator. Similar to what was reported for other insects such as the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Bolter *et al*., 1997) and the predatory bug *Perillus bioculatus* (Fabr.) (van Loon *et al*., 2000), upwind fixation reflected the strength of orientation most sensitively. The results of the double port olfactometer, contrary to the results on the locomotion compensator, suggest that the pheromone was more attractive than fermented pseudostem odours. The results of all the tests using the locomotion compensator and the two olfactometers demonstrate that responses to fermented plant volatiles and to synthetic pheromone are additive. Although the data of the double port olfactometer on enhancement of the effect of the pheromone by adding fermented volatiles is not significant, it showed the same trend as for the locomotion compensator and the double pitfall olfactometer.

Still-air pitfall olfactometers have been used to study weevil responses to different odours (Budenberg *et al*., 1993a; Cerda *et al*., 1999). In our bioassays, the mean number of non-responding weevils was greater when using the double pitfall olfactometer than the double port olfactometer (Tables 2 & 3). The time that was given to weevils to a make a choice was possibly not sufficient. Treverrow (1994) reported that up to 24 hours are needed for most weevils to respond in a double pitfall olfactometer. For the double port experiment where there is a continuous flow of air, an increase in observation time may reduce the number of non-responders. However, this may not be possible with the pitfall olfactometer since there is no exhaust extraction of volatile from the apparatus. The double port olfactometer allowed a continuous flow of air that could have led to higher responsiveness than in the pitfall olfactometer. There is a possibility for odours to mix leading to confusion in weevil response in the still-air pitfall olfactometer.

Our results generally indicate that there is a potential of combining the synthetic pheromone with fermented plant volatiles to enhance trap efficacy, as in the case of several Curculionidae species including the palmetto weevil, *Rhynchophorus cruentatus* Fabricius (Giblin-Davis *et al*., 1994a) and the western Indian weevil, *Metamasius hemipterus* sericus (Olivier) (Giblin-Davis *et al*., 1996b), where a combination of fermented host plant tissue and synthetic pheromone were deployed to create successful trapping systems. Further studies should be conducted to demonstrate the efficacy of the combination of fermented banana tissue and the synthetic pheromone under field conditions. Dose-response and longevity studies of both fermented and fresh plant extracts need to be conducted to optimise deployment of these volatiles in combination with the synthetic pheromone.

We conclude that bioassay set-up is an important factor to take into consideration when evaluating *C. sordidus* orientation response to volatile infochemicals. Steinberg *et al*. (1992) made a similar conclusion in a study in which three bioassay set-ups (i.e Y-tube olfactometer, wind tunnel and glasshouse chamber) were compared for investigating the response of a braconid parasitoid, *Cotesia glomerata* (L.), to volatile infochemicals. The locomotion compensator set-up is to be recommended as a sensitive device for evaluating weevil response to infochemicals in a no-choice situation. Both the dual port and pitfall olfactometer bioassay set-ups could be used for evaluating *C. sordidus* response to infochemicals in a dual choice situation. This study provides baseline data for our future in depth investigations of responses to mixtures of kairomones and the synthetic pheromone.

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Chapter **3**

# Host plant volatiles enhance the responses of the adult banana weevil, *Cosmopolites sordidus*, to the synthetic aggregation pheromone

*W. Tinzaara, C.S. Gold, M. Dicke, A. van Huis & P.E. Ragama*

## Abstract

Attraction of adult *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) to volatiles from banana pseudostem tissue and the synthetic pheromone Cosmolure+ presented singly or in combination was studied in the laboratory and in the field. Olfactometric studies in the laboratory showed that 50 g of fermented banana pseudostem tissue (cultivar Mbwazirume, *Musa* spp, AAA-EA group) was as attractive as pheromone (released at 3 mg/day) but more attractive than 50 g of fresh pseudostem tissue. Volatiles from pseudostem tissue had an additive effect on attraction of weevils to the pheromone in the laboratory but the effect was not significant in the field. Field trials showed that attractivity of weevils was positively correlated with the amount (dose) of fermented tissue added to the pheromone although a significant dose-effect was shown only by field experiments. There was a decrease in number of weevils caught with increasing age of pseudostem tissue that was used to enhance the pheromone for *C. sordidus* attraction. Both results of laboratory and field trials indicated an increased weevil attraction with an increase in pheromone release rate. The results generally indicate that fresh or fermented pseudostem tissue may contribute to the enhancement of pheromones but the effect was not large enough to warrant their deployment for the optimisation of the infochemical-based trapping system for the management of *C. sordidus.*

# 1 INTRODUCTION

The banana weevil, *Cosmopolites sordidus* is an important pest of highland banana in Uganda. The biology and pest status of *C. sordidus* have been reviewed by Gold *et al*. (2001). The adults are free-living, though most commonly associated with banana mats and crop residues (Gold *et al*., 2004). They crawl short distances and may be sedentary for extended periods (Gold *et al*., 1999a). Eggs are placed in the leaf sheaths and corm at the base of the banana mat (Abera *et al*. 2000). The larvae tunnel in the corm, damaging the vascular system and weakening the stability of the plant. Yield losses of up to 100% have been recorded (Sengooba, 1986). In Uganda, *C. sordidus* has been an important factor in the decline and disappearance of highland banana in the central region (Gold *et al*., 1999c).

The use of pheromone- and kairomone-based trapping systems has been suggested as a means to control *C. sordidus* (Budenberg *et al.,* 1993b; Jayaraman *et al.,* 1997; Tinzaara *et al*., 2002a). Budenberg *et al*. (1993b) first reported evidence for an aggregation pheromone released by male *C. sordidus.* Beauhaire *et al*. (1995) isolated a fraction of the major component of the pheromone, confirmed its bioactivity, named it sordidin and elucidated its structure. Mori *et al.* (1996) identified the natural configuration and enantiomeric identity of sordidin as (1S,3R,5R,7S)-(+)-1-ethyl-3,5,7-trimethyl-2,8-dioxabicyclo[3.2.1]octane. Jayaraman *et al.* (1997) synthesised four isomers (exo-B, endo-B, endo-A, exo-A) of sordidin. All four isomers occur naturally in a ratio of 1:4:4:44. Ndiege *et al.* (1996) and Jayaraman *et al*. (1997) found that a mixture of them was more attractive than the individual isomers. Currently, the lures (containing a mixture of the four sordidin isomers) are synthesized in Costa Rica by Chemtica International and sold under the trade name Cosmolure+. The pheromone attracts both males and females but the sex ratio (males: females) has been observed to range from 1:1 to 1:3 (C.A. Oehlschlager, pers. Commun.).

Alpizar *et al*. (1999) reported that pheromone-baited pitfall traps attracted 5-10 times more weevils compared to unbaited sandwich traps. Jayaraman *et al*. (1997) suggested that pheromone-enhanced mass trapping could lead to successful control of C. *sordidus*. Trials of mass trapping *C. sordidus* with Cosmolure+ baited pitfall traps (4 traps/ha, spaced at 20 m and moved to untrapped adjacent locations monthly) on plots of bananas and plantains ranging in size from 1-250 ha were reported to be effective in reducing weevil corm damage by over 60% and increasing bunch weight by 20% over a 5-6 months trapping period in Costa Rica (Alpizar *et al.,* 1999). This suggests that the use of pheromone lures may provide a weevil control option that is more effective and less labour-intensive than the use of pseudostem traps although monetary costs are likely to increase.

In a number of laboratory and field studies, male-produced aggregation pheromones for some coleopteran insects have been ineffective unless combined with host plant volatiles (Oehlschlager *et al.,* 1993; Jaffe *et al.,* 1993; Giblin-Davis *et al.,* 1994a; Giblin-Davis *et al.,* 1996b; Reddy & Guerrero, 2004). Attraction of the palm weevil (*Rhynchophous palmarum*) by synthetic pheromone rhynchophorol was synergised by the addition of palm stem or sugar cane host material (Oehlschlager *et al*., 1993), or by host odour compounds such as ethyl acetate (Jaffe *et al.,* 1993). The amount of host plant material added to traps also matters. Oehlschlager *et al.* (1993) found that pheromone traps containing 30 pieces of sugar cane captured significantly more palm weevils than traps with 7 to 15 pieces. Attraction of male and female *Rhynchophorus cruentatus* to male pheromone 5-methyl-4-octanol (cruentol) was synergised by volatiles from host plant material, such as Sabal palm stem tissue (Giblin-Davis *et al.,* 1994a, 1996b). Giblin-Davis *et al.* (1996b) reported an increased response of *Metamasius hemipterus sericeus* with increased amounts of sugarcane quantity in traps up to some threshold level. Under field conditions, the response of the West Indian sugar cane borer, *M. hemipterus* to the aggregation pheromone was enhanced by fermented and fresh plant volatiles (Giblin-Davis *et al.,* 1994a).

Budenberg *et al.* (1993b) and Jayaraman *et al*. (1997) postulated that combinations of the species-specific pheromone and host plant volatiles may have synergistic effects on *C. sordidus*, although such effects have not been well studied. Our preliminary studies in the laboratory (Tinzaara *et al*., 2003) provided an indication that host plant volatiles may enhance the aggregation pheromone. There is, therefore, a need for detailed laboratory and field investigation into the role of additional volatiles for the development of an effective pheromone-based trapping system for the control of *C. sordidus*.

In laboratory bioassays, the response level of a range of insects has been positively correlated to pheromone dose (Coffelt & Burkholder, 1972; Hardee *et al.,* 1974, Howse *et al.,* 1998). If pheromone release rate is too low, a lure may be ineffective simply because a threshold for insect response is not met. This suggests that the release rate of a lure during the trapping period is an important concern. Hallett *et al.* (1999) reported an increased response of *R. ferrugineus* with an increase in pheromone dose. Pheromone release rate and interaction with host plant material acting as kairomone in attracting *C. sordidus* has not been investigated in detail. Information on these aspects will be needed for the development of an infochemical-based trapping system for the control of *C. sordidus*.

There are studies that revealed that the efficacy of plant tissues as additives to the pheromones varies with time (Weissling *et al.,* 1992; Oehlschlager *et al.,* 1993; Rochat *et al.,* 2000). A

sugarcane additive tissue was reported to be inactive after 15 days, the optimum being one week (Rochat *et al*., 2000). Use of sugar cane as an additive to the pheromone rhynchophorol significantly increased its effectiveness but attraction decreased after 3 days (Oehlschlager *et al*., 1993). The sugarcane was only effective for 1 to 2 weeks. The banana pseudostem tissue used in conventional trapping is known to decrease in attractiveness after 3 days (Bakyalire, 1992). The longevity of banana pseudostem tissue in enhancing pheromone-baited traps has not been investigated.

The main objective of this study was to establish if kairomones (pseudostem tissue) can contribute to the attraction of *C. sordidus* to the male-produced pheromone. Experiments were conducted to examine: (i) additive effects of pseudostem tissue to pheromone, (ii) effect of the amount (dose) of pseudostem tissue and pheromone, and (iii) the age of the pseudostem tissue in enhancing pheromones, for attraction of *C. sordidus.* Experiments were carried out under controlled conditions in an olfactometer in the laboratory as well as under field conditions.

# 2 MATERIALS AND METHODS

## 2.1 Site descriptions

Laboratory studies were conducted in a dark room (5 x 3m) at the IITA Sendusu Farm ($0^{o}$32'N, $32^{o}$35'E, 1260 metres above sea level (m.a.s.l.)) located 28 km northeast of Kampala, Uganda. The windows and the door of the room were tightly sealed with black polythene sheets. Ambient temperature in the laboratory ranged from 22 to 28 $^{o}$C. During the study period, the room had a red light that was switched on during all experiments to facilitate taking data without disturbing the test insects. Bioassays were conducted between 9.00 am and 5.00 pm. An electric fan (40 cm stand fan, Evernal®, 50W) was always on during bioassays to provide aeration.

Field studies to examine the attractivity of host plant volatiles and pheromone to *C. sordidus* were conducted at Sendusu Farm and the Kawanda Agricultural Research Institute (KARI) ($0^{o}$25'N, $32^{o}$51'E, 1190 m.a.s.l.) and adjacent farmers' fields in a site (Senge) 1 km to the north of KARI. Both sites have two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and daily mean temperature of 21$^{o}$C.

## 2.2 Pheromone and pseudostem volatiles

All pheromone lures used in these studies were obtained from ChemTica International in San Jose, Costa Rica. They were sealed in plastic and sent by courier (transit time < 1 week) and subsequently

stored in a freezer upon arrival until use. Each pheromone pack contained 90 mg of Cosmolure+ with a release rate of 3 mg/day (A.C. Oehlschlager, pers. comm.). They were used in their release device packs.

Pseudostem tissue of cultivar Mbwazirume (*Musa* spp. AAA-EA group) was used in the laboratory bioassays. Pseudostem pieces (approximately 1 x 1 x 1 cm) of freshly harvested pseudostem (not infested with *C. sordidus*) were used. Fresh Pseudostem tissue was harvested on the day of the experiment. To get fermented materials, chopped pseudostem pieces were kept at room temperature in 10 litre plastic containers for 7 days. The pseudostem material was weighed using a Mettler balance to obtain standard quantities to be used in the assays. Fifty g of banana pseudostem tissue were used for laboratory bioassays (except for the experiment testing effects of different quantities of the pseudostem tissue).

### 2.3 Insects

Adult *C. sordidus* used in bioassays were trapped from farmers' banana stands in Masaka District (1200-1300 m.a.s.l) using split banana pseudostem traps (Mitchell, 1978). The weevils were brought to the laboratory and maintained in 10 litre plastic buckets, each containing about 2 kg of fresh corm of the banana cultivar Mbwazirume. The corm pieces were replaced with fresh material every 4-5 days. The buckets were covered with perforated lids to allow aeration while preventing the insects from escaping. Weevils (unsexed) were kept in the dark room with the red light on for at least 15 h prior to being used. Preliminary observations indicated that keeping the weevils in the dark room increased their response in the bioassay. The use of a red light allowed us to work during daytime hours. Each weevil was used in a single assay and then discarded.

### 2.4 Double pitfall olfactometer

All laboratory bioassays were conducted using a double pitfall olfactometer (Figure 1), adapted from Phillips and Burkholder (1981). The apparatus consisted of a round plastic basin (50 cm diameter, 30 cm deep) with two 4 cm-diameter holes at the base 2 cm from each end of the diameter line. The two flasks protruded through the two holes making sure that the rims of the flasks aligned well with the cut surface of the basin.

One weevil was placed at the centre of the olfactometer arena in each bioassay set. Each adult was exposed to the odour source(s) for a maximum of 10 minutes after which the insect was categorized as active (moved > 2 cm from the release point), semi-active (moved < 2 cm) or inactive (displayed no movement). The location of active weevils was also determined: (a) within a

pitfall; (b) < 2 cm from the release point; (c) > 2 cm from the release point. The sequence of odour sources was randomised over the experimental days. Fresh odour sources were used for every comparison. After testing each odour set, the apparatus was washed with ethanol and air-dried to eliminate cross-contamination.

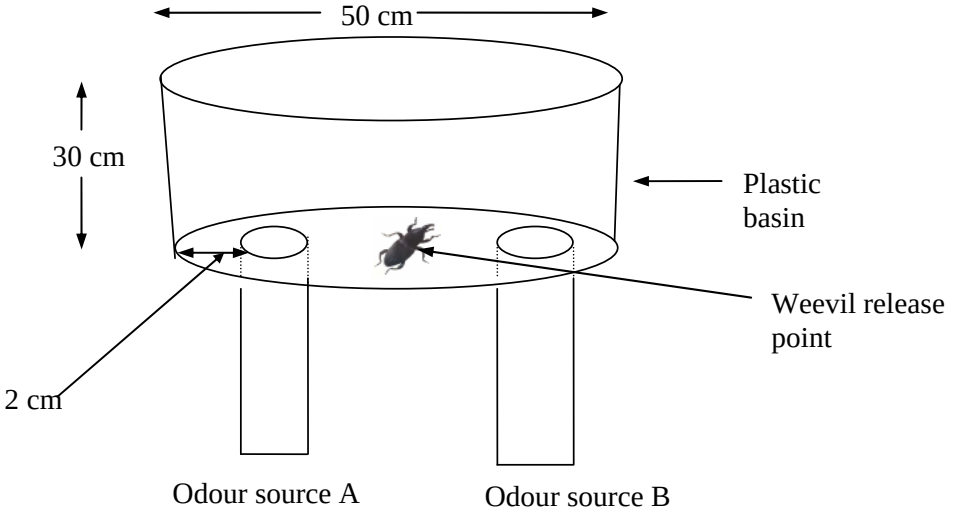


Figure 1: A double pit fall olfactometer (not to scale) set-up used in evaluating weevil response to host plant volatiles and the synthetic pheromone in the laboratory.

## 2.5 **Additive effects of pseudostem tissue and pheromone** (experiment 1)

### *2.5.1 Laboratory bioassays*

Fresh pseudostem tissue, fermented pseudostem tissue, and pheromone (Cosmolure+) were tested for their relative attractivity to *C. sordidus*. The odour sets (pairs) that were compared in the double pitfall olfactometer are presented in table 1. At least six comparison odour sets were tested per day and each comparison was repeated on six days. Twelve weevils were tested per odour set per day. The positions of the odour sources were alternated after testing six weevils to compensate for any unforeseen asymmetry of the set-up.

### *2.5.2 Field trials*

Trials were conducted to test attractivity of pheromone and kairomone (pseudostem tissue) in banana stands under field conditions. Treatments consisted of different infochemicals placed in pitfall traps that were then placed in the banana plots. In trial 1 (rainy season, March-April, 2002) treatments were: (i) pheromone, (ii) fermented pseudostem tissue, (iii) pheromone + fermented pseudostem tissue, and (iv) control (water). Treatments in trial 2 (dry season, May-June 2002) included the same four treatments plus: (v) fresh pseudostem tissue and (vi) pheromone + fresh

pseudostem tissue. Treatments for trial 3 (wet season) and 4 (dry season) were similar to those of trial 2 except that trial 4 had no control. A single Cosmolure+ package was used in pheromone treatments. Fermented pseudostem tissue and fresh tissue was obtained as described above. Treatments were assigned to plots with similar numbers of weevils. In all trials each treatment was replicated four times.

Table 1: Odour sets of fresh or fermented pseudostem tissue and the pheromone that were compared in a double pitfall olfactometer (experiment 1)

| Comparison odour sets | Fresh tissue | Fermented tissue | Pheromone | Pheromone + fresh tissue | Pheromone + fermented tissue |
|---|---|---|---|---|---|
| Air | X | X | X | X | X |
| Fresh tissue | | X | X | X | |
| Fermented tissue | | | X | | X |
| Pheromone | | | | X | X |
| Pheromone + fresh tissue | | | | | X |

The first two trials were conducted in 8-year old banana plots (30 x 18 m or 10 x 5 mats: mean 40 mats per plot) (cv Atwalira, AAA-EA) separated by 10 m alleys at IITA's Sendusu Farm. The plots were kept weed free and not mulched. At the onset of the first trial, there was a mean of 2.8 (± 0.2 s.e) *C. sordidus* per pseudostem trap (two traps of cv Atwalira per mat) in the selected plots. The third and the fourth trials were conducted at Senge in a 0.5 ha field (5-year old) planted with a mixture of East Africa highland bananas (AAA-EA types). Residue management, detrashing and weeding in the field were done. There were 1.5 (± 0.1 s.e) weevils per pseudostem trap in the field at the onset of the trials. In all trials, *C. sordidus* captured in pseudostem traps were released at the base of the mat of capture.

Pitfall traps consisted of 10-litre buckets (25 cm diameter at the top, 18 cm diameter at the base and 28 cm height) with two windows for weevil entry cut in the sides (Figure 2). One pitfall trap was placed in each plot. This was set at the base of the mat nearest to the centre of the plot. In the third trial, traps were placed at the base of banana mats approximately 20 m apart, 10 m from the stand border. Pheromone lures were suspended with a nylon string from the ceiling of the bucket. Fresh or fermented material (500 g) was placed in a half-litre-plastic cup at the bottom of pitfall traps for use in combination with pheromone or singly. Pseudostem material was changed every 3 days. A liquid detergent (1l) was added as a retaining agent and renewed after six days. Attracted

insects walking on the soil fall in the trap and their number can subsequently be determined. Traps were checked for *C. sordidus* every three days for 30 days.

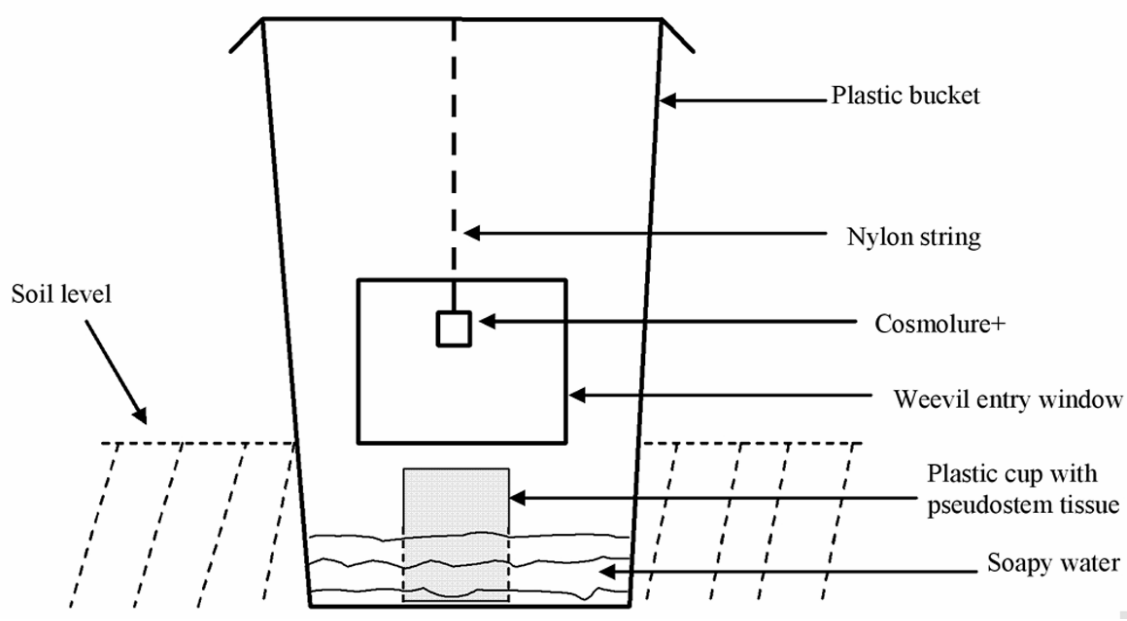


Figure 2: A bucket pitfall trap (not to scale) baited with the pheromone and banana pseudostem tissue.

## 2.6 Amounts of fermented tissue for enhancing the pheromone (experiment 2)

### *2.6.1 Laboratory bioassays*

To evaluate the effect of adding different amounts of fermented pseudostem tissue (cv Mbwazirume) to enhance pheromone effectiveness in attracting *C. sordidus* under laboratory conditions, the following comparison odour sets in a double pitfall olfactometer were tested: in the first trial: clean air vs. 10, 50, 100 and 200 g pseudostem tissue + pheromone, and pheromone + 10 g pseudostem tissue vs pheromone + 200 g pseudostem tissue were included.

Twelve weevils were tested for response per odour set per day. All comparison odour sets were tested on each day and the experiment was repeated for six and seven days for trial 1 and 2 respectively. Odour sources were alternated after testing six individuals and the apparatus was washed with alcohol and air-dried.

2.6.2 *Field trials*

Two field trials under this experiment were conducted to determine the effect of using different amounts of fermented pseudostem tissue on enhancing attractivity of pheromones to *C. sordidus.* The treatments used for trial 1 were: pheromone + 100 g, + 500 g and +1000 g fermented pseudostem tissue. The treatments of the second trial were similar to those of the first one except that the treatment of pheromone + 50 g was added. Trials were of a completely randomised design, with treatments replicated six times. The fermented tissue and pheromone lures as described above were used in this study. The pseudostem material (from the middle part of the plant) was weighed using a Waymaster balance to obtain the different amounts used for each treatment.

The first trial was conducted in the rainy season (April-May, 2003) at Senge (5 km from Kawanda) in a 0.5 ha field (5-year old) planted with a mixture of East Africa Highland bananas (AAA-EA types). Residue management, detrashing and weeding in the field was moderately done. Weevil incidence was estimated at the start of the trial using pseudostem traps (Mitchell, 1978). There were 1.5 (± 0.1 s.e) weevils per pseudostem trap in the field. *Cosmopolites sordidus* captured in pseudostem traps were released at the base of the mat of capture. The second trial was conducted using the same field in the relatively dry season (July-August 2003,). The agronomic conditions in the field during the second trial were similar to those during the first trial.

The pitfall trap described in experiment 1 was used (Figure 2). Fermented material (according to treatment) was placed in a plastic container at the bottom of pitfall traps for use in combination with pheromone. Pseudostem material was changed every 3 days. Traps were checked for *C. sordidus* every 3 days for 33 and 36 days for trial 1 and 2 respectively.

## 2.7 Pheromone release rate and interaction with fermented tissue (experiment 3)

*2.7.1 Laboratory bioassays*

A trial to evaluate the effects of pheromone release rate and fermented tissue interaction on attractivity of *C. sordidus* was conducted using a double pitfall olfactometer in the laboratory. The two treatments consisted of one and three lures, respectively, in pitfall (conical) flasks with 50 g fermented pseudostem tissue. Pheromone lures (containing 90 mg sordidin; release rate 3 mg/day) (A.C. Oehlschlager, pers. comm.) were used. Thus, it was assumed that the two odour sources were releasing pheromone at a rate of 3 and 9 mg/day respectively. Odour sources were alternated in the olfactometer after testing six individuals and the apparatus was washed with alcohol and air-dried after testing 12 weevils.

*2.7.2 Field trials*

The effect of pheromone release rate on *C. sordidus* response to pheromone traps was evaluated in a farmer's field at Senge and in an on-station field at KARI. One, two or three pheromone lures were placed in pitfall traps described in experiment 1. Pheromone traps were checked every three days and weevils found were recorded and removed from the trap. The trial was conducted in 30 days.

## 2.8 Effect of pseudostem tissue age (experiment 4)

Three trials under this experiment were conducted at KARI and Sendusu to determine the effect of age of the pseudostem tissue in enhancing pheromone for attraction of *C. sordidus*. The treatments were: (i) pheromone + 500 g of 3 days old fermented tissue, (ii) pheromone + 500 g of 9 days old fermented tissue, (iii) pheromone + 500 g of 15 days old fermented tissue and (iv) pheromone + 500 g of 30 days old fermented tissue. Trial 1 and 2 were conducted banana fields at Sendusu and KARI respectively. The third trial was conducted in the banana fields at KARI. There were six banana plots for trial 1 at Sendusu (42 mats per plot) and trial 2 at KARI (120 mats per plot), while trial 3 at KARI had 12 plots each consisting of 48 mats. Trials 1 and 2 lacked treatments (iii) and (iv) respectively while trial 3 had all treatments. Treatments for trial 1 and 2 were replicated two times while those in trial 3 were replicated three times.

The pseudostem material of cultivar Mbwazirume (Musa spp, AAA-EA group) was chopped and placed in pheromone traps in a plastic container. Pseudostem tissue was changed after 3, 9, and 15 days for treatments (i), (ii) and (iii) respectively. The pseudostem tissue for the fourth treatment was not changed during the trapping period of 30 days. The pheromone (Cosmolure+) was used in packs releasing 3 mg/day.

A pitfall trap made out of a 10-litre bucket was used (Figure 2). The pheromone was hung from the top of the bucket using a nylon string. The pseudostem tissue (500g) was placed in a plastic container and placed at the base of the bucket. A pitfall trap baited with either the pheromone or pseudostem tissue of different ages was placed at the base of the mat in the banana plots. Soapy water (1l) was added to the trap as trapping agent and was changed every 6 days. Traps were checked every three days. The number of weevils captured was recorded and discarded.

### 2.9 Statistical analyses

Numbers of responding weevils in the double pitfall olfactometer were analysed with a two-tailed binomial test (null hypothesis: both odour sources are equally attractive). Non-responding weevils were not included in the analysis. Data on field evaluation of the additive effects of host plant tissue to pheromones, and on field evaluation of pheromone release rate, pseudostem tissue amounts and longevity on enhancing weevil response to pheromones were log transformed and analyzed using the mixed model procedure of SAS (SAS® Institute Inc., 1990), and means were separated using the Student-Newman-Keuls test (SNK). A regression analysis using log-transformed data was conducted to determine a relationship between weevil catches and (i) the amount of fermented pseudostem tissue used in combination with the pheromone; and (ii) age of the pseudostem tissue used for enhancing the pheromone.

# 3 RESULTS

### 3.1 Additive effects of pseudostem tissue and pheromone (experiment 1)

*3.1.1 Laboratory bioassays*

*Cosmopolites sordidus* was strongly attracted to both host plant volatiles and the synthetic pheromone presented singly or in combination as compared to clean air in a double pitfall olfactometer (Figure 3). Fermented pseudostem tissue was more attractive to *C. sordidus* than fresh tissue. The presence of fresh or fermented pseudostem tissue enhanced attractivity of pheromone lures in comparison to pheromone lures alone. There were no differences in attractivity between pheromone + fermented tissue and pheromone + fresh tissue.

*3.1.2 Field trials*

In trials 1 and 3 under field conditions, pheromone was significantly more attractive than the fermented tissue and control but equally attractive to the combination of the pheromone and the fermented tissue (Figure 4). However, in trials 2 and 4, the addition of fermented tissue to pheromone-baited traps increased attractivity to *C. sordidus* by 50% compared to pheromone alone. Fresh pseudostem tissue contributed significantly to the attraction of *C. sordidus* only in trial 4.

## 3.2 **Amounts of fermented tissue for enhancing the pheromone** (experiment 2)

### *3.21 Laboratory bioassays*

Increasing the amount of tissue added to the pheromone did not significantly increase weevil response (Table 2). A regression analysis on percentage weevil response versus the amount of fermented pseudostem tissue added to the pheromone showed a non-significant relationship ($R^2$= 0.64, P=0.055).

### 3.2.2 *Field trials*

In the first trial, only the combination of pheromone plus 1000 g of fermented pseudostem tissue attracted significantly more weevils than the pheromone alone (Figure 5). The combination of pheromone with 100 g and 500 g of fermented pseudostem tissue attracted similar numbers of weevils among themselves and compared to the pheromone alone. In the second trial, the pheromone in combination with 500g of pseudostem tissue attracted significantly more weevils than pheromone plus 50 g of fermented pseudostem tissue, but attracted similar numbers of weevils to pheromone plus 100 g and pheromone plus 1000 g of fermented pseudostem tissue. A regression analysis on weevil catches versus the amount of fermented pseudostem tissue added to the pheromone showed a significant relationship for the first field trial ($R^2$=0.06, P=0.001), second trial ($R^2$= 0.15, P=0.001) and for both trials ($R^2$=0.10, P=0.001). An increase in the amount of fermented pseudostem tissue added to the pheromone led to an increase in weevil catches in traps although it levels off at larger pseudostem weights.

Table 2**:** Number of *C. sordidus* responding to the pheromone (PH) in combination with different amounts of fermented pseudostem (FP) tissue in the double pitfall olfactometer in the laboratory (experiment 2).

| Comparison odour sources (A vs. B) | Number of weevils responding | | % of responding weevils choosing source B | Numbers of non-responders |
|---|---|---|---|---|
| | Source A | Source B | | |
| Clean air vs. PH + 1 g FP | 10 | 36 *** | 42.9 | 38 |
| Clean air vs. PH + 5 g FP | 9 | 37*** | 44.1 | 36 |
| Clean air vs. PH + 10 g FP | 10 | 37*** | 44.1 | 35 |
| Clean air vs. PH + 50 g FP | 5 | 45*** | 53.6 | 34 |
| Clean air vs. PH + 100 g FP | 6 | 47*** | 56.0 | 31 |
| Clean air vs. PH + 200 g FP | 6 | 46*** | 32.0 | 32 |

Significantly different values are indicated with *** (P< 0.001) binomial test on individuals that responded to either odour source. Responding weevils include those found in pitfall and less than 2 cm from odour source. Seventy two and 84 weevils were tested for response in the first and second trial respectively.

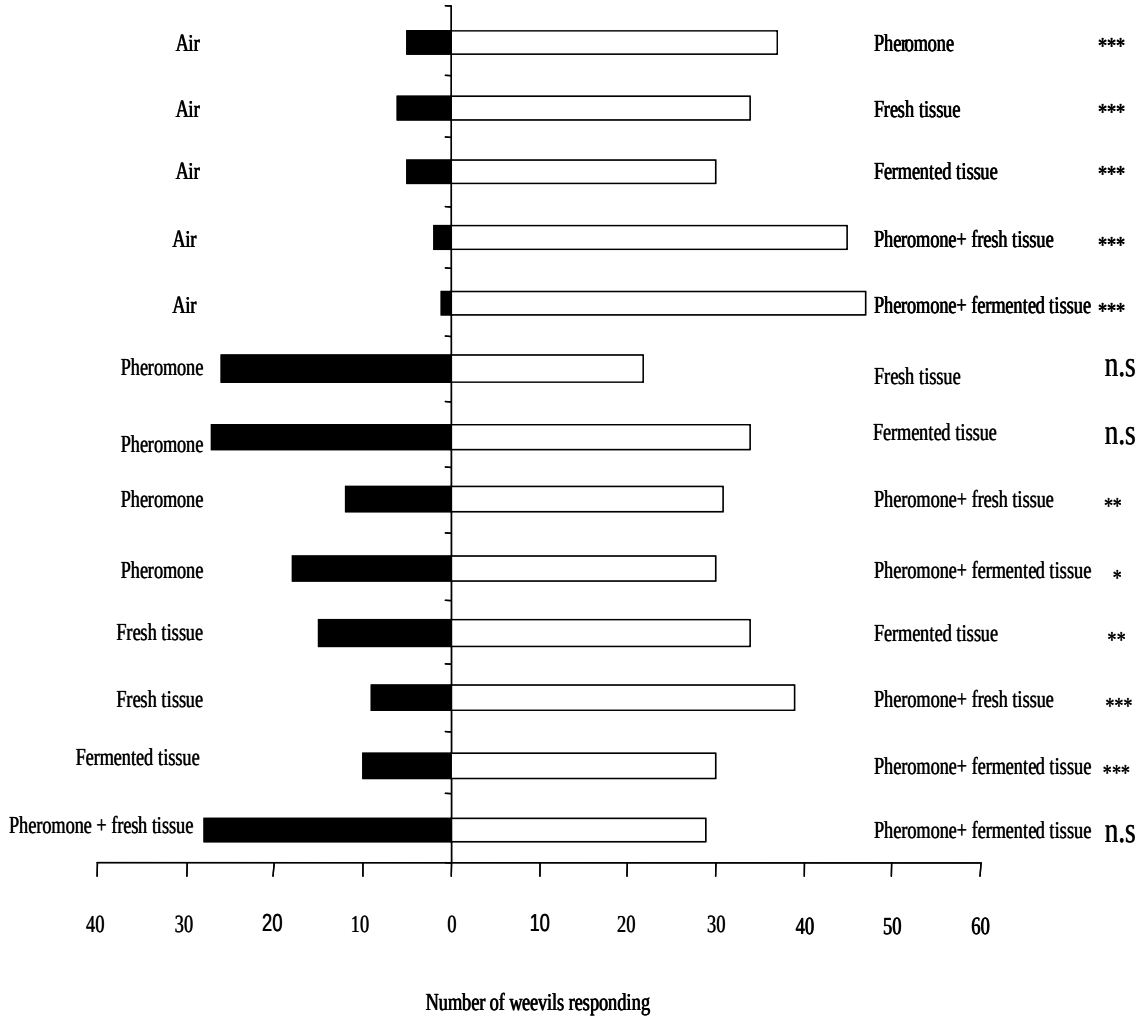


Figure 3: Number of *C. sordidus* responding to pseudostem tissue compared to synthetic pheromone in a pitfall olfactometer in the laboratory. Significant different values are indicated with: * (P<0.05), ** (P<0.01), *** (P<0.01), n.s = not significant, binomial test between individuals that responded to either odour (n=72, responding weevils including those found in pitfall and less than 2 cm from the odour source) (experiment 1)

### 3.3 **Pheromone release rate and interaction with fermented tissue** (experiment 3)

The number of *C. sordidus* (30) that responded to three pheromone lures in a double pitfall olfactometer in the laboratory was significantly greater than the number of *C. sordidus* (16) attracted to a single lure (binomial test, P = 0.027). However the number of weevils attracted per lure was greater for single (16 weevils/lure) than for multiple lures (10 weevils/lure).

In field plots on-station at KARI, slightly more *C. sordidus* were caught in pitfall traps baited with three lures than with either one or two lures (Figure 6). The traps baited with one lure captured more weevils per mg pheromone released (i.e. 1.6 weevils/mg) than traps baited with two or three lures (0.9-1.0 weevils/mg). In Senge, trap catches were low and there were no differences in the numbers of *C. sordidus* attracted to pitfall traps baited with different numbers of lures.

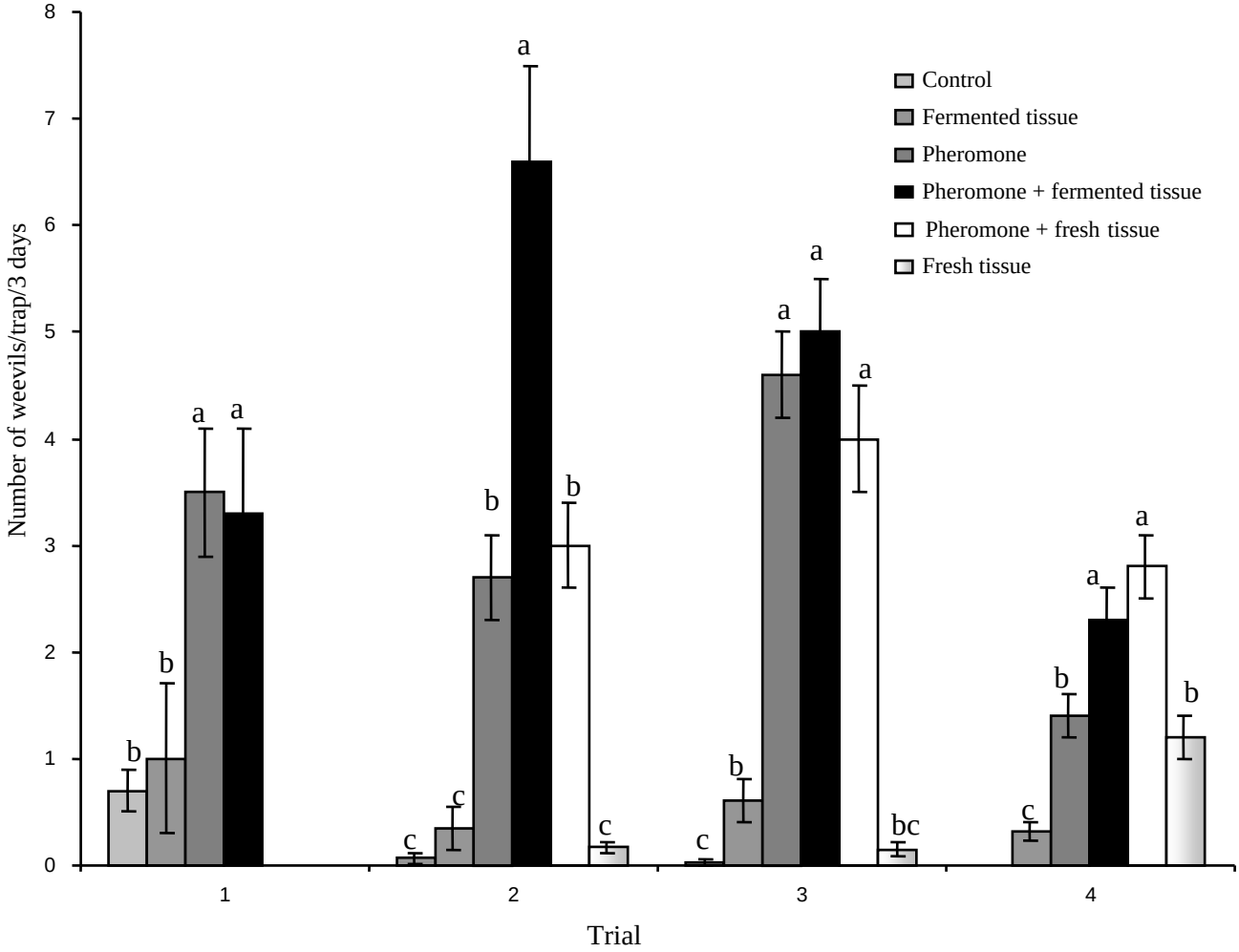


Figure 4: Mean number (±s.e.) of weevils attracted by host plant tissue and the synthetic pheromone presented singly and in combination in a pitfall trap in the field. Within a trial, bars with the same letter are not significantly different (Student-Newman-Keuls test, P<0.05) Fresh tissue and pheromone + fresh tissue treatments were not included in trial 1 and trial 4 lacked the control (experiment 1). The treatments were replicated four times in trials.

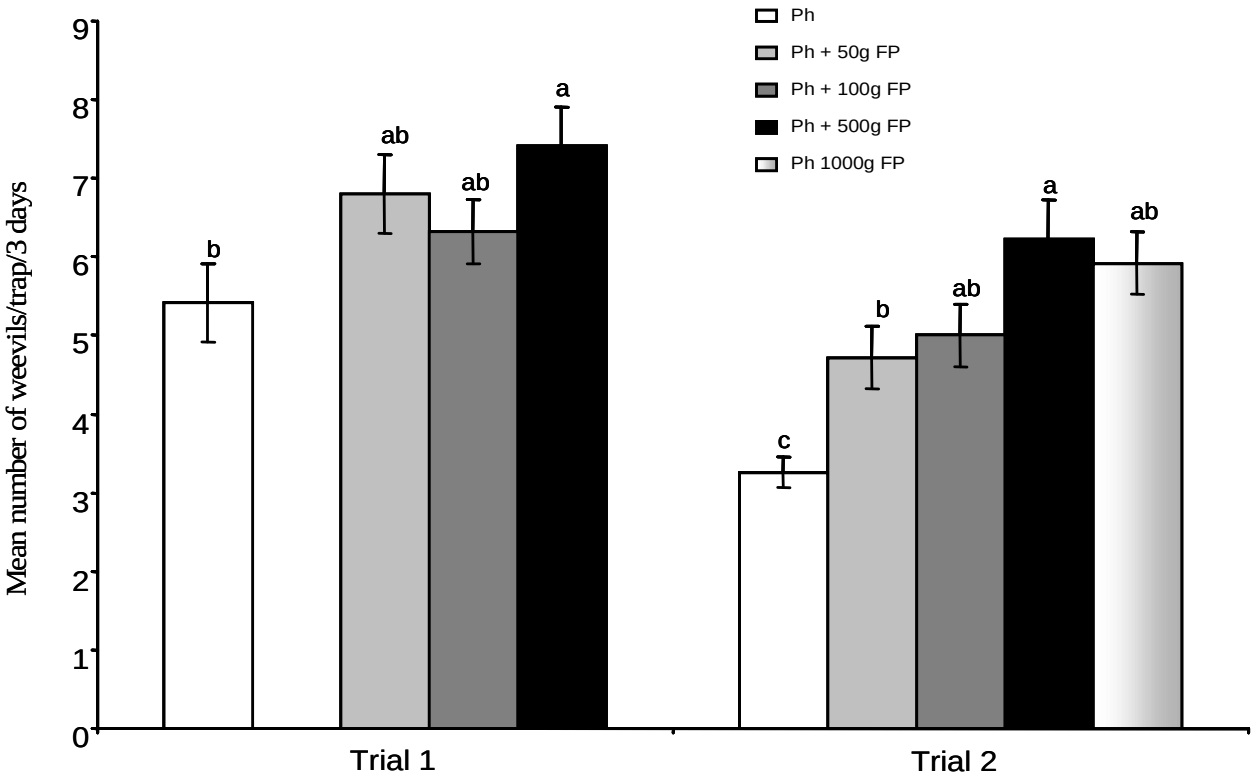


Figure 5: Mean number (±s.e.) of weevils captured in pheromone (Ph) traps baited with different amounts of fermented pseudostem tissue (FP) added under field conditions. Bars followed by similar letters within a trial are not significantly different (Student Newman-Keuls test, P<0.05) (experiment 2). The treatment Ph + 50 g FP was lacking in trial1, and treatments in both trials were replicated six times.

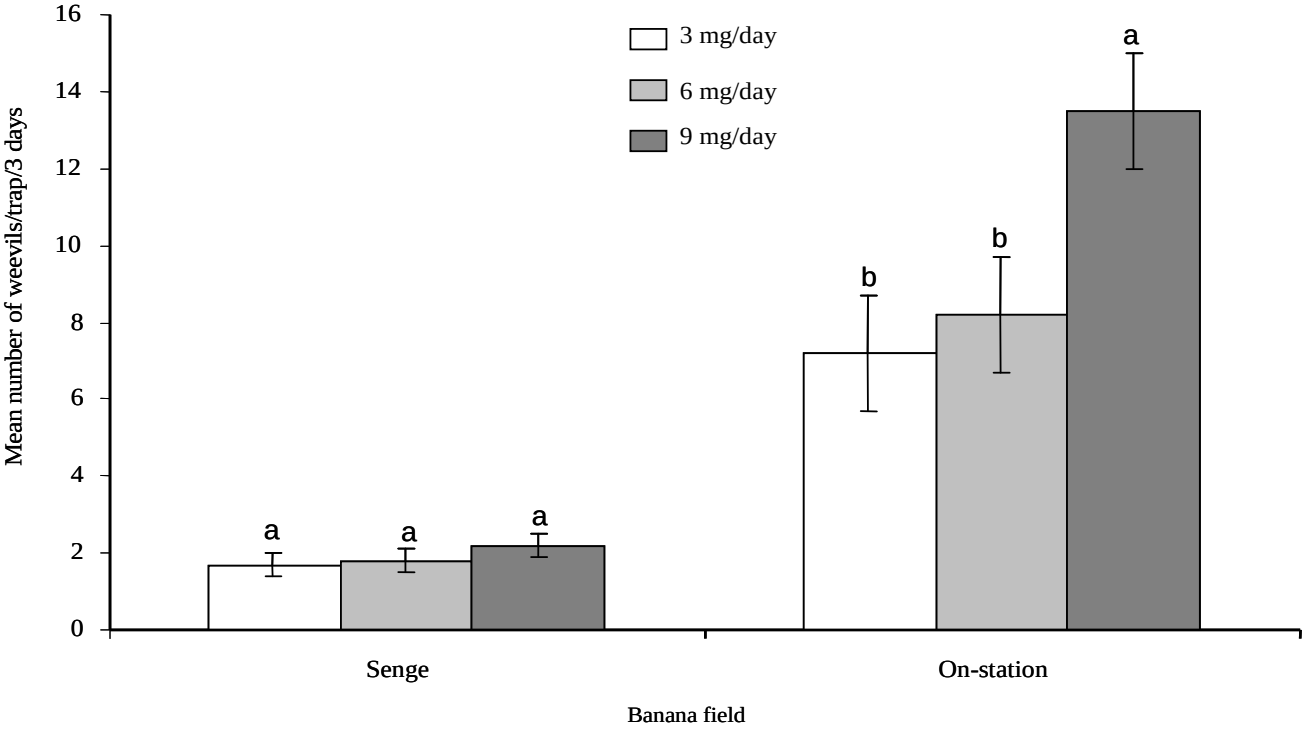


Figure 6: Mean number (±s.e.) of *C. sordidus* captured in pheromone traps releasing pheromone at different rates under field conditions. Within a field, bars followed by same letters are not significantly different, Student-Newman-Keuls test, P<0.05 (experiment 3). Treatments were replicated four times in both trials.

### 3.4 **Effect of pseudostem tissue age** (experiment 4)

The pheromone-baited traps in which the pseudostem tissue was changed every 3 and 9 days caught equal numbers of weevils (Figure 7) at both Sendusu and KARI. Significantly lower numbers of weevils were caught in the traps that had the pseudostem tissue changed every 30 days than traps that had the tissue renewed after 3 and 9 days at KARI. At Sendusu, traps in which the pseudostem tissue was changed after 3 and 9 days caught significantly more weevils than those traps that had the pseudostem tissue changed after 15 days. In trial 3, traps baited with pheromone and the tissue changed every 3, 9 and 15 days, attracted similar number of weevils. The traps that had the pseudostem tissue changed after 9 days attracted significantly more weevils than traps that had the tissue changed after 30 days ($P < 0.05$). There was general decrease in the weevils caught in the traps at both KARI ($r^2$=0.13, P=0.004) and Sendusu ($r^2$=0.21, P=0.001) with increasing period of changing the pseudostem tissue.

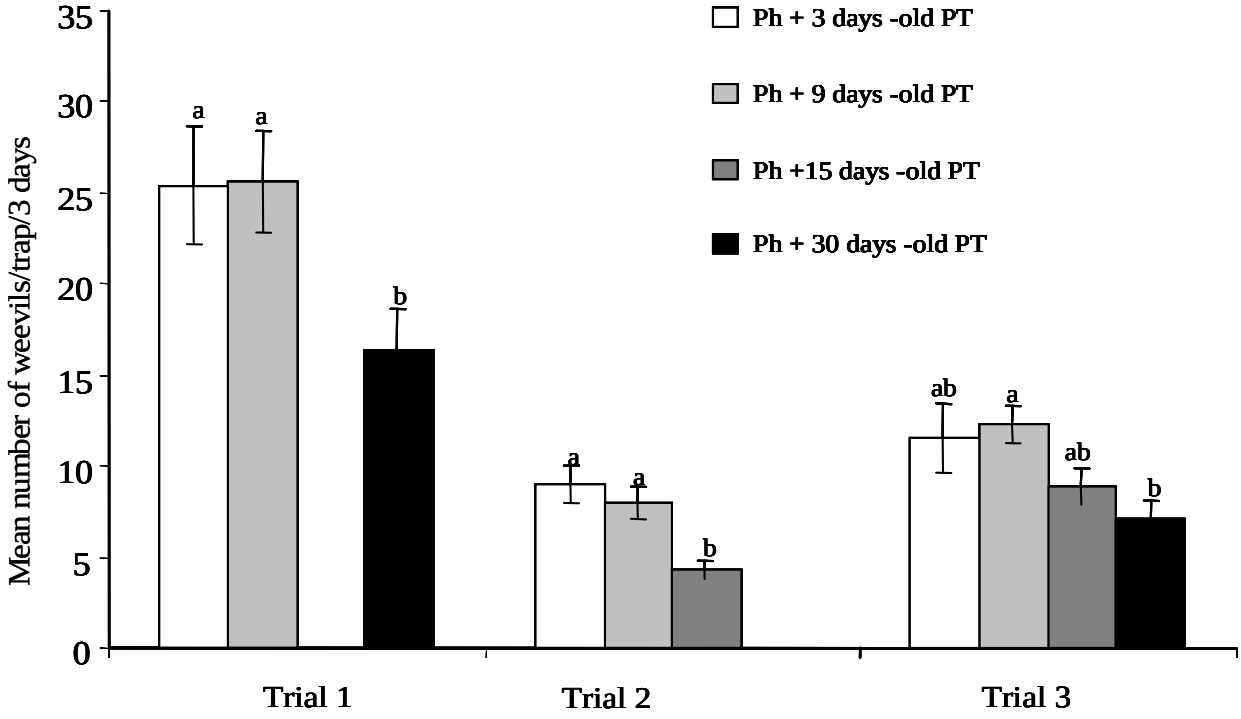


Figure 7: Mean number (±s.e.) of weevils recaptured in traps baited with pheromone (Ph) and pseudostem tissue (PT) changed after 3, 9, 15 and 30 days at KARI and Sendusu under field conditions. Means of bars within a site with similar letters are not significantly different, Student-Newman-Keuls test, P< 0.05 (experiment 4). Trial 1 and 2 did not have treatment Ph +15 days-old PT and Ph + 30 days-old respectively. Treatments for trial 1 and 2 were replicated two times while those in trial 3 were replicated three times.

# 4 DISCUSSION

Strong attraction of insects to fermented plant volatiles has been reported for other Rhynchophorinae, including *Rhynchophorous palmarum* and *R. cruentatus* to fermenting chopped sugarcane (Jaffe *et al.,* 1993; Giblin-Davis *et al.,* 1994a). Fermentation processes may affect both the quality and the quantity of volatiles released, which in turn resulted in increased insect response. Moist fermenting tissue from various palm species, fruits, sugarcane, pineapple and molasses were reported to be more attractive to *R. cruentatus* (Giblin-Davis *et al.* 1994a). In both laboratory and field studies, the synthetic pheromone and plant volatiles were each attractive to *C. sordidus*. In a direct comparison in our laboratory bioassays, the fermented pseudostem tissue showed greater attractivity *to C. sordidus* than fresh pseudostem tissue. The low attraction of fresh pseudostem tissue compared to the fermented tissue in our laboratory bioassays may have been due to low quantity of volatiles released in addition to lack of moisture in these tissues. *Cosmopolites sordidus* is known to respond positively to moisture gradients (Roth & Willis, 1963).

In many Rhynchophorinae, pheromones and kairomones produce synergistic effects (Giblin-Davis *et al.,* 1994a; Giblin-Davis *et al.,* 1996a; Cerda *et al.,* 1999; Rochat *et al.,* 2000). However, our studies in the laboratory demonstrated an additive rather than a synergistic effect. In the field, an additive effect of pseudostem tissue to the pheromone was observed in two out of four field trials. The field data suggest that it may not be necessary to use a combination of pheromone and fermented pseudostem tissue in the mass-trapping programme for the control of *C. sordidus*. It is possible that the amounts (500 g) may not have been enough to cause significant enhancement of the pheromone. The hypothesis that an increase in the amount of fermented pseudostem added to pheromone would increase weevil response was supported to a limited extent by our field data but not by the laboratory trials. An increase in insect response with increased amount of plant tissue as additives has been reported for other weevils (Giblin-Davis *et al.,* 1996b; Oehlschlager *et al.,* 1993). They argued however that increasing the amounts of plant tissue to pheromone traps would be too cumbersome and not practical.

A large number of weevils were captured in the field in traps baited with pheromone and in traps with pheromone plus pseudostem tissue in the rainy season than in the dry season. These results are consistent with other reports of greater adult activity in rainy compared to dry conditions (Gold *et al.,* 1999b). In the dry season, when weevils are inactive (and possibly buried in the soil), stronger cues might be required to attract weevils than in the rainy season and therefore adding pseudostem tissue may have more additive effect. The infochemical threshold for the response of *C. sordidus* may be higher in the dry season than rainy season.

Various authors that have reported a positive correlation between pheromone release rate and insect response (Coffelt & Burkholder, 1972; Hardee *et al.,* 1974, Howse *et al.,* 1998; Hallett *et al.,*

1999). Our data also support this for the banana weevil. In our study, traps baited with one lure captured more weevils per mg pheromone released (i.e. 1.6 weevils/mg) than traps baited with two or three lures (0.9-1.0 weevils/mg). Therefore, from an applied point of view, it is better to spread three lures in three traps rather than to concentrate them in a single trap. We also think that the modest increase in trap efficiency recorded here does not outweigh the increased costs of using greater release rates of the pheromone.

The effect of banana pseudostem tissue on *C. sordidus* when used as additives to the pheromone was observed to decrease with time. There was a higher weevil catch in traps when the plant tissue was renewed after 3 and 9 days than after 15 days and when it was not renewed at all. Our results are comparable with observations of Rochat *et al* (2000) who found for the palm weevil (*R. palmarum*) that sugarcane tissue as an additive to the pheromone rhynchophorol became ineffective after 15 days. Oehlschlager *et al* (1993) also reported that the sugarcane was effective in the traps for only 1 to 2 weeks. The decline in the attractiveness of the banana pseudostem tissue could be attributed to the rapid dehydration of the tissue in bucket traps especially during the dry days or to changes in the composition of the volatiles mixture.

In general, laboratory results showed that host plant volatiles can enhance the aggregation pheromone, but the effect was not significant in the field. Besides, including fermented or fresh pseudostem tissue, as kairomones would render the trapping system more labour intensive and costly, as fermentation requires utensils for storage. The materials would also require frequent replacement for effective trapping efficacy. Therefore, our data suggests that the banana pseudostem tissue may play a limited role in the enhancement of the pheromone for the management of *C. sordidus*.

**Acknowledgement**

The research was funded by the Rockefeller Foundation through a grant to International Institute of Tropical Agriculture (IITA) and Wageningen University Sandwich Fellowship. We are grateful to Dr A.C. Oehlschlager of Chemtica International, Costa Rica, for providing the pheromone lures used in the study and to Dr W. Tushemereirwe (NBRP-NARO) for his cooperation. David Mukasa assisted in data collection.

Chapter **4**

# The influence of age, female mating status and density of the banana weevil, *Cosmopolites sordidus*, on its response to aggregation pheromone

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## Abstract

Laboratory and field experiments were conducted to test the hypotheses that the response of the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) to aggregation pheromone is affected by age, female mating status and weevil density. Laboratory bioassays were conducted using a double pitfall olfactometer while a bucket pitfall trap was used in field experiments. Immature and mature weevils (males as well as females) responded equally to the pheromone in laboratory bioassays while in the field, 40-days-old weevils had a stronger response to the pheromone than 10-days-old weevils. The response of unmated weevils to the pheromone was stronger than the response of mated weevils both in the laboratory and field experiments. Weevil response to the pheromone was not significantly influenced by weevil density. The data provide insight in aspects of weevil biology that influence mass trapping of this insect pest using the aggregation pheromone.

# 1 INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is considered a major insect pest of bananas in East Africa (Gold *et al*., 1999). The pest can cause yield losses of up to 100% (Sengooba, 1986) and shorten plantation life span (Gold *et al.,* 2004a). C*osmopolites sordidus* adults live up to four years and females have a low fecundity (Gold *et al*., 2001). The sex ratio is 1:1 (Gold *et al*., 2001). The weevil rarely flies and seldom crawls more than 50 m in three months (Gold *et al*., 2001). Eggs are placed singly within the base of the host plant (Abera, 2000). After hatching, the larvae tunnel into the corm and pseudostem of the plant where they develop and pupate. The emerging adult is free-living but most often associated with banana mats or crop residues (Gold *et al*., 2004b).

The adults are attracted by volatiles (kairomones) emanating from banana plants, especially cut or damaged corms (Gold *et al*., 2001). This has been utilised in trapping of weevils using pseudostem material. The males produce an aggregation pheromone (sordidin) that is attractive to both sexes (Budenberg *et al*., 1993b). Currently, the pheromone is synthesized by Chemtica International and sold as lures (containing a mixture of the four sordidin isomers plus plant volatiles) under the trade name Cosmolure+.

Control by mass trapping using pheromones may be influenced by biology the pest (Hardee *et al.*, 1969; Borden, 1977; Obeng-Ofori & Coaker, 1990; Jansson *et al.,* 1991). For instance, mated females of the cotton boll weevil *Anthonomus grandis* were less responsive to male-produced aggregation pheromone (grandlure) than virgin females, both in laboratory bioassays and in field tests (Hardee *et al.*, 1969). In insect species in which virgin females are trapped, the reduction in the subsequent generation may be directly related to the proportion removed from the population. If females are trapped after mating and after having laid some eggs, the efficiency of this control method decreases. If males were trapped, a very large proportion probably would have to be captured before there is a noticeable impact on the next generation. This is true for insect species in which both males and females are capable of mating many times (Borden, 1977). The efficiency of the pheromone traps may be influenced, among other factors, by the physiological status of the pest such as reproductive maturation and the mating status (Borden, 1977; Jansson *et al.*, 1991).

Although the aggregation pheromone are known to attract both female and male weevils (Alpizar *et al.,* 1999; Tinzaara *et al.,* 2000), there is lack of information on the effect of age and female mating status of *C. sordidus* on catches in pheromone-baited traps. *Cosmopolites sordidus* become sexually mature 20 days after emergence (Uzakah, 1995). We hypothesize that if mature and mated individuals do not respond to the pheromone lures, then trapping will cause limited suppression of

the weevil population in the field. A high population density of the target insect can also limit the control efforts especially if there is competition between synthetic and insect produced plumes (Howse *et al*., 1998). Mass trapping tends to be more effective at low population densities as was reported for *Anthonomus grandis* (Legget *et al.,* 1989; Ridgway *et al.,* 1990). The relationship between weevil densities and weevil response to pheromone lures has also not been investigated for *C. sordidus*.

The current study was undertaken to test the hypotheses that the *C. sordidus* response to the aggregation pheromone is affected by differences in age, density, female mating status and density.

# 2 MATERIALS AND METHODS

## 2.1 Site description

Laboratory studies were conducted in a dark room (5 x 3m) at the IITA Sendusu Farm ($0^o32'$ $32^o35'$E, 1260 metres above sea level (m.a.s.l.)), located 28 km northeast of Kampala, Uganda. The windows and the door of the room were tightly sealed with black polythene sheets. Ambient temperatures in the laboratory ranged from 22 to 28 $^oC$. During the study period, the room had a red light that was switched on during all experiments to facilitate taking of data without disturbing the test insects. Experiments were conducted between 9.00 am and 5.00 pm. An electric fan (40 cm stand fan, Evernal®, 50W) was on during experiments to provide aeration.

Field studies to examine the attractivity of aggregation pheromone to *C. sordidus* were conducted at Sendusu Farm and the Kawanda Agricultural Research Institute (KARI) ($0^o25'$N, $32^o51'$E, 1190 m.a.s.l.) and adjacent farmers' fields in Senge, 1 km north of KARI. Both sites have two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and daily mean temperature of 21 $^oC$.

## 2.2 Pheromone sources

The pheromone lures for use in laboratory bioassays and field experiments were obtained from ChemTica International in San Jose, Costa Rica. They were sealed in plastic and sent by courier (transit time < 1 week) and subsequently stored in a freezer upon arrival until use. Each pheromone pack contained 90 mg of Cosmolure+ with a release rate of 3 mg/day (A.C. Oehlschlager, pers. comm.). They were used in their original plastic package material.

### 2.3 Insects

A culture of banana weevils was established in a shade house at KARI using weevils that were trapped using pseudostem traps (Mitchell, 1978) from banana plantations in Masaka District. Weevils were released to 30 l capacity buckets containing pared (chopping off roots and outer layer) corms. After laying eggs on corms for 5-7 days, adult weevils were removed. The corms were then placed in drums that were covered with a papyrus mat to allowing ventilation. Corms were sprayed with water when necessary to maintain an appropriate relative humidity until adult weevils emerged (after about 50 days). Weevil cultures were established at a month interval to raise weevils of different ages.

All weevils used in our studies were kept in the dark room with the red light on for at least 15 h prior to being used. Preliminary observations indicated that keeping the weevils in the dark room increased their response in the bioassay. The use of a red light allowed us to work during daytime hours. Each weevil was used in a single assay and then discarded.

### 2.4 Double pitfall olfactometer

All laboratory bioassays were conducted using a double pitfall olfactometer (Tinzaara *et al*., 2003). The apparatus consisted of a round plastic basin (50 cm diameter, 30 cm deep) with two 4 cm-diameter holes at the base 2 cm from each end of the diameter line. Two flasks protruded through the two holes with their rims aligning well with the cut surface of the basin.

One weevil was placed at the centre of the olfactometer arena in each bioassay set. Each adult was exposed to the odour source(s) for a maximum of 10 minutes after which the insects were categorized as active (moved > 2 cm from the release point), semiactive (moved < 2 cm) or inactive (displayed no movement). The location of active weevils was also determined: (a) within a pitfall; (b) < 2 cm from the release point; (c) > 2 cm from the release point. The sequence of odour sources was randomised over the experimental days. The odour sources were rotated after each bioassay to eliminate a potential location effect. Fresh odour sources were used for every comparison. After testing each odour set, the apparatus was washed with ethanol and air-dried to eliminate cross-contamination.

### 2.5 Sex and age of *C. sordidus* (experiment 1)

*Laboratory bioassays.* We tested weevils of different sex and ages for their response to pheromone lures in a double pitfall olfactometer. The treatments consisted of immature (10 days old) and mature (40 days old) weevils. Weevils used in the study were collected from the established culture that was raised as described above. Their sex was determined according to Longoria (1968). Female and male

weevils were maintained separate until required for bioassays. There were two jars connected to the arena containing odour sources, i.e. one jar had the pheromone (1 pack, releasing at a rate 3 mg/day) and the other had clean air (control). A weevil was placed individually at the centre of the arena (15 cm from the odour sources) in the bioassay set and each weevil was used in only one set of test. Eighteen weevils from each age/sex category were tested on the same day for their response to synthetic pheromones. The experiment was repeated on four days (i.e a total of 72 weevils were tested per age category per sex).

*Field experiment*. Field experiments to determine the effect of age and sex on weevil response to pheromone lures were conducted at KARI in a banana field planted with cultivar Atwalira (Musa spp, AAA-EA group). The different ages (treatments) of weevils used for the experiment were: (i) 10-days old weevils and (ii) 40-days old weevils. Each treatment was replicated in three plots. The plot size was 10 mats x 12 mats at a spacing of 3 m x 3m and plots were separated by 10 m wide alleys. The plots were cleanly weeded and self-mulching. In each plot, weevils were released on selected mats in the following distance ranges: on trap mat (0 m), and on mats less than 5 and more than 5 m from the pheromone traps.

Weevils were collected from an established weevil culture (described above) of different ages according to the treatments. Weevils were marked according to age and release distance from the pheromone traps. Eight weevils (4 males and 4 females) were released on selected mats in the evening when they are active and can escape from predators. One trap mat of 0 m, and three mats less than 5 and four mats more than 5 m from the pheromone-baited traps were used in each plot.

The 10 l bucket pitfall trap (Tinzaara *et al*., 2000) baited with pheromone was placed in the plots one day after releasing the weevils. The traps were placed at the centre of the plot and only one pheromone lure was placed in each trap. Pheromone traps were checked everyday and weevils recaptured were counted and recorded. The sex and distance of release of recaptured weevils was recorded. The experiment was conducted over a period of 30 days. At the end of the sampling period, the percentage of weevils recaptured from the different distances and of the different ages was calculated.

### 2.6 **Female mating status** (experiment 2)

*Laboratory bioassays.* The hypothesis that the mating status of females influences response to pheromones was tested in the laboratory. Weevils collected from the established culture immediately after eclosion were sexed. One set of 100 female weevils (virgin) of the same age were kept in a plastic

bucket until maturity while another set was exposed to an equal number of males and kept together until they started egg laying.

Both mated and unmated females were tested in the double pitfall olfactometer. One weevil was placed at the centre of the arena at a time. The odour sources (pheromone and clean air) were rotated after testing three weevils. The apparatus was then washed with alcohol and air-dried before subsequent use. Twelve weevils of each group (mated and unmated) were tested on the same day and the experiment was repeated on five days.

*Field experiment.* The field experiment was conducted at the KARI banana field to evaluate the effect of the mating status and ovarian development on response to pheromone-baited traps. The treatments consisted of mated and unmated female weevils. Weevils were collected from the established culture (described above) at KARI. They were sexed immediately after emergence. A batch of females was kept with males (mated weevils) and the other batch was kept without males (virgin weevils). The weevils were then kept in the laboratory for a month before release in the field. The weevils were marked according to the mating status and release mat.

Prior to releasing weevils in the field, 20 weevils of each mating status were used to determine pre-treatment oocyte numbers. The weevil ovary was dissected under a microscope and the numbers of small, medium and mature oocytes were counted. Six marked females of each mating status were released per mat in each of the four plots in the evening (7.00-8.00 p.m) on the following mats: (i) one trap mat at 0 m, (ii) four mats at less than 5, and (iii) eight mats at more than 5m from the pheromone trap.

Pitfall traps baited with the pheromone were placed in the plots as described in experiment 1. Traps were checked every day and weevils recaptured were recorded according to distance/ mat of release.

### 2.7 *Cosmopolites sordidus* density (experiment 3)

*Laboratory bioassays*. The influence of weevil density on response to pheromone was evaluated using a double pitfall olfactometer in the laboratory. Weevil densities (treatments) tested for each sex were 4 (low density), 8 (medium density) and 16 (high density) per 100 gm corm. Weevils collected from the established culture and separated by sex were placed in plastic containers (diameter 30 cm, height 20 cm) under three density conditions. Weevils were held under these conditions for 48 h until use in the bioassay. During the bioassays, one weevil was released at the centre of the arena at a time. Different density treatments were tested on the same day while one sex category was tested per day.

The individuals (8 weevils) tested per sex constituted a replicate and there were six replicates. Observations for response were taken after 10 minutes. The odour sources (pheromones and clean air) were rotated after testing four weevils in the bioassay to eliminate the location effect. After testing 8 weevils (one replicate), the apparatus was washed with alcohol and air-dried to eliminate cross contamination.

*Field experiment.* The treatments were: (i) 4 weevils/mat, (ii) 8 weevils/mat, and (iii) 16 weevils/mat. The experiment had a randomized design with three replicates. The experiment was conducted at Sendusu in 8-year old banana plots planted with cultivar Atwalira and separated by 10 m wide alleys. The plot size was 5 mats x 5 mats at a spacing of 3 m x 3m. The plots were well weeded and unmulched. The resident weevil population size was estimated before releasing the weevils. The mean resident weevil incidence per mat in plots used was 0.1, 1.8 and 4.0 for low, medium and high density plots respectively. The weevils were released to supplement the existing resident weevil population. Weevils were marked prior to release according to sex and released per mat in the ratio 1:1 (male: female).

A pitfall trap baited with the pheromone as described in experiment 1 was then placed in the plots to capture weevils. Traps were checked every three days for 30 days and weevils recaptured were recorded according to distance of release and sex for different densities.

### 2.8 Statistical analysis

In all experiments, data for weevils responding to the odour sources or percentages of the recaptured weevils in the field were analysed using a $\chi 2$-test for contingency of the Minitab statistical package (Minitab, 1995). The mean number of oocytes of mated and unmated females before their release was compared using a student's t-test. The numbers of weevils recaptured from plots of low, medium and high weevil densities were log transformed and analysed using analysis of variance with the SAS statistical package (SAS, 1990). The means were compared using student-Newman-Keuls (SNK) test.

# 3 RESULTS

## 3.1 Sex and age of *C. sordidus* experiment 1)

*Laboratory bioassays*: Orientation responses by males and females to the pheromone in the laboratory setup were similar (Table 1). The response by males ($\chi 2 = 0.22$, $P = 0.64$) and females ($\chi 2 = 0.05$, $P=0.82$) to the pheromone was not dependent on maturity of the weevils. There was no association between sex and maturity of weevils responding to the pheromone ($\chi 2 = 0.49$, $P = 0.48$) (Table 1). Sexual maturity and age of the weevils (mature weevils being 30 days older than immature ones) appeared not to influence weevil response to pheromones in the laboratory.

*Field experiment*: In the field trials, larger proportions of the 40-day old weevils were captured in the pheromone-baited traps than for 10-day old weevils, at all distances of release from the pheromone-baited trap (Figure 1). However, the differences were only significant for weevils recaptured from mats >5 m from the pheromone trap ($\chi 2 = 4.06$, $P = 0.04$). The percentage of weevils recaptured dropped considerably when the weevils were released on the mat near the pheromone trap compared to those released further away. The sex ratio (male: female) of weevils that responded to the pheromone was 1:2.3 and 1:1.4 for immature and mature weevils respectively.

Table 1: Number of sexually immature and mature adult *C. sordidus* responding to pheromones in double pitfall olfactometer.

| Weevil sex | Maturity | Pheromone | Clean air | % of weevils that responded |
|---|---|---|---|---|
| Males | Immature | 46 | 2 | 66.6 |
| | Mature | 41 | 1 | 58.3 |
| Females | Immature | 36 | 3 | 54.2 |
| | Mature | 40 | 4 | 61.1 |

Only weevils found inside the pitfall and less than 2 cm from the pitfall are presented in the table; Total numbers of weevils tested were 72. Immature weevils were 10 days old while mature weevils were more than 40 days old.

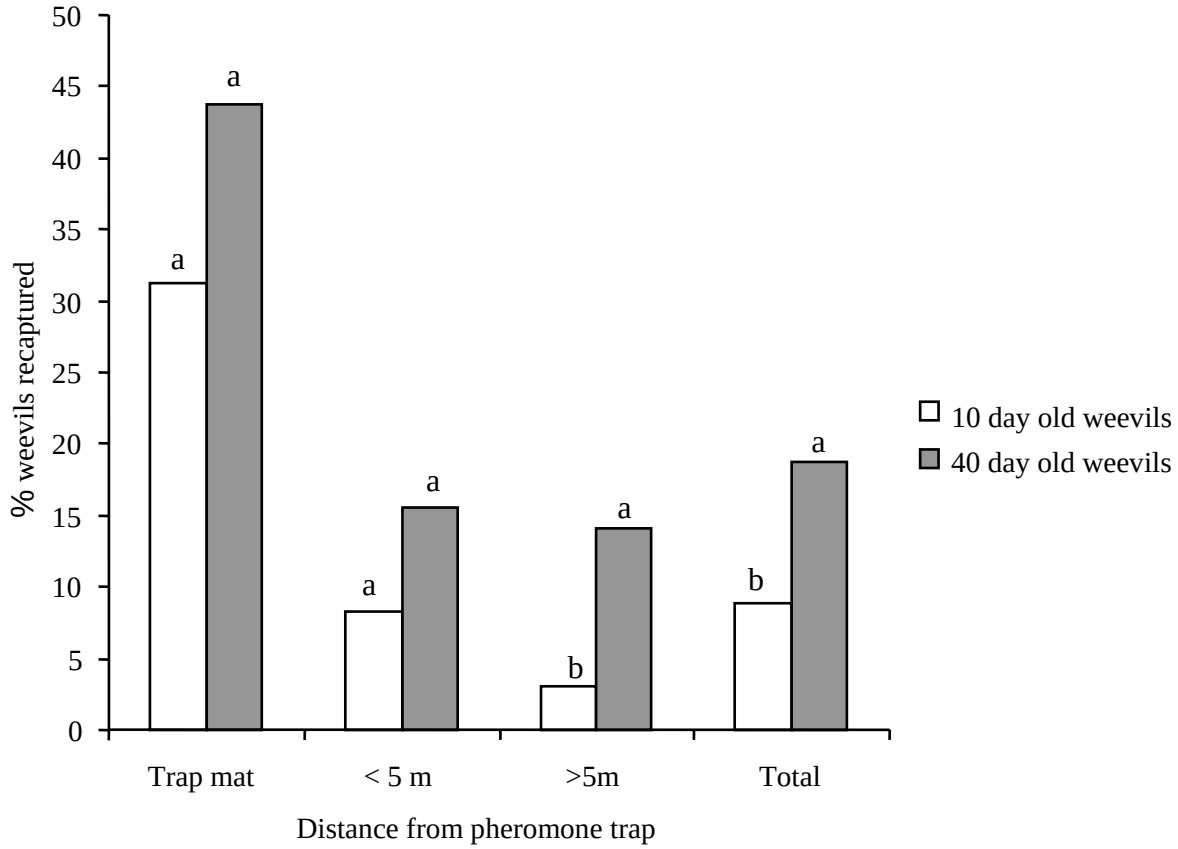


Figure 1: Percentage number of weevils of different ages recaptured from different distances relative to the pheromone-baited trap. Sex ratio of the recaptured weevils was 1: 2.3 for 10-days old, and 1:1.4 for 40-days old weevils. Bars with the same letters are not significantly different ($\chi$2-test, P > 0.05).

### 3.2 **Female mating status** (experiment 2)

*Laboratory bioassays*: Unmated female weevils responded significantly more to pheromone than mated ones in laboratory bioassays (Table 2). The percentage of non-responders was, however, relatively high for both mated (52%) and unmated (40%) weevils.

Table 2: Number of mated and unmated female *C. sordidus* weevils found at different positions from the odour sources in the double pitfall olfactometer (N = 60).

| Mating status | Responding weevils | | Non-responders |
|---|---|---|---|
| | Pheromone | Air | |
| Mated | 21 | 7 | 32 |
| Unmated | 34 | 2 | 24 |

Unmated females responded significantly more than unmated females ($\chi$2 = 4.9, d.f. = 1, P=0.03, 2x2 contingency table test).

*Field experiment*: Significantly more small and mature oocytes were observed in mated than in unmated weevils prior to release into the field (Figure 2). The percentages of unmated (24.1%) and mated (19.2%) weevils recaptured in pheromone-baited traps in the field were similar ($\chi 2=0.61$, P=0.44) (Figure 3). The percentage recapture of both mated and unmated weevils was similar from the trap mats and mats less than 5 m from the trap but was significantly higher for unmated weevils from mats greater than 5 m ($\chi 2=4.06$, P=0.04) from the pheromone-baited trap (Figure 4).

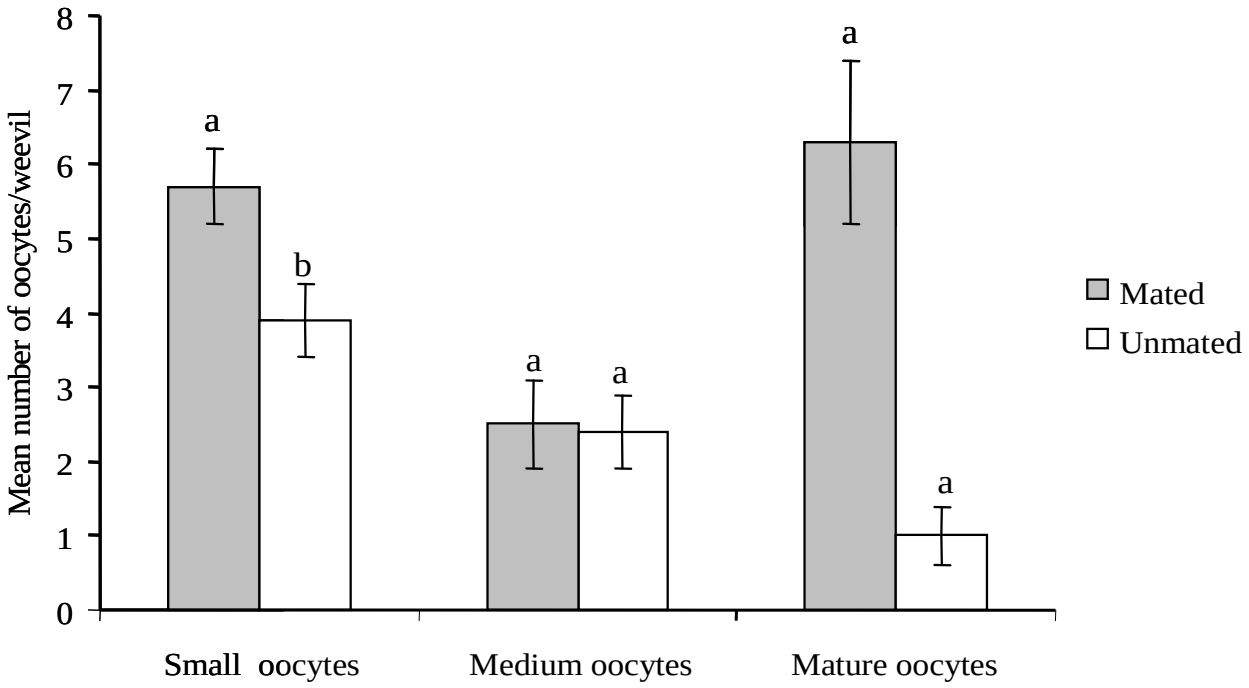


Figure 2: Mean (± s.e.) number of oocytes for mated and unmated female weevils used in the experiment to determine the effect of the mating status on weevil response to the pheromone. Bars for each oocyte category with similar letters are not significantly different (t-test, P > 0.05).

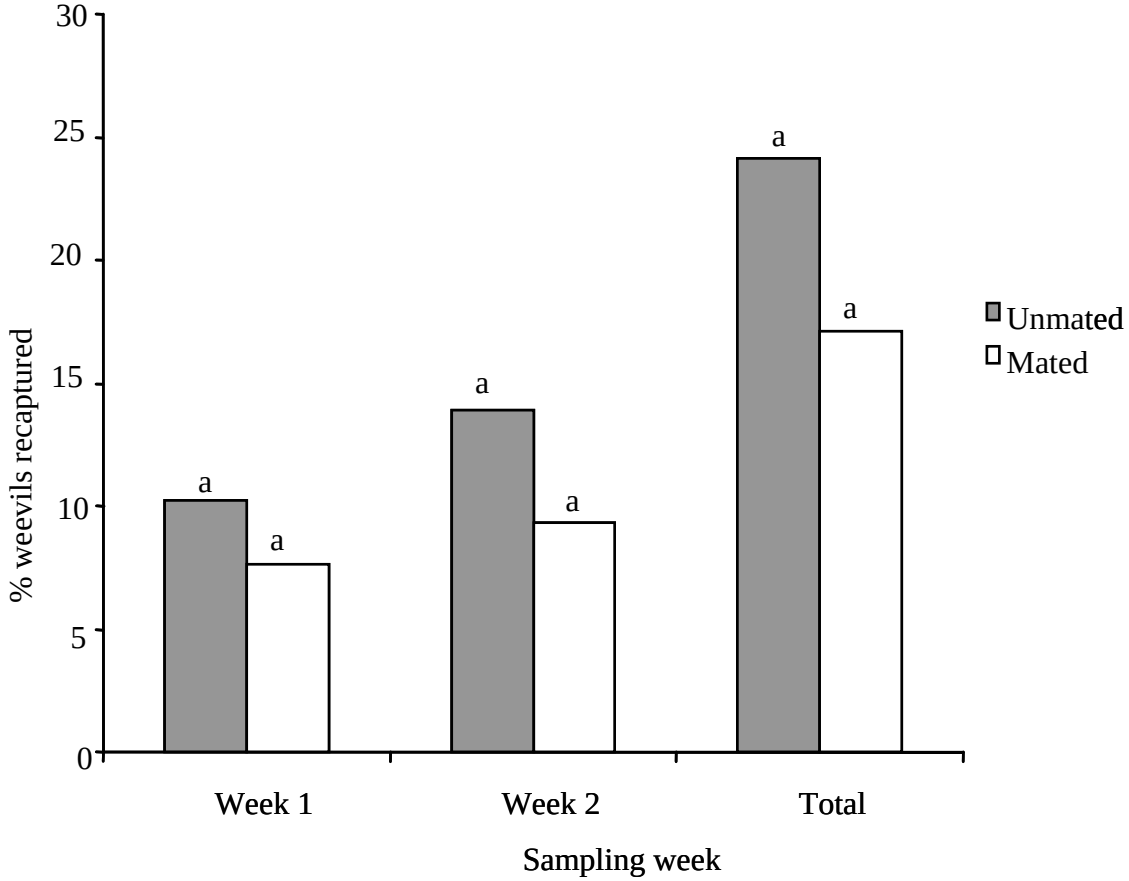


Figure 3: Percentage of mated and unmated *C. sordidus* recaptured in pheromone-baited traps. A total of 216 weevils were released for each mating status in the four plots. Bars with similar letters are not significantly different (P>0.05, $\chi$2-test).

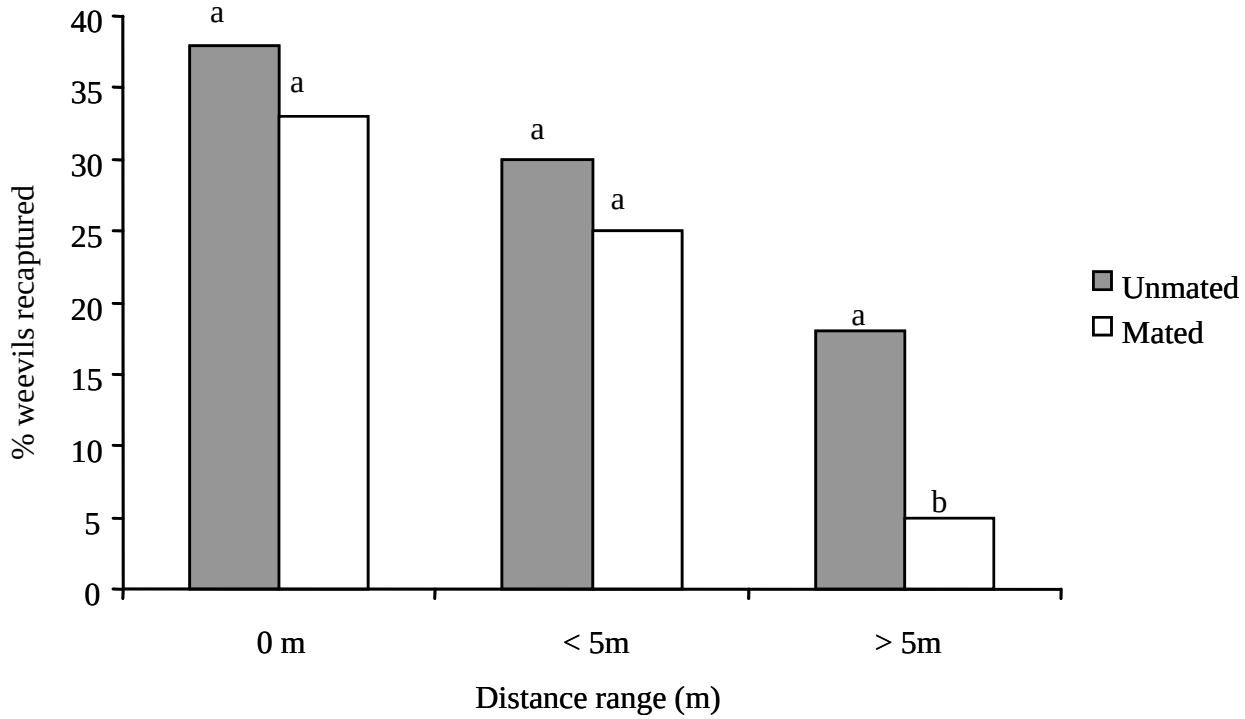


Figure 4: Percentage of mated and unmated weevils recaptured from different distances from the pheromone- baited trap. Bars with similar letters are not significantly different (P>0.05, $\chi$2-test).

### 3.3 *Cosmopolites sordidus* density (experiment 3)

*Laboratory bioassays:* Males, females and mixed weevil groups under each density treatment responded similarly to the pheromone in a double pitfall olfactometer (P> 0.05, $\chi$2-test), and therefore data of the three sex groups were pooled for final analysis. Weevils that had been kept at each of the three different densities prior to the bioassay did not differ in the degree of attraction to the pheromone (Table 3). Previous weevil density appears not to have significant effect on weevil response to the pheromone.

*Field experiment*: In the field experiment, the percentages of weevils recaptured (only marked weevils) from plots with low weevil density (7.8%), moderate density (7.5%) and high density (6.6%) were similar ($\chi$2=3.33, d.f.=2, P=0.19) (Table 4). Sex ratios of recaptured weevils from the three density plots were similar ($\chi$2=5.2, d.f.=2, P=0.73), although more females than males were recaptured in all densities.

Table 3. Number of *C. sordidus* of different densities that responded to the pheromone in a double pitfall olfactometer

| Weevil density | Number responding | | Non-responders |
|---|---|---|---|
| | Pheromones | Clean air | |
| Low | 74 | 5 | 65 |
| Moderate | 82 | 13 | 49 |
| High | 59 | 8 | 77 |

A total of 144 weevils (72 per sex) were tested per density. The number weevils responding to the pheromone of different densities was not significant ($\chi$2=3.65, d.f.=2, P=0.16, 3x2 contingency table).

Table 4. Number and sex ratio of weevils recaptured in pheromone-baited traps from field plots with different densities at Sendusu, Uganda.

| Treatment[1] | Mean resident weevils/ mat | Number of weevils recaptured | | | | Unmarked |
|---|---|---|---|---|---|---|
| | | Males | Females | Sex ratio (M:F) | % of released weevils | |
| 4 weevils/mat (400) | 0.9 | 24 | 70 | 1:2.9 | 7.8 | 228 |
| 8 weevils/mat (800) | 1.8 | 49 | 132 | 1:2.7 | 7.5 | 305 |
| 16 weevils/mat (1600) | 4.0 | 112 | 206 | 1:1.8 | 6.6 | 471 |

[1]The total number of weevils that were added in each of the banana plots per treatment is indicated in brackets.

# 4 DISCUSSION

The physiological state of *C. sordidus* may influence its response to the aggregation pheromone. Males and females of *C. sordidus* of different ages were evaluated for response to the pheromone in a double pitfall olfactometer. Both males and females responded to the pheromone although the sex ratio was skewed for females especially in field experiments. A sex ratio of captured insects which is skewed for females has been previously observed for weevils in the subfamily Rhynchophorinae (e.g *Metamasius hemipterus*) (C.A Oehlschlager, pers. comm.). As females are known to be more active and move greater distances than males as they search for oviposition sites and mates (Gold *et al*., 2001), more females than males could have been captured in pheromone-baited traps.

Receptivity of *C. sordidus* to the aggregation pheromone source may vary with age. Immature and mature weevils responded equally in a double pitfall olfactometer. Weevils that were mature had a stronger response to the pheromone than immature weevils in our field experiments. Increased response with age has been previously reported for other coleopteran insects such as *Anthonomus grandis* (Borden, 1977; Obeng-Ofori & Coaker, 1990; Jansson *et al.*, 1991). On the other hand, a decrease in sensitivity to aggregation pheromone with increasing age has been recorded for many other beetles. Walgenbach and Burkholder (1986) found that adult maize weevils S*itophilus zeamais* Motschulsky, up to one week old, showed a significant response to the male-produced aggregation pheromone (Sitophilate), while weevils from 2 to 6 weeks old showed no response to pheromone, and weevils from 8 to 10 weeks old were significantly repelled by Sitophilate pheromone. The increased response observed in *C. sordidus* could be explained by changes in the importance of particular behaviour with increasing age. Mature weevils may benefit from the aggregation if it leads to increased mating chances of individuals.

In both laboratory and field experiments we observed that unmated females had a stronger response to the pheromone than mated weevils. Similarly, unmated females of *Metamasius hemipterus* were attracted more by aggregation pheromone in laboratory bioassays (Ramirez-lucas *et al*., 1996). Searching for males to mate with could be the reason why more unmated female *C. sordidus* responded to the aggregation pheromone. Capturing unmated and fertile females could play a significant role in slowing down the population build up of the pest in a mass trapping approach. Capturing female *C. sordidus* when they have laid eggs on the host plant would contribute less to suppressing the pest.

In our laboratory studies, there was no significant difference between weevils that responded to the pheromone at low, medium and high weevil density. In our field experiments, the proportion of

weevils recaptured from the different density plots were similar. The results did not support our hypothesis that weevils respond less in conditions with high weevil density where the pheromone trap may compete with natural pheromone sources. Crowding and starvation are some of the factors that have been reported to influence response of insects to pheromones (Walgenbach & Burkholder, 1986). Pierce *et al* (1983) found reduced responses by the beetle *Oryzaephilus surinamensis* to beetle or frass volatiles with increasing population density. Insects in crowded cultures did not respond to volatiles but transfer to fresh medium at lower densities restored response.

In general, our results showed that the physiological state of *C. sordidus* influences their responsiveness to the aggregation pheromone. This will affect trap efficacy as well as laboratory bioassay results. The interpretation of laboratory data and trap catches in the field situation must, therefore, depend on knowledge of the behaviour and physiology of insects. Information on the effect of the sex, age, female mating status, and pest density on pheromone traps catches may be necessary in planning mass trapping programmes using the aggregation pheromone.

**Acknowledgement**

The research was funded by the Rockefeller Foundation through a grant to International Institute of Tropical Agriculture (IITA) and Wageningen University Sandwich Fellowship. We are grateful to Dr A.C. Oehlschlager of ChemTica International, Costa Rica, for providing the pheromone lures used in the study. Francis Sebulime and David Mukasa assisted in data collection.

Chapter 5

# Factors influencing pheromone trap effectiveness in attracting the banana weevil, *Cosmopolites sordidus*

*W. Tinzaara, C.S. Gold, M. Dicke, A. van Huis & P.E. Ragama*

## Abstract

Studies were conducted in Uganda to evaluate the influence of distance, environmental factors, trap location and trap type on *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) catches in pheromone-baited traps. A pheromone-baited bucket pitfall trap was used in the studies. Trap efficiency decreased with distance from the pheromone trap. Of the weevils released within a distance of 0.5, 1, 2 or 4 m from the pheromone-baited traps, 9-13% were recaptured within a trapping period of 30 days at all places, which suggests a low efficacy of the pheromone lures. Relative humidity showed a significant positive relationship to *C. sordidus* catches in pheromone traps, while wind speed, temperature and rainfall did not have an effect. Pheromone-baited traps with banana leaves covering around the trap captured higher numbers of *C. sordidus* compared to uncovered traps. More weevils were captured in pheromone-baited traps placed in alleys than on mats. Information on how various factors influence pheromone-baited trap effectiveness will assist in designing a pheromone-based mass trapping strategy for the control of *C. sordidus*.

# 1 INTRODUCTION

Pheromones and other behaviour modifying chemicals hold a great potential as tools for pest management (Cardé & Minks, 1995, 1997; Phillips, 1997; Agelopoulos *et al.,* 1999; Suckling, 2000). Pheromones have been used in both monitoring insect populations and in direct control (Phillips, 1997; Agelopoulos *et al.,* 1999). Pheromone traps provide an easy and efficient way of detecting insect populations in the field and in storage facilities (Phillips, 1997). Control of insect pests can be achieved by mass trapping using pheromone-baited traps that lure insects to their death (Cardé & Minks 1995, 1997, Giblin-Davis *et al.,* 1994a). The application of pheromone trapping requires optimization of the trapping method.

A number of factors are known to influence the effectiveness of pheromone traps in capturing insects, such as distance of insects to traps (Schlyter, 1992; Byers, 1999; Laboke e*t al*., 2000), trap location (McNeil, 1991; Muirhead-Thompson, 1991), weather (e.g. rainfall, wind speed, temperature) (Jansson *et al.,* 1989; Laboke *et al*., 2000; Sappington & Spurgeon, 2000), and trap type (Murad, 2001). Here, we investigate the trapping of the banana weevil (*Cosmopolites sordidus* Germar) (Coleoptera: Curculionidae) with its aggregation pheromone that is available in synthetic form (Budenberg *et al*., 1993b). Information on the effects of the above-mentioned factors on trapping effectiveness would be useful in developing a pheromone-based trapping system for the control of this pest. In this paper we evaluate how the different factors influence trap effectiveness in capturing *C. sordidus.*

*Cosmopolites sordidus* feed on banana corm and are attracted by volatiles (kairomones) emanating from banana plants, especially from cut or damaged corms (Treverrow, 1994). This feature has been used to make pseudostem traps (Gold *et al*., 2002). The males produce an aggregation pheromone (sordidin) that is attractive to both sexes (Budenberg *et al*., 1993b). The role of infochemicals (kairomones and pheromones) in the management of *C. sordidus* has been reviewed by Tinzaara *et al*. (2002a). Both kairomones and pheromones have been studied for application in a trapping system to control *C. sordidus* (Alpizar *et al*., 1999; Tinzaara *et al*., 2000, 2003; Kagezi *et al*., 2002). In Costa Rica, traps baited with Cosmolure+ (a mixture of four isomers of sordidin) were 5-10 times more effective at collecting *C. sordidus* adults than conventional pseudostem traps (Jayaraman *et al*., 1997; Alpizar *et al*., 1999). In on-station trials in Uganda, pheromone-baited traps captured up to 18 times as many weevils/day as pseudostem traps in a field heavily infested with weevils (Tinzaara *et al*., 2000). Trapping with pheromones, however, resulted in only limited population reductions in on-farm evaluation studies conducted in Uganda (Kagezi *et*

*al.,* 2002). Factors such as trap maintenance, cropping systems, residue management and environmental conditions were postulated to have affected pheromone efficacy. Studies conducted in Australia showed that to maintain the traps working well it may be necessary to cover them with fresh banana leaves to reduce the temperature and to keep the area around the trap moist to encourage movement of the weevil (Murad, 2001).

Seasonal differences in trap catches of *C. sordidus* most likely reflect weather effects on weevil activity patterns rather than population dynamics (Gold *et al*., 2001). In the laboratory, Uzakah (1995) found activity to be positively correlated to relative humidity and negatively correlated with temperature and light intensity. While *C. sordidus* adults appear more active in moist conditions (Gold *et al*., 2001), it remains unclear how soil moisture and other environmental factors might affect catches in pheromone-baited traps.

The objectives of this study were to determine the efficacy of pitfall traps baited with Cosmolure+ in relation to: (1) the distance between trap and weevil source; (2) the proximity of the trap to banana plants; (3) environmental factors; and (4) covering traps with banana leaves.

# 2 MATERIALS AND METHODS

## 2.1 Site descriptions

Field studies were conducted at IITA's Sendusu Farm ($0^{o}$32'N, $32^{o}$35'E, 1260 metres above sea level) (m.a.s.l.), located 28 km northeast of Kampala (Uganda) and Kawanda Agricultural Research Institute (KARI) ($0^{o}$25'N, $32^{o}$51'E, 1190 m.a.s.l.) located 12 km north of Kampala, and farmers' fields at Senge (adjacent to KARI). Both sites have two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and daily mean temperature of $21^{o}$C.

## 2.2 Pitfall trap design

Pitfall traps were made from 10-liter buckets (39 cm height, 30 cm rim diameter). The sides of the buckets were cut to allow adult *C. sordidus* to enter the traps (Tinzaara *et al*., 2000). The buckets were then buried such that the cuts were flush against the soil. In each trap, a single Cosmolure+ lure was suspended by a nylon string 3 cm over the soapy water. Each lure contained 90 mg of pheromone with a release rate of 3 mg/day (C. Oehlschlager, pers. comm.). The lures were obtained from Chemtica, International in San Jose, Costa Rica. These were shipped in closed polythene bags by express mail to Uganda and stored in a freezer until use.

## 2.3 **Range of trap effectiveness** (experiment 1)

*2.3.1 Attraction of C. sordidus released at different distances from traps*

Pheromone-baited pitfall traps were placed in the centre of six subplots (441 $m^2$) consisting of 7 rows of 7 banana mats (cv Atwalira, AAA-EA) spaced in a 3 x 3 m arrangement in an established banana trial (Figure 1). The plots were well weeded and without mulch. *Cosmopolites sordidus* infestation levels were estimated by placing a 30-cm pseudostem piece on each of the mats (Mitchell, 1978) prior to the initiation of this experiment and an average of 4.9 (± 0.2 s.e.) *C. sordidus* adults were trapped per piece. Field collected *C. sordidus* of unknown age distribution collected from farmers' fields were marked and released at a rate of 5 males and 5 females at the base of each of the 49 mats. Sex was determined on the basis of punctuation on the rostrum (Longoria, 1968). The weevils were marked by gently scratching the elytra with a dissecting blade. Distinct marks were made to indicate release points (mats) and sex. The weevils were released at dusk on 2 December 2002.

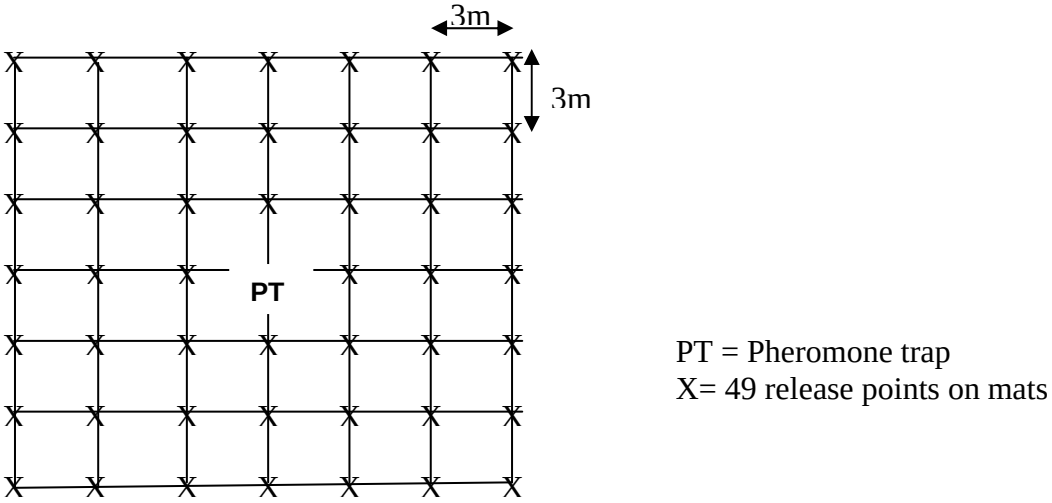


Figure 1: A schematic diagram of the banana mats in the plot where weevils were released.

A pheromone-baited pitfall trap containing a single Cosmolure+ lure was placed at the centre of each subplot one day after weevil release (Figure 1). The traps were checked daily for 30 days and captured weevils were recorded according to sex, release point and direction of release mat. Distances of mats from the pheromone traps on which weevils were released were measured.

The numbers of marked insects captured from each release point were used to compute the trap performance within a given distance from the trap. The average performance (P) was determined directly from the total number of marked insects caught and the total of marked insects released within a given distance (radius) from the pheromone-baited trap dt (performance (P) = the number of marked

insects caught divided by the number of insects released within a given distance from the trap dt.). The proportion of female and male weevils recaptured in all plots from < 5 m, 5-10 m and > 10.1-15 m was estimated.

*2.3.2 Attraction of C. sordidus released in vicinity of trap*

The objective of this trial was to determine the capture rate of C. sordidus released in the vicinity of pheromone-baited pitfall traps. The experiment was conducted in unmulched banana plots at Sendusu (5 mats x 10 mats cv Atwalira, spacing 3 by 3 m). Pheromone-baited pitfall traps containing a single Cosmolure+ lure and liquid detergent as a retaining agent, were placed in the center of the plots. The release distances from the pheromone trap were 0.5, 1, 2 and 4 m in each of the four cardinal directions (i.e. 16 release points) in each of six plots. Four males and four females (bearing distinctive marks) were placed in the field at each release point at dusk one day after placement of traps. Traps were checked daily and captured weevils were identified to their release point. The proportion of weevils that were recaptured from each distance was calculated and subjected to analysis of variance (ANOVA) with the use of SAS software (SAS, 1990). Means were separated by the Student-Newman-Keuls (SNK) test.

**2.4 Environmental factors** (experiment 2)

The influence of environmental factors (rainfall, humidity, temperature, wind speed and direction) on pheromone trap captures was studied in the plots used in experiment 1 between December 2002 and May 2003. Pitfall traps were maintained at the same location with pheromone lures being renewed monthly. Traps were checked daily for captured weevils. Both marked and unmarked weevils were counted. Wind speed, temperature, rainfall and relative humidity were recorded daily using an automated weather instrument (CR-10X, Campbell Scientific Inc.) that was placed 300 m from the experimental plots. A linear regression analysis using the SAS statistical package (SAS, 1990) was done to determine the relationship between weevil catches and wind speed, temperature, rainfall and relative humidity per day.

**2.5 Covering the trap with banana leaves** (experiment 3)

*2.5.1 Covering the whole trap*

The objective of this experiment was to determine if covering the entire pheromone-baited pitfall traps with banana leaves would increase captures of *C. sordidus*. The treatments consisted of covered and uncovered pheromone-baited traps. Trials were run in two established banana stands in Senge (Senge 1

and Senge 2) and one at KARI in the wet season (April–May 2002). The banana stands (approximately 2.5 x 2.5 m spacing) in Senge were three years old, consisted of mixed highland banana cultivars and had moderate levels of management (residue pseudostems were present but trash removal and weeding were well done). The KARI trial was placed in three-year old banana plots (36 mats, cv Atwalira, 3 x 3 m spacing, 5 m grass alleys between plots) with high densities of *C. sordidus*. The plots were well managed and mulched with elephant grass (*Pennisetum purpureum*).

Prior to the placement of pheromone-baited pitfall traps, pseudostem traps (2/mat) were placed in the field to give an indication of *C. sordidus* abundance. *Cosmopolites sordidus* captured in pseudostem traps were counted and released. Trap captures averaged 2.0 (± 0.8 s.e) adults in the Senge stands and 6.0 (± 2.2 s.e) adults in the KARI plots. Different treatments were assigned to plots with similar numbers of weevils. The experiment at KARI was repeated in the dry season (January-February 2003).

Four pheromone-baited traps were placed in Senge 1, eight traps in Senge 2, and six traps in the field at KARI. Traps were spaced at least 20 m apart. A liquid detergent (liquid soap) was put in traps as a retaining agent and was changed every six days. Alternate traps were covered with four fresh banana leaves that touched on the ground at 0.5 m from the trap. Each trap was considered a replicate; thus, there were 2, 4, and 3 replicates at Senge 1, Senge 2 and KARI, respectively. Seven replicates of each treatment (with and without banana leaves) were used when the experiment was repeated at KARI in the dry season.

Traps were checked for weevils every three days, at which time the leaves would be replaced with fresh ones. The trial was conducted for 30 days. Data within a field were compared using a student's t-test. The fields used were different in terms of management and size of resident weevil population, so it was not possible to compare the effect of trap covering between fields.

### *2.5.2 Covering around the trap*

Two trials were held concurrently to determine the effect of covering with banana leaves around the pitfall trap baited with the pheromone. In these trials the covering was only done around the pitfall trap and did not include the pheromone. These trials were conducted at Kawanda banana plots in the wet season (April–May 2004). The treatments were pitfall traps with and without banana leaves around the trap and both types of traps were baited with the pheromone. The first trial was conducted in banana plots (36 mats, cv Atwalira, 3 x 3 m spacing, 5 m grass alleys between plots) that were well managed and mulched with elephant grass (*Pennisetum purpureum*). The second trial was conducted in banana plots about 500 m away with similar conditions except that they were not mulched.

Prior to the placement of pheromone-baited pitfall traps, pseudostem traps (2/mat) were placed in the field to give an indication of *C. sordidus* abundance. *Cosmopolites sordidus* captured in pseudostem traps were counted and released. Trap captures averaged 4.4 (± 1.2 s.e) adults in the stands for the first trial and 2.4 (± 0.7 s.e) adults in the plots for the second trial.

Four and three pheromone-baited traps were placed per treatment in trial 1 and 2 respectively. Traps were placed in the central mat per plot. A liquid detergent (liquid soap) was put in traps as a retaining agent and was changed every six days. Alternate traps were covered with fresh banana leaves such that the area of about 0.5 m radius around the trap was covered. Each trap was considered a replicate; thus, there were 4 and 3 replicates for trial 1 and 2 respectively.

Traps were checked for weevils every three days, at which time the leaves would be replaced with fresh ones. The trials 1 and 2 were conducted for 33 days and 30 days respectively. Data within a trial were compared using a student's t-test.

### 2.6 **Trap location relative to the host plant** (experiment 4)

To evaluate the effect of trap location (relative to the host plant) on trap catches, the following treatments were used: a) pheromone-baited trap between mats (1.5 m from mats), and b) pheromone-baited trap on mats. The traps were placed in selected plots at KARI (as described for experiment 3) and at Sendusu. The plots at Sendusu were planted with banana cultivar Atwalira (*Musa* spp, AAA-EA group). There were six traps in the field at KARI and four traps in the field at Sendusu for each of the two treatments. The plots at KARI were mulched with *Pennistem purpereum* and the plots at Sendusu were not mulched. The experiment was run for 30 days in each site. The number of weevils captured was recorded, and the weevils were taken to the laboratory to determine their sex. The number of weevils captured from different locations according to their sex category was compared using a $\chi 2$ test. The numbers of weevils attracted to the two trap locations relative to banana mats were compared using a student's t-test.

# 3 RESULTS

### 3.1 **Range of trap effectiveness** (experiment 1)

Trapping efficiency decreased with release distance from the pheromone-baited trap (Figure 2). Trap catches contained significantly ($P < 0.05$) more females than males at each of the different release distance ranges (Figure 3). When weevils were released in the close vicinity of the trap, more weevils were recaptured from 0.5 m (14.1%) compared to those recaptured from 4 m (7.3%)

(Figure 4). The percentage of weevils recaptured in the pheromone-baited trap at all release distances was generally low.

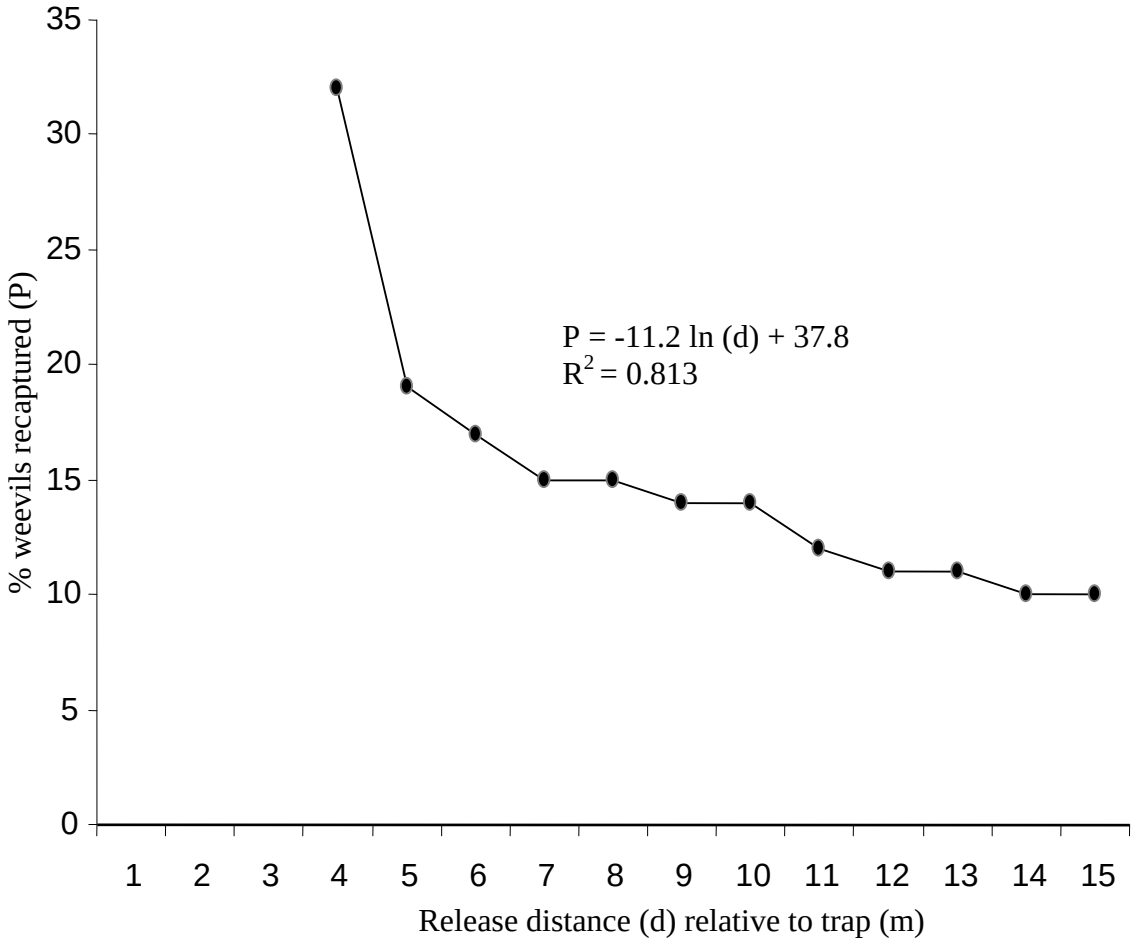


Figure 2: Percentage of weevils recaptured when released at different distances from the pheromone-baited trap.

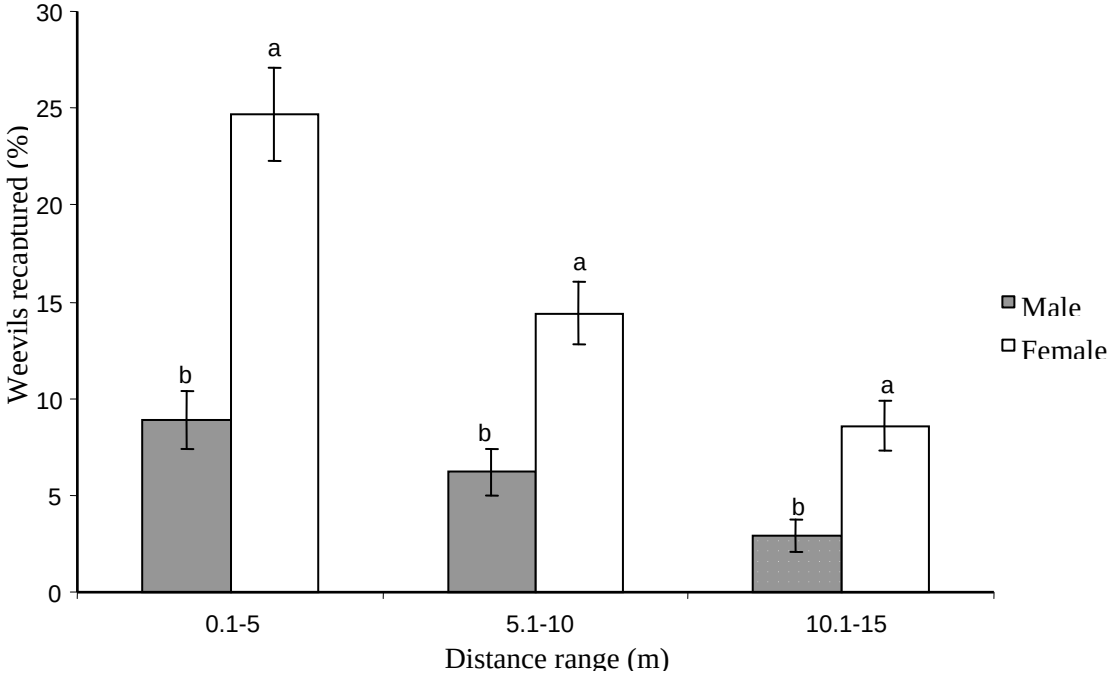


Figure 3.Percentage of male and female weevils recaptured following release at different distances from the pheromone trap. Different letters indicate that means of females and males recaptured from each distance category were significantly different (t-test, P<0.05).

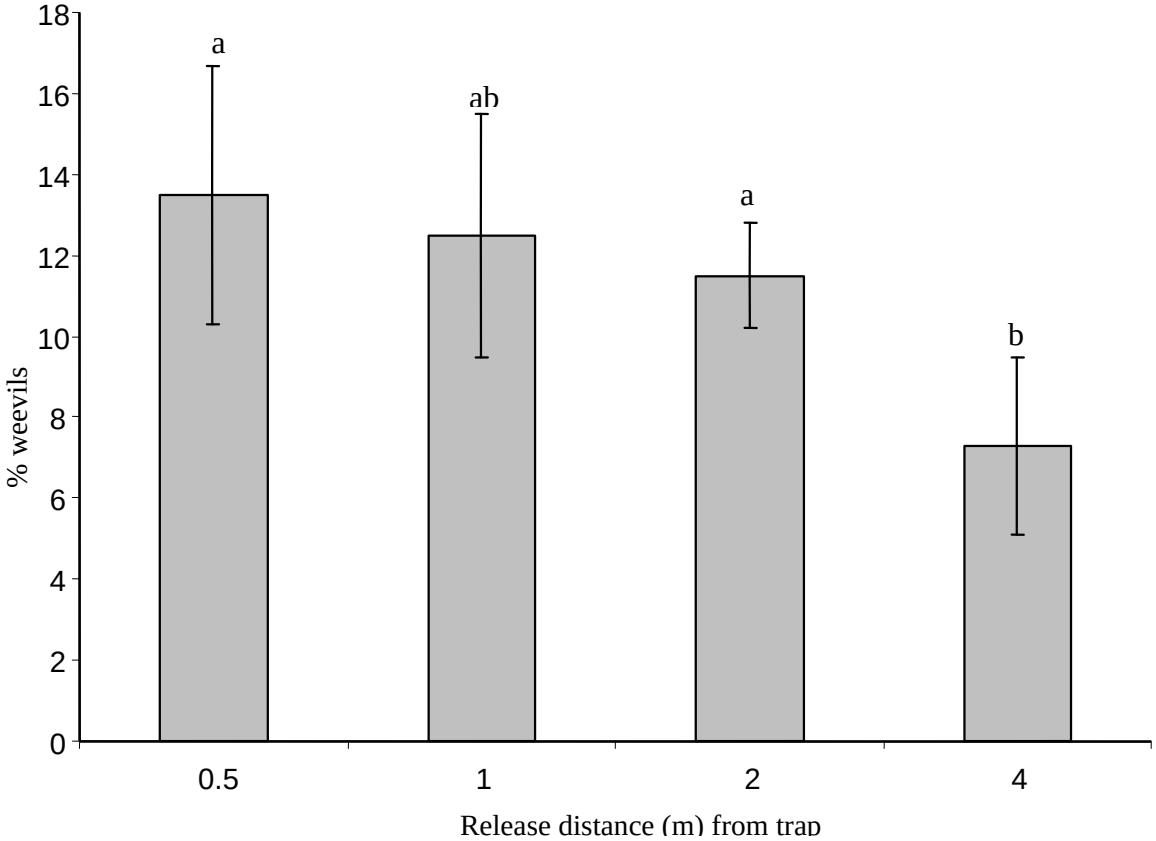


Figure 4: Mean (±SE) percentage number of weevils recaptured in pheromone-baited traps when released at different distances from the traps. Different letters indicate that means of weevils recaptured from each distance category were significantly different (SNK test, P<0.05).

### 3.2 **Environmental factors** (experiment 2)

The percentages of weevils recaptured from different directions relative to the pheromone-baited trap were similar (Table 1). Weevil attraction was not influenced by the direction of release relative to the pheromone trap. There was no association between sexes of recaptured weevils with the direction of release (P = 0.18, d.f. =3, $\chi 2$ = 5.098). A regression analysis of weevil catches in pheromone-baited traps with relative humidity ($R^2$=0.11, P=0.001) showed a significant, positive relationship (Figure 5). Rainfall ($R^2$ = 0.009, P=0.36), wind speed ($R^2$= 0.013, P=0.26) and temperature ($R^2$=0.026, P=0.11) did not show a significant relationship to weevil trap catches in pheromone-baited traps.

Table 1: The percentage of weevils recaptured in pheromone-baited trap in different directions from the point of release.

| Direction/location from trap | Number of weevils released * | Number of weevils recaptured | | |
|---|---|---|---|---|
| | | Males | Females | % of total |
| Pheromone trap mat | 40 | 4 | 10 | 35.0 |
| North | 200 | 4 | 19 | 12.0 |
| East | 200 | 9 | 14 | 12.0 |
| South | 200 | 5 | 14 | 9.5 |
| West | 200 | 8 | 18 | 13.0 |
| Total | 840 | 30 | 75 | 12.5 |

* Sex ratio =1:1. The association between sex of recaptured weevils with direction was not significant (P=0.17, d.f.=3, $\chi 2$=5.098, 4x2 contingency test).

### 3.3 **Covering the trap with banana leaves** (experiment 3)

*Covering the whole trap*: In all banana fields, uncovered pheromone-baited traps captured more weevils compared to wholly covered traps although the differences were statistically significant in only 2 out of 4 trials (P<0.05) (Figure 6). There were more weevil catches in pheromone traps in the first trial that was conducted in the rainy season than in the second trial that was conducted in the dry season at KARI.

*Covering around trap*: The pitfall-pheromone-baited traps where the covering was done only around the trap captured significantly (P< 0.05) more weevils than uncovered traps for both trials (Figure 7).

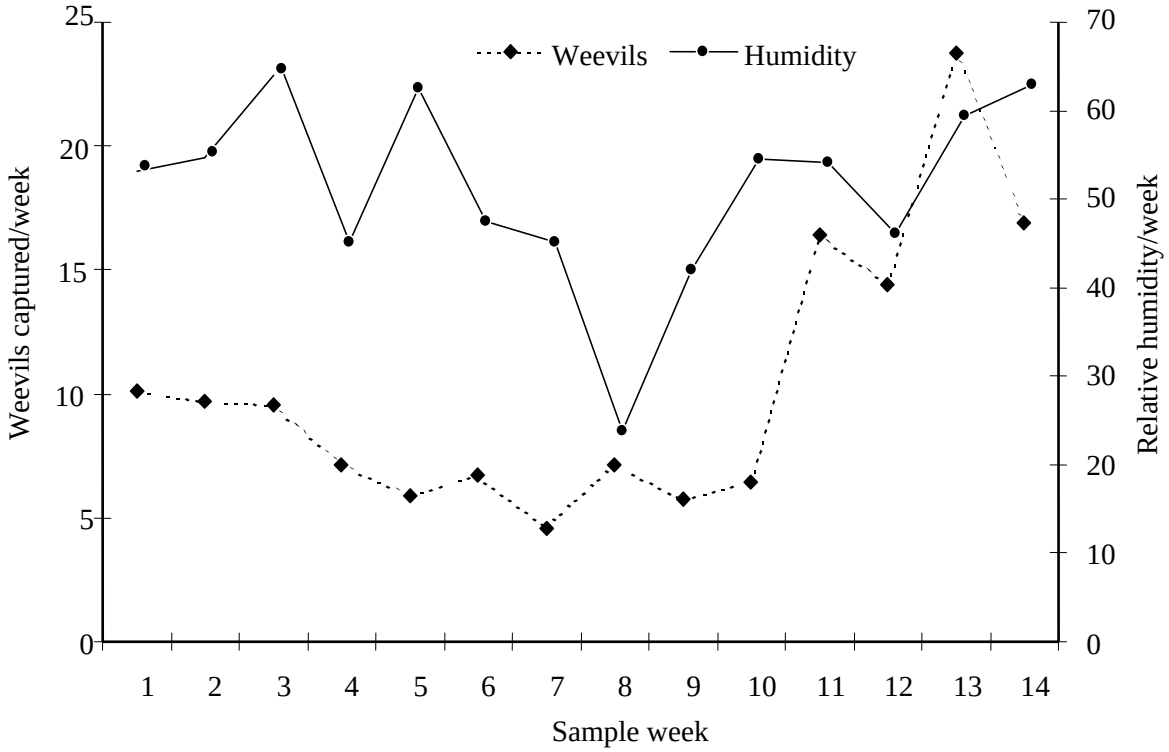


Figure 5: Variation of *C. sordidus* captured in pheromone-baited traps with relative humidity in the banana field at KARI ($R^2$=0.11 P=0.001).

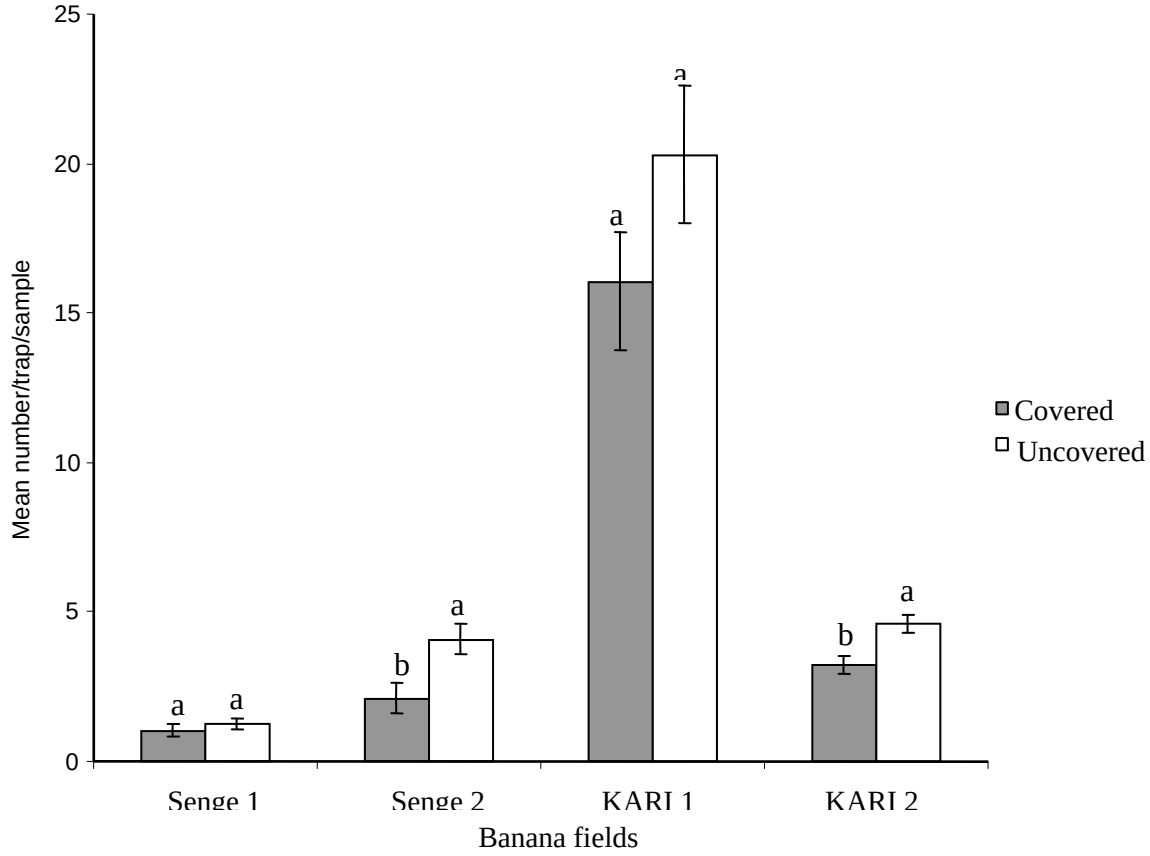


Figure 6: Mean (±SE) number of weevils caught in pheromone traps wholly covered by banana leaves compared to uncovered traps. Within a field, means with the same letter are not significantly different (t-test, P> 0.05).

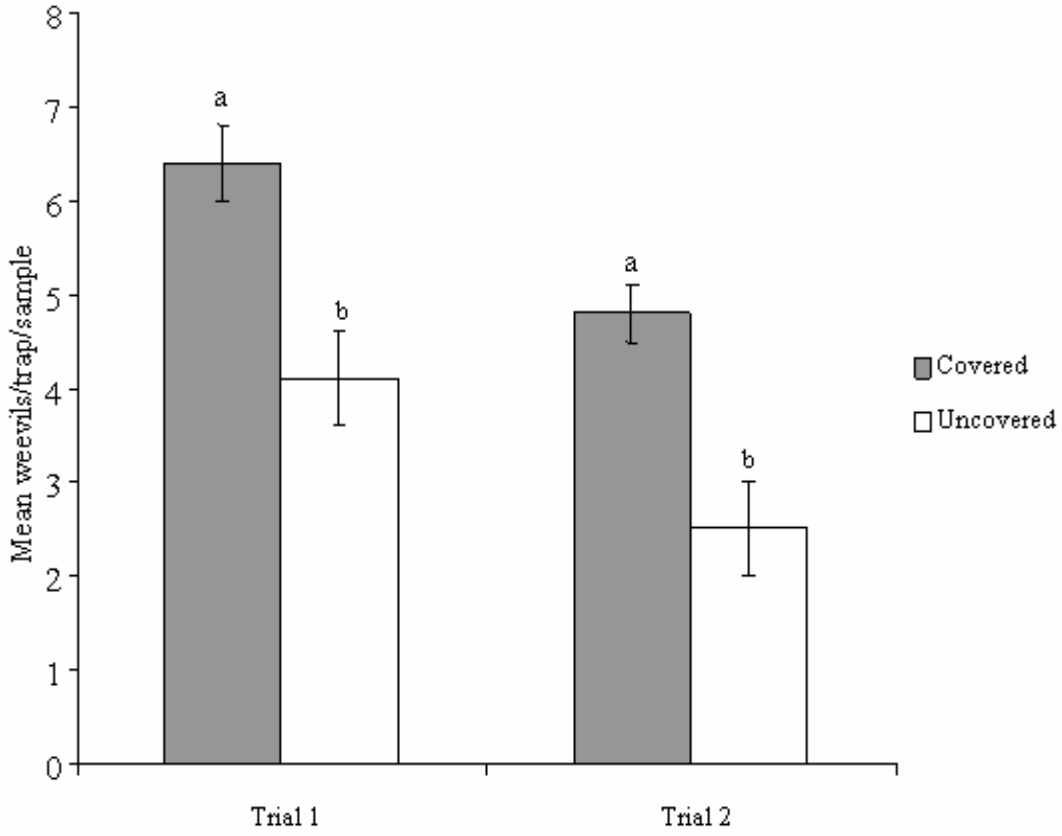


Figure 7: Mean number (±s.e) of weevils captured in pheromone-baited traps covered with banana leaves around the trap compared with uncovered traps. Means within a trial with similar letters are not significantly different (t-test, P>0.05)

**3.4 Trap location relative to the host plant** (experiment 4)

More weevils were collected in traps placed in the alleys (2.8 weevils/trap/day) than at the base of mats (1.7 weevils/trap/day) at KARI. There were significantly more females and males that responded to traps in alleys than on the mat in the KARI experiment (t-test, $P<0.05$). In Sendusu, however, similar numbers of weevils were caught in alleys (1.8 weevils/trap/day) and on the mats (1.7 weevils/trap/day). Relative trap catches on the mat and between mats were similar for both weevil sexes at Sendusu ($\chi 2=0.552$, d.f.=1, $P=0.35$) and at KARI ($\chi 2=0.025$, d.f.=1, $P=0.87$). Equal numbers of males and females were captured in traps that were placed in alleys and on the mats in the experiment at Sendusu (Figure 8).

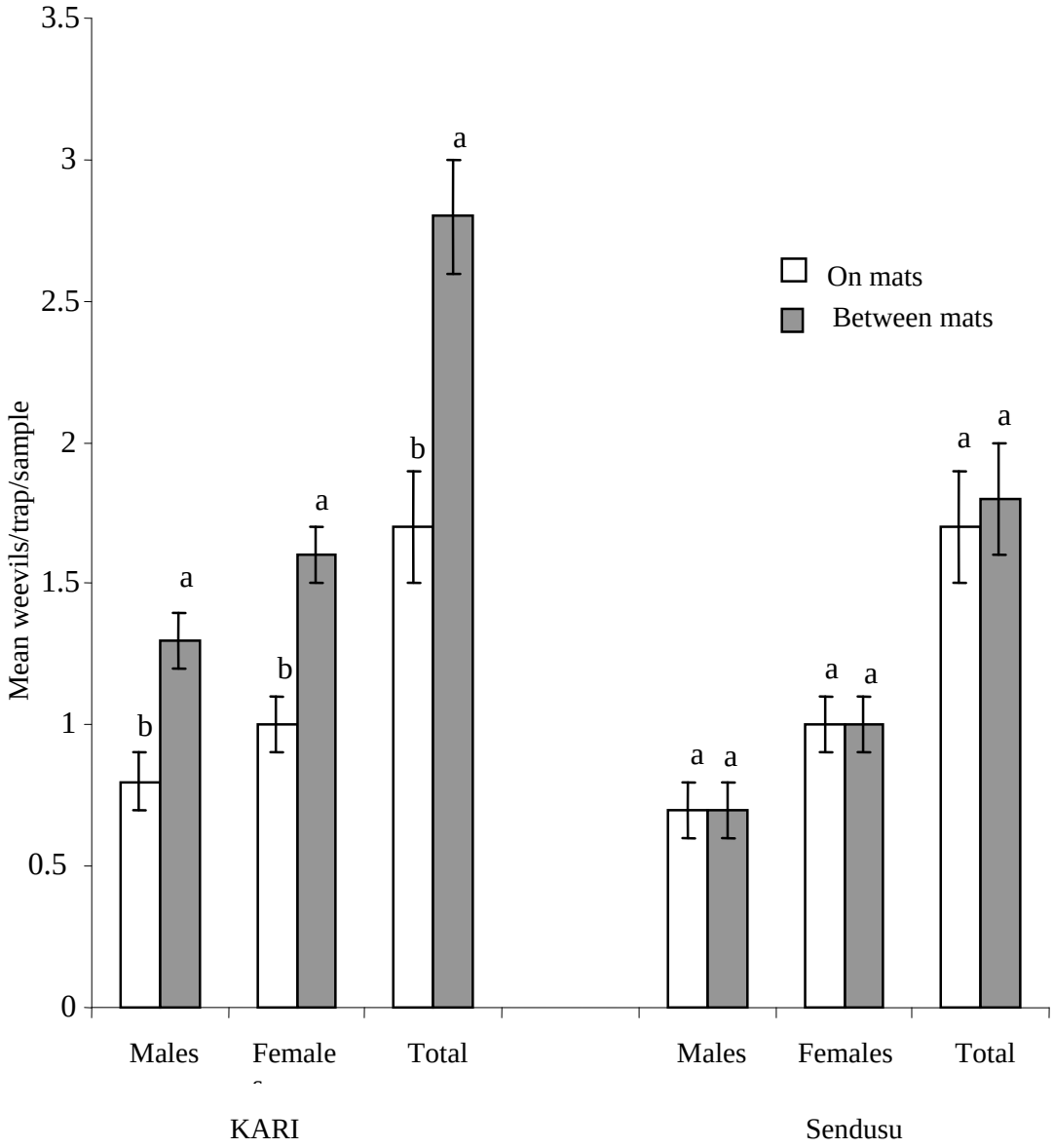


Figure 8: Mean number (±s.e) of weevils caught in pheromone traps at different locations relative to the host plant in banana fields at KARI and Sendusu. Within a weevil sex category means with the same letter are not significantly different (t-test, P>0.05).

# 4 DISCUSSION

Several factors (such as the pheromone efficacy, trap parameters and environmental factors) may influence the pheromone trap effectiveness (Schlyter, 1992; Byers, 1999; Jansson *et al.*, 1989; Laboke *et al.*, 2000; Sappington & Spurgeon, 2000; Murad, 2001). In the present study, we found that *C. sordidus* catches in pheromone traps were influenced variously by the distance from the trap, placement and trap type, and environmental factors (e.g relative humidity, wind speed and rainfall).

There were generally low proportions of released weevils that were recaptured from the different release distances. One possible explanation for such low recapture rates is that weevils may be attracted to within the vicinity of the trap but may not necessarily enter it (Tinzaara *et al.*, 2002b). An alternative explanation may be that a large proportion of the released weevils dispersed out of the attraction range. In our studies, there was a continuous decline of weevil catches with increasing release distance from the pheromone-baited trap. Previous studies estimated the pheromone trap effective attraction radius to range from 5-15 m using interference studies (A.C. Oehlschlager, pers. comm.). Our data suggests that the trap may be more effective in the range up till 10 m.

More females were attracted to the pheromone-baited traps than males in our studies. A sex ratio of captured insects which is skewed for females has been previously observed for weevils in the subfamily Rhynchophorinae (e.g *Metamasius hemipterus*) (C.A Oehlschlager, pers. comm.). As females are known to be more active and move greater distances than males as they search for oviposition sites and mates (Gold *et al*., 2001), more females than males could have been captured in pheromone-baited traps.

A relationship between climatic factors (rainfall, relative humidity and temperature) and pseudostem trap catches of *C. sordidus* was reported by Arleu (1982). Uzakah (1995) found weevil activity in the laboratory to be positively correlated to relative humidity and negatively correlated with temperature and light intensity. In our field studies, relative humidity but not rainfall showed a strong relationship with *C. sordidus* catches in pheromone-baited traps. Although weevils are known to be active in moist conditions (Gold *et al*., 2001), the dissemination of pheromone may be hampered during the rainy season. The mean temperature during our study was 19.5 $^{o}$C (±1.8 S.D). Temperature is known to increase the dissemination rate of the pheromone and insect activity (Mason *et al*., 1990; Howse *et al.*, 1998) and therefore a positive relationship with weevil catches was expected. However, no relationship between temperature and catches of *C. sordidus* in pheromone traps was observed. Also, there was no effect of wind speed on weevil catches in our studies, which may be attributed to

the wind speed being negligible at the soil level in the field. A non significant effect of wind speed on pheromone trap catches of the sweet potato weevil (*Cylas brunneus*) was reported in Uganda (Laboke *et al*., 2000). Recapture rates of weevils that were released in different (wind) directions from the trap were similar, suggesting that there was hardly any wind influence on weevil movement to the pheromone trap. The results may be attributed to the biology (sedentary, hidden in residues and corms) of the weevil.

Pheromone-baited pitfall traps that were not covered with banana leaves tended to capture more weevils than traps that were wholly covered. Our results suggest that covering the pheromone-baited trap interferes with the pheromone dissemination and hence affected the number of weevils that respond. Possible hindrance of the weevils to enter the traps could also have led to the lower catches in traps covered with banana leaves. Studies conducted in Australia argued that coving with fresh leaves keeps the area around the trap moist to encourage movement of the weevil and eventual capture in traps (Murad, 2001). When considering moisture, our experiment was conducted both in the wet and the dry season, and in both conditions, uncovered traps captured more weevils than covered traps. This suggests that moisture played a non significant role in influencing the number of weevils captured in covered pheromone traps.

When the experiment was conducted with the covering done only on soil around the pheromone-baited trap, higher numbers of weevils were captured in covered compared to uncovered traps. This result agrees with observations from Canary Islands (A. Carnero, pers. comm.) and from Australia (Murad, 2001). The possible explanation is that covering may maintain moisture around the trap and may also limit creation of gaps between the trap and the soil on drying which may prevent weevil entry into traps.

Placement of pheromone traps near or away from the host plant may influence trap catches depending on the relationship between host and mate finding (De Groot and Debarr, 1998). We thought that if a pheromone trap is placed near a host plant, volatiles from the host plant may synergise or enhance trap catches. In our study, there was no evidence to support our expectation of finding more weevils on the mats. Weevils are usually near banana mats which are associated with sites for oviposition and mates (Bakyalire, 1992; Gold *et al*., 2004b).

In general, our study demonstrated that trap parameters as well as environmental factors influence the numbers of *C. sordidus* captured in pheromone baited traps. An understanding on how these factors influence trap catches can assist in effective trap deployment for the control of *C. sordidus*.

**Acknowledgements**

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Chapter **6**

# The effect of mulching on banana weevil, *Cosmopolites sordidus*, movement relative to pheromone-baited traps

*W. Tinzaara, C.S. Gold, M. Dicke, A. van Huis & P.E. Ragama*

## Abstract

A study was conducted in Uganda to determine the effect of mulching on banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), movement relative to pheromone-baited traps. Three banana treatments were used to create different mulching levels: (1) bananas without mulch (control); (2) bananas with thin mulch (< 3 cm thick); and (3) bananas with thick mulch (6 cm thick). Pheromone-baited traps were placed in the plots and weevil trap catches were monitored. Weevil catches in pheromone-baited traps from both mulched and unmulched plots were generally similar. The mulching level did not influence the ratio of males to females recaptured. The numbers of weevils captured in pheromone traps at different distances from their release point were lower in the dry season than in the wet season and they were not influenced by mulch levels. Mulching levels in plots did not influence the numbers of weevils recaptured from different directions. The results generally indicate that mulching had no effect on weevil catches in pheromone-baited traps. Mulching is therefore compatible with the use of pheromone-baited traps in the control of the banana weevil.

# 1 INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), is a major pest of bananas in East Africa. Weevil larvae cause damage by boring into the corm which results in reduced nutrient uptake of the plant. The adult's biology is characterised by nocturnal activity, hydrotropism, long life span (up to four years), low fecundity and limited mobility (Gold *et al*., 2001). The insect rarely flies and moves only short distances by walking. The weevil tends to be more active in moist than in dry conditions and to move further in mulched than unmulched systems (Gold *et al*., 1999b). Mulched systems often support larger populations than unmulched systems (Price, 1993; Rukazambunga *et al.*, 2002).

Mulching has been a widely recommended practice to farmers in Uganda as a means of conserving moisture and reducing soil erosion in banana plantations. For example, Rukazambuga *et al.* (2002) found a yield increase in mulched farms. Nevertheless, the banana weevil pest status may be greater in mulched than unmulched systems. Yield loss to banana weevil was 14 tonnes/ha in mulched systems compared to 8 tonnes/ha where mulching was not applied (Rukazambuga *et al*., 2002).

The use of the banana weevil aggregation pheromone Cosmolure+ is currently being studied in Uganda for the control of *C. sordidus.* Results of laboratory and field experiments are promising (Tinzaara *et al*., 2000, 2003). The pheromone trap efficiency is also influenced by an interaction of the cropping system (including mulching) and the biology of an insect pest (Hebblethwaite, 1989). Mulching influences weevil movements in banana plantations (Gold *et al*., 1999b), but to what extent this would affect pheromone trap catches of *C. sordidus* is not known. Therefore, the influence of farm management practices such as mulching on pheromone trap catches needs to be studied.

Mulching may have several effects on efficacy of pheromone lures in attracting *C. sordidus*. First, the mulch may impede dissemination of the pheromone through the field, as well as the weevil's ability to detect the pheromone's presence (i.e. the mulch may put an extra layer between the weevil and the odour). Second, weevils tend to be more active and move further in mulched fields, presumably due to differences in soil moisture. Such activity may make weevils more responsive to the lures, as well as increase the likelihood of the weevils coming into contact with it (i.e. move through the effective range of the lure).

The objectives of this study were to: (i) determine the effect of mulching on number of weevils captured in pheromone-baited traps; (ii) determine distances moved by weevils relative to the

pheromone-baited traps in mulched and unmulched plots; and (iii) determine the effect of direction from the pheromone-baited trap on weevil catches in mulched and unmulched plots.

# 2 MATERIALS AND METHODS

## 2.1 Site description

The experiments were conducted in a field trial at the IITA Sendusu Farm ($0^{o}32'$N, $32^{o}35'$E, 1260 metres above sea level), located 25 km north east of Kampala, Uganda. The site has two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and daily mean temperature of $21^{o}$C.

## 2.2 Experimental design

Pheromone efficacy in attracting *C. sordidus* under different mulching regimes was studied in a trial planted at Sendusu. The trial consisted of three treatments: (1) bananas without mulch (control); (2) bananas with thin mulch; and (3) bananas with thick mulch (see next paragraph for details). Plots (306.3 $m^2$) consisted of 7 rows of 7 banana mats (*Musa* spp., cv Kibuzi, AAA-EA type) planted in a 2.5 x 2.5 m arrangement. Plots were separated by 5 m alleys. The treatments were placed in a completely randomised design with four treatments.

## 2.3 Field history, planting and management

The experimental field was previously planted with banana cultivar Atwalira (*Musa* spp, AAA-EA type) which was wiped out by weevil infestation. The field was sprayed with Chlorpyrifos in August 2002 before ploughing. The field was planted in October 2002. The planting material was obtained from farmers' fields in the Masaka District, Uganda. Prior to planting, the pseudostems were cut 15 cm above the collar. The suckers were then pared (cleaned by chopping off roots and the outer layer of the corm) and those showing heavy weevil damage were rejected. The remaining suckers were immersed in a solution of Chlorpyrifos (1.5 ml per litre of water) for 30 minutes to reduce banana weevil and nematode infestations (Tinzaara *et al*., 2002c). Suckers were placed 10 cm below the soil surface in planting holes (60 cm diameter, 60 cm deep) containing soil and 20 kg of cow dung manure. Replanting where suckers did not germinate was done in December 2002. Plant density was maintained at three plants per mat. Weeds were controlled by spraying with Roundup (glyphosate) every 2-3 months. Plant desuckering and detrashing (removal of old leaves) were conducted when needed.

Grass mulches (mixtures of *Panicum maximum, Imperata cylindrica* and *Brachria spp.*) were first applied when the crop started to flower (May 2003). Five trailers (200-300 kg per trailer) of mulch were applied in the treatment (per plot) for thick mulch and two trailers of mulch were applied in the treatment for thin mulch. Supplementary mulching was done every four months using the mulch material in the ratio of 5: 2: 0 for thick, thin and no mulch respectively. The mulch was approximately 6 cm and < 3 cm thick for thick and thin mulch respectively. No mulch was applied in the control plots.

### 2.4 *Cosmopolites sordidus* release

Adult *C. sordidus* were collected from farmers' fields in Masaka District using pseudostem traps (Mitchell, 1978). Weevil sex was determined using curvature of the last abdominal segment (Roth & Willis, 1963) and punctuation on the rostrum (Longoria, 1968). Prior to release, weevils were marked by making scratches on the elytra with distinct marks for each banana mat per treatment. Weevils were released in the evening (7.00-8.00 pm) at the base of the mat in each plot by placing them in shallow holes around the base of each mat. Ten weevils (5 females and 5 males) were released per mat. The distance of mats from the pheromone-baited trap was recorded.

### 2.5 Pheromone source and trap placement

Cosmolure+ (Chemtica International, San Jose, Costa Rica) was used as a pheromone lure in all experiments. It is comprised of a polythene pack of pure sordidin (1S,3R,5R,7S)-(+)-1-ethyl-3,5,7-trimethyl-2,8-dioxabicyclo[3.2.1]octane) (Mori *et al*., 1996) that is released at 3 mg / day (A.C. Oehlschlager, pers. comm.). For storage, packs were tightly sealed and kept in a dark cupboard at 21-25 $^{0}$C. After a container with pheromone had been opened, the pheromone packs were stored in the freezer at –5 $^{o}$C.

Banana weevil aggregation pheromone was placed in each plot in pitfall traps. A pitfall trap prepared out of a 10-litre bucket was used in this experiment (Tinzaara *et al*., 2000). The trap was placed at the base of the banana mat that was at the centre of the plot, one week after releasing the weevils. The pheromone lure was hung from the top of the bucket. One trap per plot was placed at the central mat. Traps were checked every three days to make sure that they flushed well with soil level. A liquid detergent solution (1 litre) was placed in the trap as a trapping agent and was changed every five days. Pheromone traps were first placed during the wet season in August-October 2003 (trial 1). The experiment was repeated in the dry season between December 2003 and February 2004 (trial 2). Between trials, split pseudostem traps were placed on each of the banana

mats in the plot (two pieces per mat) once per week to remove weevils and reduce existing populations.

## 2.6 Sampling and data collection

*2.6.1 Pheromone trap catches and sex ratio of weevils*

Pheromone-baited traps were checked every three days for 60 and 66 days for trials 1 and 2 respectively. Recaptured weevils were recorded, placed in vials and taken to the laboratory for differentiation according to their sex and mat of release.

*2.6.2 Weevil movement relative to the pheromone-baited trap*

The number of weevils recaptured in pheromone-baited traps within 18 days of trap placement was used to determine weevil movement relative to the trap in plots of different mulch levels. Numbers of weevils recaptured in pheromone-baited traps were recorded according to the mat of release. To calculate distances moved by weevils, each banana mat in the plot was allotted co-ordinates. Data on weevil captures were grouped before analysis according to distance ranges moved relative to the trap.

*2.6.3 Effect of direction on weevil catches*

The direction of banana mats relative to the pheromone-baited traps on which weevils were released was recorded. The direction from where the weevils came was determined only for the weevils found in pheromone-baited traps in the first 18 days of pheromone trap placement. The data were then grouped into direction quarters represented as East (SE -NE), North (NE-NW), West (NW-SW) and South (SW-SE) for analysis.

## 2.7 Data analysis

The mean weekly number of weevils captured from plots of different mulch levels was analyzed using ANOVA of SAS (1990) and means were separated using a Student-Newman-Keuls (SNK) test. The association of weevil trap catches with direction and distances moved in the different mulch levels was analyzed using a contingency table test on numbers, followed by Bonferroni correction, $\alpha = 0.05/3 = 0.017$, for multiple comparisons for those that showed significant differences.

# 3 RESULTS

## 3.1 Pheromone trap catches and sex ratio of weevils

*In the wet season (Trial 1),* significantly more weevils (marked and unmarked) were captured in pheromone-baited traps in thick mulch plots in the first week than in thin and no mulch plots ($P < 0.05$) (Figure 1). In all subsequent sampling weeks the catches were similar among treatments ($P > 0.05$). There was an observed decline of weevil catches with time from all mulch levels. The total number of marked weevils that were recaptured from thick mulch and thin mulch plots were significantly higher than from unmulched plots ($\chi^2 = 18.4$, d.f. = 2, $P = 0.0001$) (Table 1). More females were captured in mulched (=thin or thick mulch) plots than control plots ($\chi^2 = 19.7$, d.f. = 2, $P = 0.0001$), while for males the numbers recaptured between treatments were similar ($\chi^2 = 1.49$, d.f. = 2, $P = 0.48$).

*In the dry season (Trial 2)***,** weevil catches were similar for the three mulching levels ($P > 0.05$) in all sampling weeks except in the seventh week when the catches were higher from no mulch plots compared to thin mulch plots ($P < 0.05$) (Figure 1). There were no differences in total numbers of weevils recaptured from plots of different mulch levels ($\chi^2 = 2.36$, d.f. = 2, $P = 0.31$) (Table 1). The numbers of males ($\chi^2 = 0.15$, d.f. = 2, $P = 0.93$) and females ($\chi^2 = 2.73$, d.f. = 2, $P = 0.26$) recaptured from the different mulch levels were similar.

*Comparison between seasons (Trial 1 and 2)***.** The total numbers of weevils captured in pheromone traps were higher in the wet (trial 1) than in the dry season (trial 2) for thick ($\chi^2=68.9$, d.f.=1, $P<0.001$), thin ($\chi^2 = 51.0$, d.f. = 1, $P < 0.0001$) and no mulch levels ($\chi^2 = 8.7$, d.f. = 1, $P = 0.003$) (Table 1). The ratio of males to females recaptured in both seasons for each of the mulch levels was similar. In all instances more females than males were recaptured ($P < 0.05$).

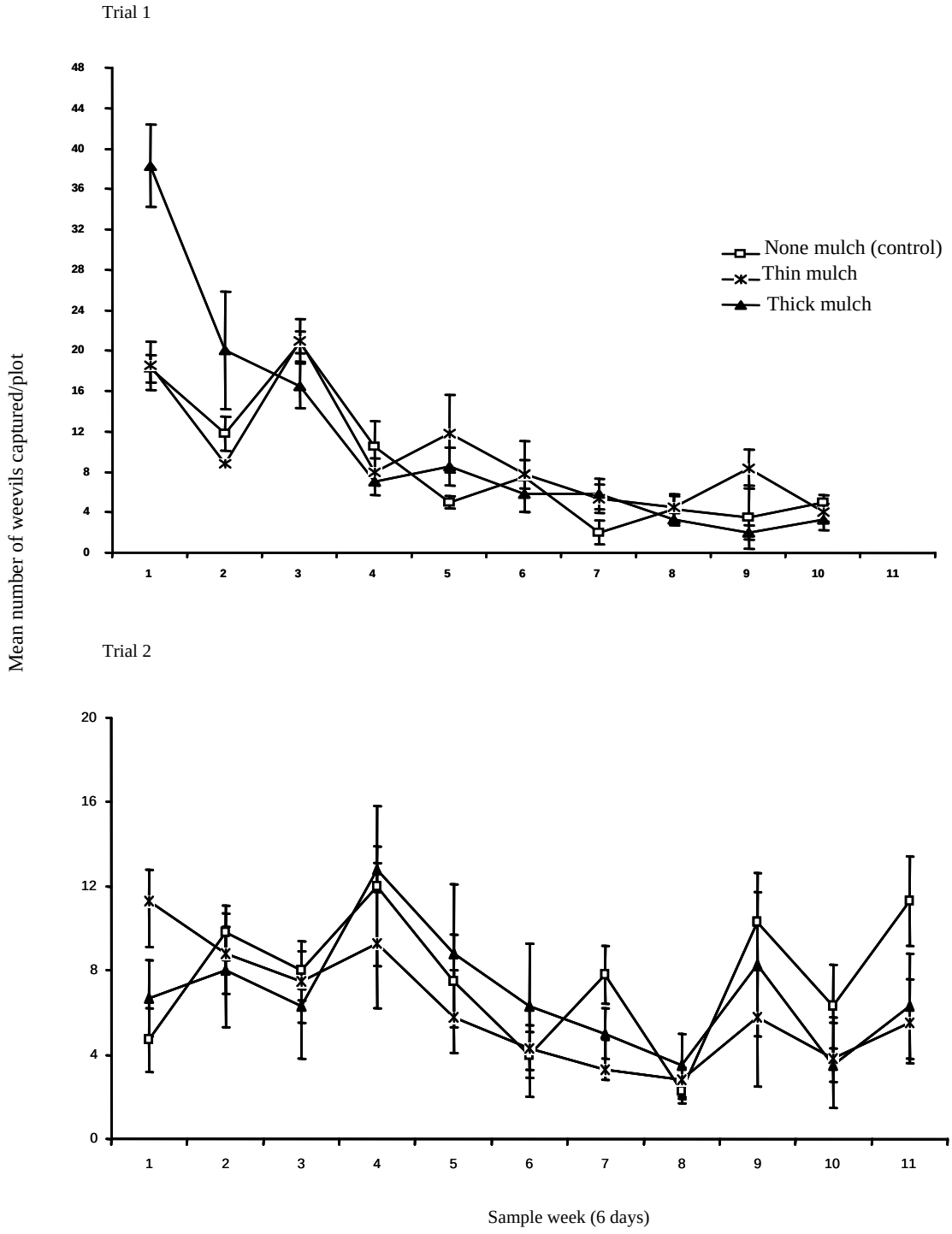


Figure 1: Mean number (± S.E.) of banana weevils captured in pheromone-baited traps in mulched and unmulched plots during the wet (trial 1) and the dry season (trial 2).

Table 1: Total number and sex ratio of weevils attracted to pheromone-baited traps in plots of different mulching levels (after 60 and 66 days for trial 1 and 2 respectively) at Sendusu, Uganda. In each treatment, a total of 490 marked weevils (245 males and 245 females) were released per plot, one week before a pheromone-baited trap was placed in the centre of each plot.

| Trial | Treatment: | Number of weevils captured | | | | Male: |
|---|---|---|---|---|---|---|
| | Mulch layer | Males[1] | Females[1] | Unmarked | Total recaptured[1] | females[2] |
| 1 (Wet season) | Thick | 67a | 158a | 209 | 225a | 1 : 2.4 |
| | Thin | 61a | 143a | 184 | 204a | 1 : 2.4 |
| | None (control) | 54a | 94b | 167 | 148b | 1 : 1.7 |
| 2 (Dry season) | Thick | 17a | 67a | 148 | 84a | 1 : 3.9 |
| | Thin | 19a | 66a | 121 | 85a | 1 : 3.4 |
| | None (control) | 19a | 83a | 126 | 102a | 1 : 4.4 |

[1]Numbers in a column, per trial, followed by the same letter are not significantly different (contingency table test followed with Bonferroni correction of $\alpha = 0.05/3 = 0.017$ for multiple comparisons).
[2]The ratio of males to females recaptured was significantly different from 1:1 in all mulch levels ($P<0.05$, contingency table test).

### 3.2 Weevil movement relative the pheromone-baited trap

Of weevils released at distances of 3.1- 6.0 m and 6.1-9.0 m from the pheromone-baited trap more individuals were recaptured in the thick mulch than in the no-mulch treatment during the wet season (trial 1) (Table 2). Recapture of weevils from other distances from the trap were similar for the different mulch treatments. During the dry season (Trial 2) the recapture of weevils per release distance were similar between mulch levels (Table 2). The number of weevils recaptured per release distance was higher in the wet season (trial 1) than in the dry season (trial 2).

### 3.3 Effect of direction on trap catches

During the wet season (trial 1) the numbers of weevils that were recaptured in pheromone traps from the south and east directions from the trap were larger than those that were recaptured from north and west directions in all mulch levels (Table 3). More weevils were recovered in thick mulch than no mulch plots for the eastern and western directions. The numbers of weevils recovered from mulched and unmulched plots were similar for the southern and northern directions. During the dry season (trial 2), the numbers of weevils recaptured in mulched and unmulched plots were similar for southern, eastern and western directions (Table 3), but for the northern direction more weevils were recovered from thick mulch than no mulch plots.

Table 2: Number of weevils recaptured in pheromone-baited traps after release at different distances from the traps in mulched and unmulched plots during the first 18 days of trap placement.

| Trial | Release distance from pheromone trap (m) | Number of weevils released | Number of weevils recaptured from a distance | | | P (Contingency table test) |
|---|---|---|---|---|---|---|
| | | | Thick mulch | Thin mulch | None mulch | |
| 1 (Wet season) | 0.0- 3.0 | 200 | 32 | 18 | 24 | 0.1 |
| | 3.1- 6.0 | 640 | 62a | 39ab | 38b | 0.014 |
| | 6.1- 9.0 | 960 | 64a | 41ab | 38b | 0.011 |
| | 9.1-12.0 | 160 | 9 | 8 | 2 | 0.094 |
| 2 (Dry season) | 0.0- 3.0 | 200 | 8 | 2 | 4 | 0.13 |
| | 3.1- 6.0 | 640 | 14 | 17 | 24 | 0.22 |
| | 6.1- 9.0 | 960 | 15 | 14 | 14 | 0.98 |
| | 9.1-12.0 | 160 | 1 | 6 | 2 | 0.09 |

Numbers in a row that are followed by the same letter are not significantly different (contingency table test followed with Bonferroni correction of $\alpha = 0.05/3 = 0.017$ for multiple comparisons).

Table 3: Percentage of weevils recaptured from different directions relative to the pheromone-baited trap in plots with different mulching levels. The number of weevils released per direction in each of the four plots was 480 weevils (240 males: 240 females) while 10 weevils were released per pheromone trap mat.

| Trial | Direction of weevil recaptured | % of weevils recaptured | | | P(Contingency table test) |
|---|---|---|---|---|---|
| | | Thick mulch | Thin mulch | None mulch | |
| 1 (Wet season) | Pheromone trap mat | 25.0 | 20.0 | 17.5 | 0.70 |
| | South | 14.2 | 11.9 | 9.8 | 0.11 |
| | East | 12.5a | 9.4ab | 7.0b | 0.017 |
| | North | 7.9 | 4.2 | 5.2 | 0.036 |
| | West | 7.9a | 6.0ab | 3.5b | 0.015 |
| 2 (Dry season) | Pheromone trap mat | 2.5 | 5.0 | 5.0 | 0.81 |
| | South | 3.3 | 3.1 | 9.0 | 0.04 |
| | East | 6.0 | 6.3 | 5.4 | 0.85 |
| | North | 2.9b | 5.4ab | 8.8a | 0.0003 |
| | West | 5.0 | 2.5 | 4.6 | 0.11 |

Direction quarters represent East (SE -NE), North (NE-NW), West (NW-SW) and South (SW-SE). Numbers in a row followed by similar letters are not significantly different (contingency table test analysis on numbers followed with Bonferroni correction of $\alpha = 0.05/3 = 0.017$ for comparisons).

# 4 DISCUSSION

The amounts of mulch in plantations have been reported to affect activity patterns of *C. sordidus*. Gold *et al.* (1999b) found greater weevil movement in mulched than unmulched banana stands. Rukazambuga *et al.* (2002) and Price (1993) recaptured more weevils in mulched banana plots compared to unmulched plots. This is likely to reflect increased survivorship of adults and/or altered movement patterns (i.e. tenure time) within the field as a result of mulching (Rukazambuga, *et al.*, 2002). This may have been mediated by a better micro-environment (higher soil moisture levels, more constant temperature and relative humidity, and improved refuge against enemies) in mulched plots. In our study, weekly pheromone trap catches from mulched and unmulched plots were generally similar. However, the total numbers of weevils recaptured in pheromone-baited traps in mulched plots were higher in thick mulched plots than unmulched plots in the wet season but catches were similar in the dry season (Table 1). The data did not agree with our hypothesis that mulching may affect trap catches. The higher recapture in thick mulch plots during the wet season could be related to the higher weevil activity and movement during the wet conditions. Weevils are active in wet/moist conditions and become sedentary during dry conditions (Gold *et al.*, 1999b) and their ability to detect pheromones may be decreased.

Equal numbers of males and females were released in our trials. In both mulched and unmulched plots, more females than males were recaptured. Similarly, Delattre (1980) caught more female weevils than males in pseudostem traps in the field during the rainy season when moisture was high. Mulching did not affect the sex ratio of the captured weevils. Female weevils may move more and may have greater activity as compared to males as they search for oviposition sites and mates (Gold *et al.*, 1999b). Indeed, it has been reported that female weevils are more attracted by the male produced aggregation pheromones than males (Budenberg *et al.*, 1993b; Tinzaara *et al.*, 2004c).

Weevils are reported to be more active and move longer distances in mulched than unmulched areas (Gold *et al.*, 1999a). Our results indicate that more weevils were captured from thick mulch than no mulch plots only in the distance ranges of three to nine meter during the wet season. In the dry season there was no effect of distance on the weevil catch. Therefore, the effect of mulch level on the distance the weevil covered towards the pheromone baited traps was not substantial. The results do not support the hypothesis that during the wet season the pheromone may adsorb to mulch leading to reduced catches in the traps. The numbers of weevils captured in pheromone traps from different distances in the dry season were lower than in the wet season and not influenced by

mulch levels. This implies that weevils are less active during the dry season. Weevils become sedentary in the dry season (Gold *et al*., 2001) and chances of coming in contact with the pheromone lures are low.

There was a limited effect of mulch level on the direction from which weevils were recaptured relative to the pheromone-baited traps. Weevil movement from a given direction relative to the pheromone trap has an association with the wind direction. The results of our study imply that wind direction did not substantially influence trap catches. Our recent studies showed that wind speed was reported to have a limited effect on weevil trap catches (Tinzaara *et al*., 2004c). Moreover, wind speed at the ground level in banana plantations is expected to be low.

The proportion of adult *C. sordidus* recaptured in pheromone traps in the different mulch levels was 7-11% in the wet and 4-5% in the dry season. Factors that contribute to the low pheromone trap catches in our banana cropping system have been discussed (Tinzaara *et al*., 2004c). Trap catches are likely to be influenced by pheromone efficacy. However, since the pheromone traps in this study were not very effective in recapturing high numbers of weevils, the effect of mulching on trap catches may have been underestimated.

The results of this study generally indicate that mulch levels did not have a substantial effect on weevil catches in pheromone-baited traps. The different mulch levels did not have an effect on the sex ratio of weevils captured. Mulch level had a non significant effect on the distances moved by weevils towards the pheromone-baited traps. Although the number of weevils caught in pheromone traps in the dry season was numerically lower, no effect of mulching was observed on trap catches. Mulching therefore is compatible with use of pheromone traps in banana plantations.

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Chapter 7

# Effects of two pheromone trap densities against banana weevil, *Cosmopolites sordidus*, populations and their impact on plant damage in Uganda

*W. Tinzaara, C.S. Gold, G.H. Kagezi, M. Dicke, A.van Huis, C.M. Nankinga, W. Tushemereirwe & P.E. Ragama.*

## Abstract

An on-farm study to evaluate the effect of pheromone trap density on the population of the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) was conducted in the Masaka district, Uganda. The pheromone used was the weevil's aggregation pheromone that is commercially available. Forty-two farms were assigned to one of three treatments: 0, 4 and 8 pheromone traps/ha. Pheromone lures were changed monthly at which time the traps were moved to a different location within the stand. Adult weevil population densities were estimated by using mark and recapture methodology at 0, 6, 12, 18 and 21 months, while damage to the banana corm was assessed at 0, 3, 6, 12, 18 and 21 months since the start of the experiment. Pheromone trap captures were generally low: about 10 weevils per trap per month. There was no significant differences in mean catches of C. *sordidus* per trap per month except for February 2002 where doubling the pheromone trap density decreased weevil catches. Although not significant, decreased efficiency was also the trend in higher trap densities over all the data sets. Doubling the number of traps however increased the number of weevils caught per ha per month from 11.2% to 18.4%. There was no significant difference in plant damage between the pheromone treatments in low compared to high trap densities. Possible reasons for the low trap efficacy in this study are discussed.

# 1 INTRODUCTION

Trapping with pseudostems to control the banana weevil, *C. sordidus*, has been recommended by national research and extension programmes (Gold *et al.,* 1993; Ndege *et al*., 1995) although the efficacy of this method remains a subject of controversy (Gowen, 1995). Nevertheless, reductions in *C. sordidus* populations following trapping with pseudostems have been reported by Koppenhofer *et al*. (1994), Masanza (1995), Ndege *et al.* (1995), Seshu Reddy *et al*. (1995), Ngode (1998) and Gold *et al*. (2002). Yet, farmers' adoption of this method has been limited due to trapping materials not being available (Gold *et al*., 2002), high labour requirements (Seshu Reddy *et al*., 1999; Gold *et al*., 2002), and lack of confidence in its effectiveness (Ssennyonga *et al*., 1999).

The limitations associated with pseudostem trapping have led to attempts to develop more effective means of trapping, such as the use of synthetic pheromones. Budenberg *et al*. (1993b) were the first to report evidence for an aggregation pheromone released by male *C. sordidus.* Subsequently, Beauhaire *et al*. (1995) isolated a fraction of the major component of the pheromone, confirmed its bioactivity, named it sordidin and elucidated its structure including stereochemistry. Ndiege *et al*. (1996) and Jayamaran *et al.* (1997) developed a large-scale synthetic racemic sordidin that made field-testing possible. Currently, the pheromone is synthesized in Costa Rica by Chemtica International and sold as lures under the trade name Cosmolure+. This pheromone has been recently tested in the laboratory and in the field for *C. sordidus* response (Tinzaara *et al*., 2002b, 2003).

Compared to pseudostem trapping, pheromone lures have the advantage of increased trap efficiency with less labour requirement. Pseudostem traps normally last for only 3-7 days and require frequent visits to remove and destroy adult *C. sordidus,* which may enter and leave the traps (Gold *et al*., 2001). By contrast, pheromone lures last one month. These are most commonly placed above pitfall traps that drown entering weevils (Alpizar *et al*., 1999; Tinzaara *et al*., 1999a).

The use of aggregation-pheromone lures for trapping adult *C. sordidus* has been reported in Costa Rica (Alpizar *et al*., 1999) and in Uganda (Tinzaara *et al*., 1999a) as a promising option. These researchers employed a mass trapping strategy using four pheromone-baited pitfall traps per ha, in which traps were initially spaced in a single line 20 m apart and 10 m from the border of the plantation. Each month the traps received new lures and were moved 20 m further into the banana stand. This procedure reduced corm damage by more than 60% in 4-5 months, while bunch weight increased by 20% (Alpizar *et al*., 1999).

This trapping method (Alpizar *et al*., 1999; Tinzaara *et al*., 1999a) employs three tacit assumptions: (1) in one month the traps remove a high proportion of the weevils within 20 m of the

trap; (2) adult *C. sordidus* are sedentary and there is limited immigration of weevils to those parts of the field that have earlier been treated by trapping; (3) there is a low reproductive potential leading to limited population build up. Studies in Uganda have also shown that *C. sordidus* is a sedentary insect although some adults may move up to 60 m in five months (Gold *et al*., 2001).

*Cosmopolites sordidus* is an important pest of highland cooking banana (*Musa* spp, AAA-EA) in East Africa. The results from the study by Alpizar *et al* (1999) suggest that pheromones might offer promise in helping control this pest in this region. Although *C. sordidus* biotypes may exist (Ochieng, 2001), preliminary on-station trials in Uganda showed Cosmolure+ lures to be highly attractive to *C. sordidus* (e.g. collecting up to 18 times as many weevils as pseudostem traps) (Tinzaara *et al.*, 1999a).

In Africa, cropping systems are more diverse and management less intense than in Costa Rica, suggesting that field conditions affecting pheromone trap efficacy may not be comparable. An additional constraint to using pheromones in Africa is that they are not manufactured locally, entailing high costs and problems with importation, distribution and storage. High pheromone trap densities and high trapping frequency are often needed to obtain satisfactory levels of pest population suppression (Wolf *et al*., 1971; Lloyd *et al.*, 1981). Yet, Alpizar *et al.* (1999) employed a relatively low number of traps, i.e. 4 per hectare, to control the banana weevil in Costa Rica. For the American palm weevil, *Rhynchophorus palmarum* (L.), Oehlschlager *et al*. (1992) suggested that a low trap density (2 traps/ha) would be as effective as a high density (6 traps/ha). In contrast, with low population levels, a density of 2.5 traps /ha in cotton fields resulted in a high rate of elimination of the boll weevil, *Anthonomus grandis grandis* Boheman (Legget *et al*., 1989). There are no data available on the effect of pheromone trap density on *C. sordidus* populations in Ugandan conditions. Therefore, the objective of this study was to evaluate the effectiveness of two densities of pheromone traps in reducing weevil populations and damage in farmer's fields.

# 2 MATERIALS AND METHODS

## 2.1 Site description

The study was conducted in Kiseeka sub-county, Masaka district situated 30 km southwest of Masaka town, Uganda from August 1999 to March 2002.. The site was at 1200-1300 m above sea level with two rainy seasons (March to May and September to December) and with mean annual rainfall of 1300 mm. The site consisted of seven parishes (i.e Busubi, Kakamba, Kiwangala, Kikenene, Nakalembe, Nakateete and Ngereko) that were used for the study.

## 2.2 Farm selection

Forty-two farms (6 per parish) were selected to participate in this study. Selected farms had banana stands that were at least two years old and contained at least 100 mats. A baseline survey was undertaken on selected farms to determine the size of *C. sordidus* populations, damage levels, plantation residue management standards, banana stand sizes and farm isolation.

## 2.3 Treatments and experimental design

The three treatments in this study were: 0 (control), 4 (low-density) and 8-pheromone traps/ha (high-density). Each farm was considered a replicate. Farmers were selected on a volunteer basis for trial participation during a stakeholders meeting to ensure cooperation during the study. The allocation of the farmers to treatments was done randomly.

The number of traps for individual farms was calculated by multiplying trap density times the size of the banana stand. When this produced fractions of traps per farm, we used a mean density over the course of the study (e.g. if a farm was to receive 2.5 traps per month, we would place 2 traps the first month and 3 traps the next month).

## 2.4 Pheromone trap design and placement

Pitfall traps were made from 3-litre buckets 18 cm diameter, 16 cm height (Tinzaara *et al*., 1999a). The sides of the buckets were cut to allow adult *C. sordidus* to enter the traps. The buckets were then buried such that the cuts (lower edge of the windows) were level with the soil surface. In each trap, a single lure (Cosmolure+) was suspended 3 cm over the water by a nylon string. Each lure pack contained 90 mg of pheromone at a release rate of 3 mg/day (Oehlschlager, pers. commun.). Soapy water (1 L) was placed in the trap to facilitate drowning of the weevils.

The first traps were placed at the base of banana mats approximately 20 m apart, starting 10 m from the stand border. Each month we replaced the lures and moved the traps 20 m further into the stand. Farmers were advised on the importance of maintaining the pitfall traps lower edge window flush against the soil surface. In addition, our field assistant based at the site checked the traps every five days to make sure that the traps were correctly placed against the soil, and the water in the traps was replenished if necessary.

### 2.5 *Cosmopolites sordidus* collection from traps

The field assistant removed and counted the *C. sordidus* adults that had entered each trap every five days. The date, trap number and number of weevils were recorded on a data sheet.

### *2.6 Cosmopolites sordidus* adult population estimates

Adult *C. sordidus* populations were estimated at 0, 6, 12, 18 and 21 months using the mark and recapture methods described for banana weevil by Price (1993) and Gold and Bagabe (1997). Split pseudostem traps (30 cm long) (Mitchell, 1978) were placed at the base of every mat in smaller fields, and alternate mats in larger fields. Three days after placement the traps were checked for adult *C. sordidus*. These were counted, marked by scratching the elytra with a surgical blade (distinct marks were made for each sampling date), and released at the same spot. A second set of pseudostem traps was placed two weeks later with subsequent checking of traps after three days. The numbers of marked and unmarked *C. sordidus* adults were recorded. Weevil adult populations were estimated through mark and recapture using the Lincoln index (Gold & Bagabe, 1997): N=m*n/r, where N is the population estimate; m is the number of released (marked) individuals; n is the total number of collected weevils; and r is the number of marked individuals which were recaptured (Southwood, 1978). Population estimates of *C. sordidus* were converted from numbers per farm to density per hectare.

Previous mark and recapture studies using sequential trapping at the base of banana mats demonstrated considerable mixing of released adults with the rest of the population (Rukazambunga, 1996; Gold & Bagabe, 1997). Moreover, *C. sordidus* biology (i.e long life span, low fecundity and limited mobility) further suggest that the assumptions of the Lincoln index were met.

### 2.7 Banana corm damage

*Cosmopolites sordidus* damage on the corms of recently harvested plants was assessed at 0, 3, 12, 18 and 21 months using the cross section method of Gold *et al.* (1994). Cross sections were made through the collar (corm/pseudostem junction) and through the corm 5 cm below the collar. In each cross cut, the percentage of surface area consumed by *C. sordidus* larvae was estimated for the central cylinder and outer cortex. The mean of the four scores was calculated to estimate total cross section damage.

### 2.8 Data analysis

Data on pheromone trap catches, weevil population and total cross section damage scores were subjected to analysis of variance (ANOVA) using the GLM procedures of SAS (SAS, 1990). Means were separated by the Student-Newman-Keuls (SNK) test. A regression analysis was conducted to determine the relationship between the mean number of weevils captured per month and the average rainfall (mm).

# 3 RESULTS

Baseline data collected on study farms with the mark-release-recapture method revealed a mean initial population of 15,000 *C. sordidus* adults/ha on control farms, 11,000/ha on low-trap density farms and 12,000/ha on high-trap density farms (Figure 1). At the beginning of the experiment, the number of *C. sordidus* adults /ha in control, low and high trap density farms were statistically similar ($P > 0.05$). On subsequent sampling occasions in May 2000, December 2000 and June 2001, the number of estimated adults/ha were significantly lower in low pheromone trap density farms compared to control farms ($P<0.05$). In May and December 2000 the estimated number of weevils at the high trap density farms was similar to that in the control farms. The number of weevils/ha in low and high-trap density farms were statistically similar on all sampling occasions ($P > 0.05$). Only on one sample occasion (June 2001) both low and high trap density farms had significantly lower number of weevils than the control farms. The control and pheromone treated farms showed similar numbers of adult *C. sordidus*/ ha during the last sampling occasions (December 2001 and March 2002) of the experiment.

The pheromone pitfall traps caught a mean of 9.7 (range = 7.1 – 13.9) *C. sordidus* adults per month under low-trap density conditions, and a mean of 8.7 (range = 6.4 – 11.6) in high-trap density treatments (Figure 2). The mean of *C. sordidus* per trap per month in low and high-trap density farms were statistically similar except during the sampling month of February 2001 when mean catches were significantly higher ($P<0.05$) in low-trap density farms than high-trap density farms. The relationship between the mean number of weevils caught per month and average rainfall (mm) was not significant ($r^2=0.16$, $P=0.13$) (Figure 2).

Pheromone trap captures in this study correspond to a mean monthly removal of 39 weevils/ha at low-trap density (0.4% of estimated field populations) and 70 weevils/ha at high-trap density (0.6% of estimated field populations). Interpolation of baseline population estimates suggests initial population densities of 345 and 377 weevils within a 10 m radius of traps in low- and high-trap

density treatments. This would indicate that doubling the number of traps increased the number of weevils caught per ha per month from 11.2% to 18.4%. The trap efficiency however decreased from 2.8% and 2.3% by doubling trap density within the reported 10 m (314 $m^2$) radius, which is the most effective range of attraction by the pheromone traps (A.C. Oehlschlager, pers. commun.).

Weevil corm damage was generally low and statistically similar ($P>0.05$) among treatments throughout the experiment except in September 2000 and December 2000 when the mean damage difference in controls farms compared to pheromone treated fields was higher (Figure 3). In September 2000 and December 2000, weevil corm damage in low- and high-trap density farms were statistically similar ($P> 0.05$).

# 4 DISCUSSION

The mean monthly removal of *C. sordidus* from farmers' fields was generally low in this study. It was therefore not surprising that there were limited reductions in adult *C. sordidus* population density or in weevil damage compared to in the control fields. In Cameroon, Messiaen (2001) similarly observed that pheromone traps were not effective in reducing *C. sordidus* populations. Alpizar *et al*. (1999) on the other hand reported significant population and damage reduction in Costa Rica. In laboratory studies using olfactometric methods and a locomotion compensator, the pheromone was observed to be attractive to *C. sordidus* and the effect was higher when presented in combination with fermented pseudostem tissue (Tinzaara *et al*., 2002b, 2003).

Doubling the number of traps in the present study increased the number of weevils caught per ha per month but did not reduce damage to the plants. The trap efficiency per month (within the 10 m radius) however decreased from 2.8% to 2.3% by doubling the trap density. Catch per trap may be lower when the pheromone traps are placed at high than at low densities (Schlyter, 1992). In preliminary studies conducted in Costa Rica, the pheromone traps captured more *C. sordidus* at low than at high trap density and this was attributed to trap interference (C.A. Oehlschlager, pers. Commun.). However, since pheromone traps in our study were not very effective in capturing high numbers of weevils, the effect of doubling trap density on the number of weevils captured per ha may have been underestimated. Theoretically, the effectiveness of mass trapping could be improved by increasing the density of traps (Knipling, 1979), but densities higher than eight traps per ha are neither economical nor practical. Future research in Uganda should focus on improving the efficiency of pheromone traps, before further on-farm studies are undertaken.

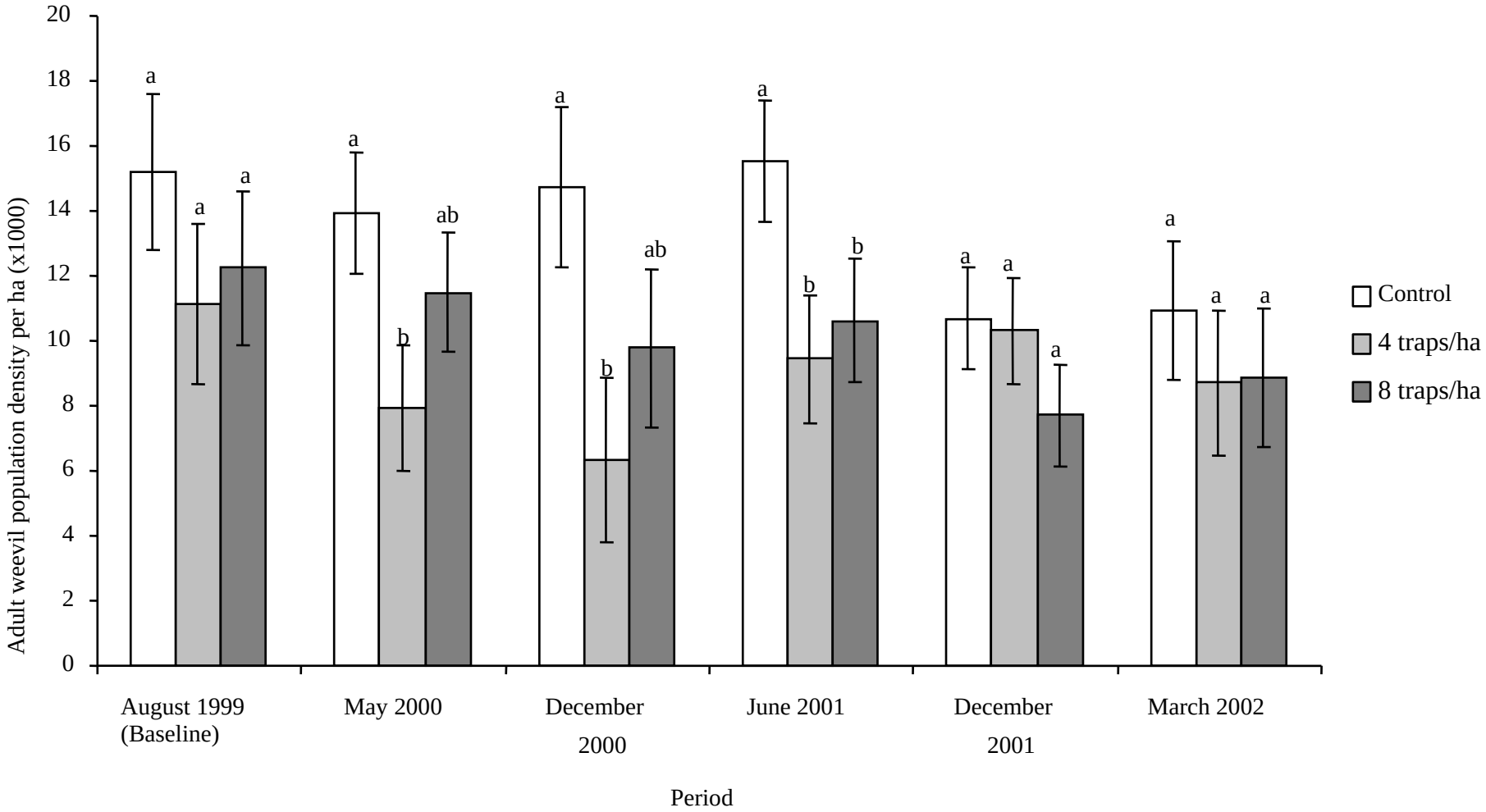


Figure 1: Adult *C. sordidus* population density on farms employing different pheromone trap densities in Masaka district, Uganda. (N = 14 per treatment). Means (±SE) of bars per sampling month with similar letters are not significantly different (P>0.05, Student-Newman-Keuls (SNK) test).

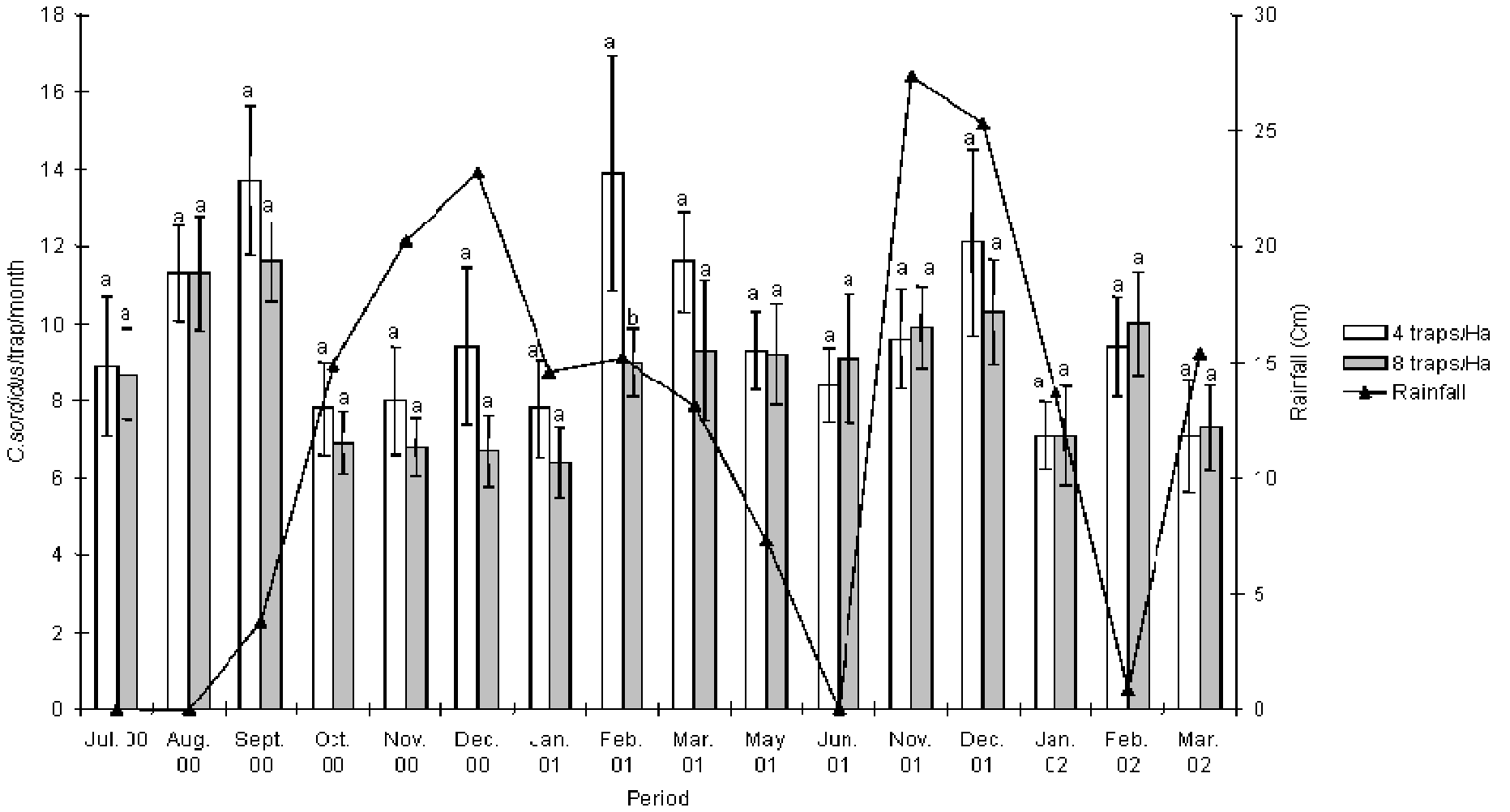


Figure 2: Mean number of *C. sordidus* adults (±SE) captured in Cosmolure+ pheromone traps on farms employing different pheromone trap densities, Masaka district, Uganda (N = 14 farms per treatment). Means of bars per sampling month with similar letters are not significantly different (P>0.05, SNK test).

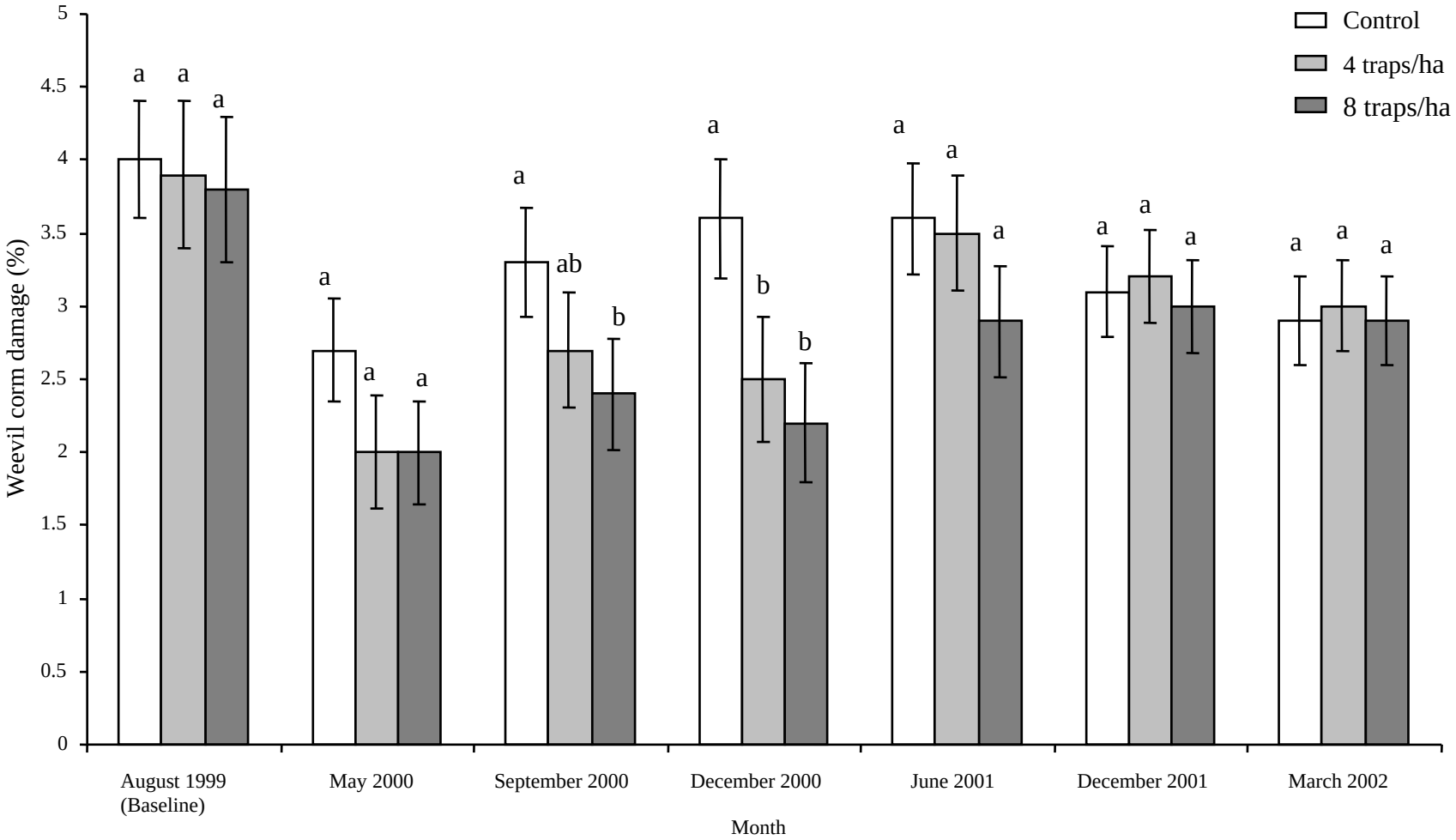


Figure 3: Mean percent *C. sordidus* total cross section damage (±SE) over time on farms employing different pheromone trap densities, Masaka district, Uganda (N = 14 farms per treatment). Means of bars per sampling month with similar letters are not significantly different (P>0.05, SNK test). The relationship between the mean number of weevils caught per month and average rainfall (mm) was not significant ($r^2$=0.16, P=0.13).

The poor performance of pheromone traps in Uganda may have resulted from several factors that might be improved upon. The success of suppressing pest populations depends on the efficacy of the trap used. Pheromone release rate may influence how traps perform relative to the natural pheromone sources and thus determine how well they attract insects (Mason & Jansson, 1991; Oehlschlager *et al*., 1995; Smit *et al*., 1997; De Groot & De Barr, 1998). Cosmolure+ is packed in plastic containers of 90 mg releasing at a rate of 3 mg per day (Oehlschlager pers. comm.). This release may not have been sufficient to stimulate weevil response in our cropping system. The number of weevils attracted by Cosmolure+ may be enhanced by increasing the release rate of the pheromone or by using two lures per trap. Increasing the number of lures per trap may increase the number of weevils captured but the efficiency (number captured per lure) may be decreased. Increasing the number of lures has the disadvantage of making the trapping system costly. There are also cases where reduced pheromone trap catches due to higher release rates of the pheromone were observed (Howse *et al.*, 1998; Hardie & Minks, 1999). In this case the insect may reach a response threshold before reaching the pheromone trap.

Pheromone pitfall traps need to be placed tight against the soil surface to allow *C. sordidus* to enter. A gap between the pitfall trap was often observed in our study, especially during dry conditions. If there is a gap between the trap and the soil surface or if the lower edge of the cut window is not level with the soil surface, weevils will find it difficult to enter into the trap resulting in reduced trap catches. Chemtica International has designed a ramp trap to avoid this problem (Alpizar *et al*., 1999). Alternatively, researchers in Australia cover the tops of the pitfall traps with banana leaves to maintain moisture and reduce trap temperature. This leads to increased weevil activity and eventually higher catches (Murad, 2001; Tinzaara *et al*., 2004c). Higher capture rates of *C. sordidus* were observed in the wet season (February to April and August to November) than in the dry season (December to January and May to July). However, this difference was not statistically significant.

Pheromone trap efficacy can be influenced by interaction of the cropping patterns (monocropping or mixed cropping) and farm management levels (Hebblethwaite, 1989). In contrast to plantain and Cavendish banana production systems in Costa Rica, highland banana in Uganda is most often produced in complex crop mixtures with low management levels. During the course of this study, we observed that weedy fields and fields with poor sanitation (i.e. poor removal of banana residues) tended to have lower trap catches than cleaner fields. Pheromone trapping may not result in the suppression of the pest populations especially when host plant residues provide a basis for pest multiplication (Weslein, 1992; James *et al*., 1996). In banana fields that are poorly

managed, the pheromone may have to compete with volatiles from residues and stumps. Non-host plant volatiles (such as from weeds) have also been reported to have inhibitory effects that interfere with insect responses to pheromones (Dickens *et al*., 1992; Byers *et al*., 1998; Reddy & Guerrero, 2004).

Even with a possible improvement of field results, farmers may only be willing to accept the technology if their perception of costs is addressed. The lack of immediate and obvious effects of pheromone trapping on banana weevil populations and corm damage is likely to discourage farmers from using this method. For example, farmers in some parts of Uganda were reported to have abandoned pseudostem trapping after a few weeks because they observed no immediate reduction in weevil numbers or crop improvement (Gold *et al*., 1993).

In conclusion, pheromone-trapping system on farmers' fields was not effective. Preliminary observations in on-station and on-farm trials suggest that adult *C. sordidus* may aggregate at mats adjacent to pheromone traps without entering the trap (Tinzaara *et al*., 2002b). If this is the case, pheromone traps might be integrated with the delivery of entomopathogens (e.g. *Beauveria bassiana* and *Metarhizium anisopliae*). Application of these microbial control agents could then be concentrated at mats adjacent to pheromone traps, rather than applied throughout the field. These aspects are currently being investigated in Uganda.

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Chapter **8**

# Olfactory responses of banana weevil predators to volatiles from banana pseudostem tissue and synthetic pheromone

*W. Tinzaara, C.S. Gold, M. Dicke & A. van Huis*

## Abstract

As a response to attack by herbivores, plants can emit a variety of volatile substances that attract natural enemies of these insect pests. Predators of the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) such as *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) and *Pheidole megacephala* (Hymenoptera: Formicidae) are normally found in association with weevil-infested rotten pseudostems and harvested stumps. We investigated whether these predators are attracted to such environments by volatiles produced by the host plant, by the weevil or by the weevil-plant complex. We evaluated predator responses towards volatile kairomones from banana pseudostem tissue (kairomones) and the synthetic banana weevil aggregation pheromone Cosmolure+ in a two-choice olfactometer. The beetle *D. abdominale* was attracted to fermenting banana pseudostem tissue and Cosmolure+, while the ant *P. megacephala* was attracted only to fermented pseudostem tissue. Both predators were attracted to banana pseudostem tissue that had been damaged by weevil larvae irrespective of weevil presence. Adding pheromone did not enhance predator response to volatiles from pseudostem tissue fed on by weevils. The results show that the predators are able to perceive volatiles from banana pseudostem tissue. The number of both predators recovered with pseudostem traps in the field from banana mats with a pheromone trap was similar to those in pseudostem traps less or more than 5 m from the pheromone. Thus, there was no clear evidence that the weevil's aggregation pheromone influences predator distribution around the trap in the field.

# 1 INTRODUCTION

During host searching, natural enemies of herbivorous insects (predators and parasitoids) are known to utilise volatile chemicals emitted by plants or herbivorous insects (Vinson, 1976; Vet & Dicke, 1992; Dicke & Vet, 1999; Turlings *et al*., 1995). Natural enemies that forage for herbivorous hosts by using infochemicals may have a problem concerning reliability and delectability of these stimuli (Vet & Dicke, 1992; Wiskerke *et al*., 1993). Stimuli from the host's food are well-detectable but are not reliable in indicating host presence. Host-derived stimuli are generally the most reliable sources of information but are not easy to detect by natural enemies especially at long distances (Vet & Dicke, 1992). Therefore, natural enemies such as parasitoids when faced with the reliability-detectability problem have evolved mechanisms of linking easy-to-detect stimuli to reliable but hard-to-detect stimuli (Vet & Dicke, 1992). Natural enemies may also exploit pheromones of their victim as kairomones in long distance herbivore location (Wiskerke *et al*., 1993). The use of chemical information that is both reliable and easy to detect enhances natural enemy searching efficiency (Vet & Dicke, 1992).

Infochemicals, both those used within and between species, can be utilised in pest management by either exploiting the way the natural enemy responds or by manipulating the source of the infochemical (Dicke *et al.,* 1990; Vite & Baader, 1990; Foster & Harris, 1997). For example, infochemicals can be used to enhance the searching efficiency, host utilisation and reproductive capacity of natural enemies (Renwick, 1992; Turlings *et al*., 1995; Scutareanu *et al*., 1997; Steidle & van Loon, 2003; McGregor & Gillespie, 2004). There are few studies on the application of infochemicals to manipulate the behaviour of predators or parasitoids in the field (e.g., Drukker *et al*., 1995; Shimoda *et al*., 1997; Bernasconi *et al*., 2001). However, data on the role of infochemicals in predator foraging have become available for several groups such as predatory mites (Sabelis & Dicke, 1985), pentatomids (Van Loon *et al.*, 2000), anthocorids (Drukker *et al*., 1995; Dwumfour, 1992), chrysopids (Reddy *et al*., 2002) and coccinellids (Steidle & van Loon, 2002). The predator *Rhizophagus grandis* (Gyll.) (Coleoptera: Rhizophagidae) is attracted to traps baited with a kairomone produced by the bark beetle *Dendroctonus micans* Kug (Coleoptera: Scolytidae) (Aukema *et al.,* 2000) and this can be exploited to monitor the predator's distribution in the field.

The banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), a major pest of bananas in Uganda has predators that have been mostly found in environments harbouring weevils

such as pseudostem traps and rotten pseudostem tissue, often in larval tunnels (Koppenhofer *et al.*, 1992; Koppenhofer, 1993; Tinzaara *et al.*, 1999b; Abera, 2004). Some ant species that have the potential to control *C. sordidus* include *Pheidole megacephala*, and *Tetramorium guineese* (Mayr) (Hymenoptera: Formicidae) (Gold *et al*., 2001, Abera, 2004). Non-ant predators known to prey on weevil eggs and larvae include *Dactylosternum abdominale* (Fabricius) (Coleoptera: Hydrophilidae), *Euborellia annulipes* (Lucas) (Dermaptera: Carcinophoridae) and *Thyreocephalus interocularis* (Eppelscheim) (Coleoptera: Staphylinidae) (Koppenhoeffer *et al*., 1992). Of these three, *D. abdominale* and *P. megacephala* are the most abundant predators in environments preferred by weevils in Uganda (Tinzaara *et al*., 1999b; Gold *et al.*, 2001; Abera, 2004).

*Dactylosternum abdominale* and *P. megacephala* are generalist predators that feed on micro-fauna and –flora of decomposing plant tissues, eggs and small larvae of insects. Decomposing tissue is more attractive to these predators than fresh ones (Kopenhoefer, 1993). Use of infochemicals by generalist predators during prey location have been documented (Dwumfour, 1992; Scutareanu *et al.*, 1997; Haberkern & Raffa, 2003; Steidle & van Loon, 2003; McGregor & Gillespie, 2004). Information on how volatiles from decomposing banana pseudostem tissue influence these predators in their host searching and host location is not available. Therefore, we have investigated the behaviour of D. *abdominale* and *P. megacephala* predators under laboratory and field conditions to assess whether they use volatile infochemicals associated with banana weevils.

An aggregation pheromone has been identified for *C. sordidus*, which is specific to the weevil (Jayaraman *et al*., 1997). A synthetic pheromone source containing a mixture of the four sordidin isomers is sold under the trade name Cosmolure+. The pheromone has been studied in the laboratory and in the field for the management of *C. sordidus* (Tinzaara *et al*., 2000, 2003), and attracts both males and females (Alpizar *et al*., 1999; Tinzaara *et al*., 2000). The pheromone-baited trap captures up to 18 times more weevils than a conventional split pseudostem trap (Tinzaara *et al*., 2000). Information on the effect of this aggregation pheromone on the behaviour of the weevil's predators has not been investigated. Several species of predators have been reported to use the aggregation pheromones of their hosts during host searching and location (Dwumfour, 1992; Vet & Papaj, 1992; Hedlund *et al*., 1996; Haberkern & Raffa, 2003; Steidle & van Loon, 2003).

The objectives of this study were to determine whether: (i) volatiles from banana pseudostem tissue and *C. sordidus* pheromone attract the predators *D. abdominale* and *P. megacephala*, (ii) the predators respond to host plant volatiles and whether this response is dose dependent, (iii) the pheromone enhances the predators' response to weevil-damaged pseudostem tissue, and (iv) the pheromone affects the predators' distribution around pheromone-baited traps in the field.

# 2 MATERIALS AND METHODS

## 2.1 Site description

Laboratory and field studies were conducted at Kawanda Agricultural Research Institute (KARI) ($0^{o}25'$N, $32^{o}51'$E, 1190 metres above sea level), 13 km north of Kampala, Uganda. The site has two rainy seasons (March-May and September-November) with an average precipitation of 1180 mm per year. Average daily temperatures range between 16 and 29$^{o}$C. Relative humidity in the laboratory ranged from 60 to 80%.

A field experiment was conducted in banana plots at KARI planted with cultivar Nabusa (*Musa* spp, AAA-EA group). The plot size was 10 x 12 mats (a banana mat consists of plants arising from a common corm/rhizome) at a spacing of 3 x 3 m. The plots were weeded after every two months and were not mulched.

## 2.2 Volatiles

Pieces of fresh pseudostem (less than a week after harvest) from the banana cv Nabusa collected from banana fields at KARI, were placed in plastic containers for seven days at room temperature to get fermented pseudostem tissue. Fresh pseudostem tissue was collected at the time of the bioassays. Fifty grams of either fresh or fermented pseudostem tissue was used for bioassays. This same dose was previously successfully used for studies of the weevil's response to infochemicals (Tinzaara *et al.*, 2003).

The pheromone lures for use in laboratory bioassays and field experiments were obtained from ChemTica International in San Jose, Costa Rica. They were sealed in plastic and sent by courier (transit time < 1 week) and subsequently stored in a freezer at -5 $^{o}$C upon arrival until use. Each pheromone pack contained 90 mg of Cosmolure+ with a release rate of 3 mg/day (A.C. Oehlschlager, pers. comm.). The pheromone packs were individually used as odour sources in their original plastic package material.

## 2.3 Predators

Adults of the beetle *D. abdominale* and the ant *P. megacephala* were selected for use in laboratory bioassays to assay their response to infochemicals. Predators were collected by hand searching in rotten banana pseudostems and corms from the field and kept on a non-substrate tissue (wetted tissue paper) in the laboratory for 24-48 hours before use in bioassays. Neither age nor sex of the collected beetles was known. Worker ants of unknown age were used.

## 2.4 Olfactometer

An olfactometer similar to that employed by Lofgren *et al.* (1983) and Cordova-Yamauchi *et al.* (1998) to study laboratory response of ants to banana weevil aggregation pheromone was used in all our experiments. The apparatus consists of a Petri dish 19 cm in diameter and 4 cm in height, without a lid (Figure 1). Two holes were made through the sides of the dish close to the base and two delivery tubes were inserted into them. A filter paper was placed at the floor of the Petri dish and wetted with about 50 ml of water before each test. One of the (arms) tubes of the olfactometer was connected to a jar (125 ml) containing an odour source to be tested and the other to a jar containing clean air (as control). Volatiles entered the arena by diffusion for jars.

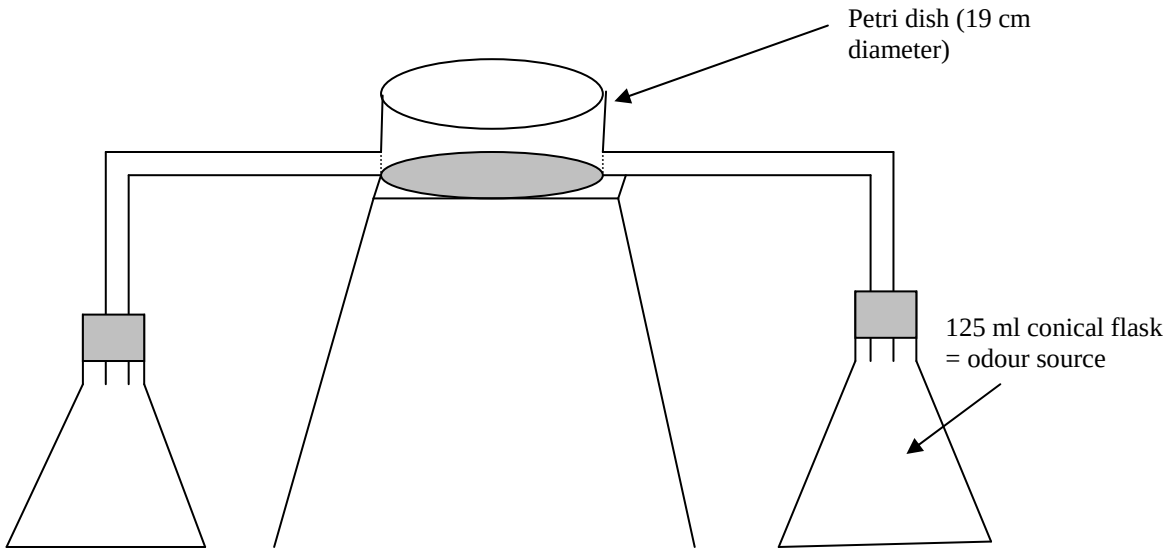


Figure 1: An olfactometer (not to scale) that was used for evaluating banana weevil predators for response to infochemicals in the laboratory.

A single predator was placed at the centre of the olfactometer arena. Each predator was observed for a maximum of 10 minutes and was considered to have responded when it entered one arm of the olfactometer or when at the end of ten minutes, the predator was within less than one cm from the entry port of the arms. After testing five individuals for each odour set, the odour sources were replaced with fresh ones. For all experiments, five individuals of each predator species were tested for all odour sets per day. The first experiment was repeated during six days (n=30, total number of individuals per predator species per odour set) while the rest of the laboratory experiments were repeated on 10 days (50 individuals per predator species per odour set). Each predator individual was tested only once and then discarded. Treatment and control arms were

exchanged after testing each predator by connecting the tubes at the opposite side to avoid trail formation. This had been observed to occur especially in the case of *P. megacephala* in preliminary tests. The apparatus was washed with ethanol and air-dried before using a new predator.

## 2.4 Experiments

We conducted six experiments with *D. abdominale* and *P. megacephala*. The first five experiments were done in the laboratory using a two-choice olfactometer and the sixth experiment was done in the field.

### *2.4.1 Testing the symmetry of the olfactometer*

This experiment was conducted to test whether any directional bias interfered with the responses of the two predator species in the olfactometer. The following odour sets were compared in this experiment: (i) clean air vs. clean air (ii) fermented pseudostem tissue vs. fermented pseudostem tissue.

### *2.4.2 Predator response to pseudostem tissue and weevil pheromone*

The response of the predators to banana pseudostem tissue and the weevil's aggregation pheromone was evaluated in this experiment. The odour sets that were tested in the olfactometer were (i) fresh pseudostem tissue vs. clean air, (ii) fermented pseudostem tissue vs. clean air, (iii) pheromone vs. clean air, and (iv) fermented vs. fresh pseudostem tissue.

### *2.4.3 Predator response to different dosages of fermented pseudostem tissue*

This experiment was conducted to determine whether predator response to infochemicals was dose dependent. The odour sources were: 1, 5, 25 and 125 g of fermented pseudostem tissue. Predator response to volatiles emanating from these amounts of tissue was compared to clean air in the olfactometer.

### *2.4.4 Predator response to pseudostem tissue fed upon by weevils*

This experiment was conducted to determine whether feeding by weevil larvae influences predator response to the banana pseudostem tissue. The treatments were: (i) fresh pseudostem, fed on by weevil larvae for 48 hours and larvae removed (F-LR), (ii) fresh pseudostem, fed on by weevil larvae for 48 hours and larvae present (F-LP), and (iii) weevil larvae alone. The following odour sets were compared: (i) F-LR vs. clean air, (ii) F-LP vs. clean air (iii) larvae vs. clean air, (iv) F-LP

vs. larvae, and (v) F-LR vs. F-LP.

Pseudostems of the cultivar Nabusa were collected from the fields at KARI. Weevil larvae (3rd-5thinstar) collected from the field were allowed to feed on fresh pseudostem tissue for 48 h. Five larvae were placed on a pseudostem piece measuring 30 x 10 cm. After 48 h at 22-28 $^{o}$C, the tissue had been tunnelled and tissue had turned dark brown and was used in bioassays with or without the larvae present. The larvae that were tested without food were collected from the field 24 hours before bioassays. They were placed in Petri dishes (9-cm diameter) with a non-substrate food material (moist tissue paper).

*2.4.5 Predator response to weevil-damaged pseudostem tissue in the presence of pheromone*

This experiment was conducted to evaluate whether the presence of pheromone enhances the predators' responses to volatiles from banana pseudostem tissue without weevil larvae feeding. The following odour sets were comparatively tested: (i) pheromone vs. clean air, (ii) F-LR vs clean air, (iii) F-LR + pheromone vs. clean air (iv) F-LR + pheromone vs. F-LR.

*2.4.6 Field distribution of predators around the pheromone-baited traps*

A field experiment was conducted at KARI to determine the distribution of banana weevil predators around pheromone-baited traps. We evaluated the hypothesis that predators aggregate around the trap mat as a result of a response to the pheromone and/or weevil-related volatiles. A pitfall pheromone-baited trap (Tinzaara *et al*., 2000) was placed at the centre of each plot. Soapy water was placed in the trap to retain the predators that had entered. The soapy water was renewed at every sampling occasion. The pheromone traps were checked every three days and predators captured in the traps were recorded and taken to the laboratory in vials for sorting and identification. Soapy water was renewed at every sampling occasion.

Ten fresh split pseudostem pieces (each 30 cm long) were placed in the plots at the same time of installing the pheromone traps. In each plot, pseudostem pieces were placed on the pheromone trap mat and on four mats in each of the distance range of 0.1-5 m and 5.1-10 m from the pheromone trap. Selection of the distance ranges was base on previous data on response by the weevil to the aggregation pheromone (Tinzaara *et al*., 2000). Six replicate plots were used. To determine the distribution of predators around the pheromone-baited traps, predators were searched for in banana pseudostem pieces after 30 days at different distances from the trap.

### 2.5 Statistical analysis

The $\chi$2-test for goodness of fit was used to determine the preference for one of the stimuli tested during the olfactometer bioassays (distribution of expected values 50:50). Field data on the number of predators and the weevils distributed around the pheromone-baited traps relative to distance were subjected to analysis of variance (ANOVA) using the GLM procedures of SAS software (SAS, 1990). The means were compared using the Student-Newman-Keuls (SNK) test. A regression analysis was used to determine the relationship between weevil and predator catches in pheromone-baited traps.

## 3. RESULTS

*3.1 Testing the symmetry of the olfactometer*

Both predator species *D. abdominale* and *P. megacephala* responded equally to clean air vs clean air and to fermented pseudostem tissue vs. fermented pseudostem tissue (P>0.05) (Table 1). There were fewer non-responders for *P. megacephala* (22%) than D. *abdominale* (40%) in the olfactometer apparatus used. The data indicate that the apparatus showed no symmetrical bias in evaluating responses of these predators to banana pseudostem tissue and the pheromone.

Table 1. Number of predators responding to clean air and fermented banana pseudostem odour sources in a two choice olfactometer in the laboratory at KARI, Uganda.

| Comparison odour sources | *Dactylosternum abdominale* | | | *Pheidole megacephala* | | |
|---|---|---|---|---|---|---|
| (A/B) | A | B | No response | A | B | No response |
| Clean air/clean air | 10 | 9 | 11 | 12 | 11 | 7 |
| Fermented/fermented tissue | 9 | 8 | 13 | 11 | 13 | 6 |

A total number of 30 individual predators were tested per comparison set. The response of the predators to the two odour sources did not differ significantly (P>0.05, $\chi$2- test)

*3.2 Predator response to pseudostem tissue and weevil pheromone*

Both *D. abdominale* and *P. megacephala* preferred fermented pseudostem tissue over clean air (P<0.001 and P<0.01 respectively). Both predators chose equally for fresh tissue and clean air (Figure 2). In a direct comparison beetles chose in similar numbers for the fermented and the fresh pseudostem tissue. Significantly more beetles moved to the side of the olfactometer with the pheromone (P<0.01) than to the one with clean air. More *P. megacephala* ants chose for the fermented tissue than for fresh tissue (P<0.05), while their choice between clean air and pheromone was similar.

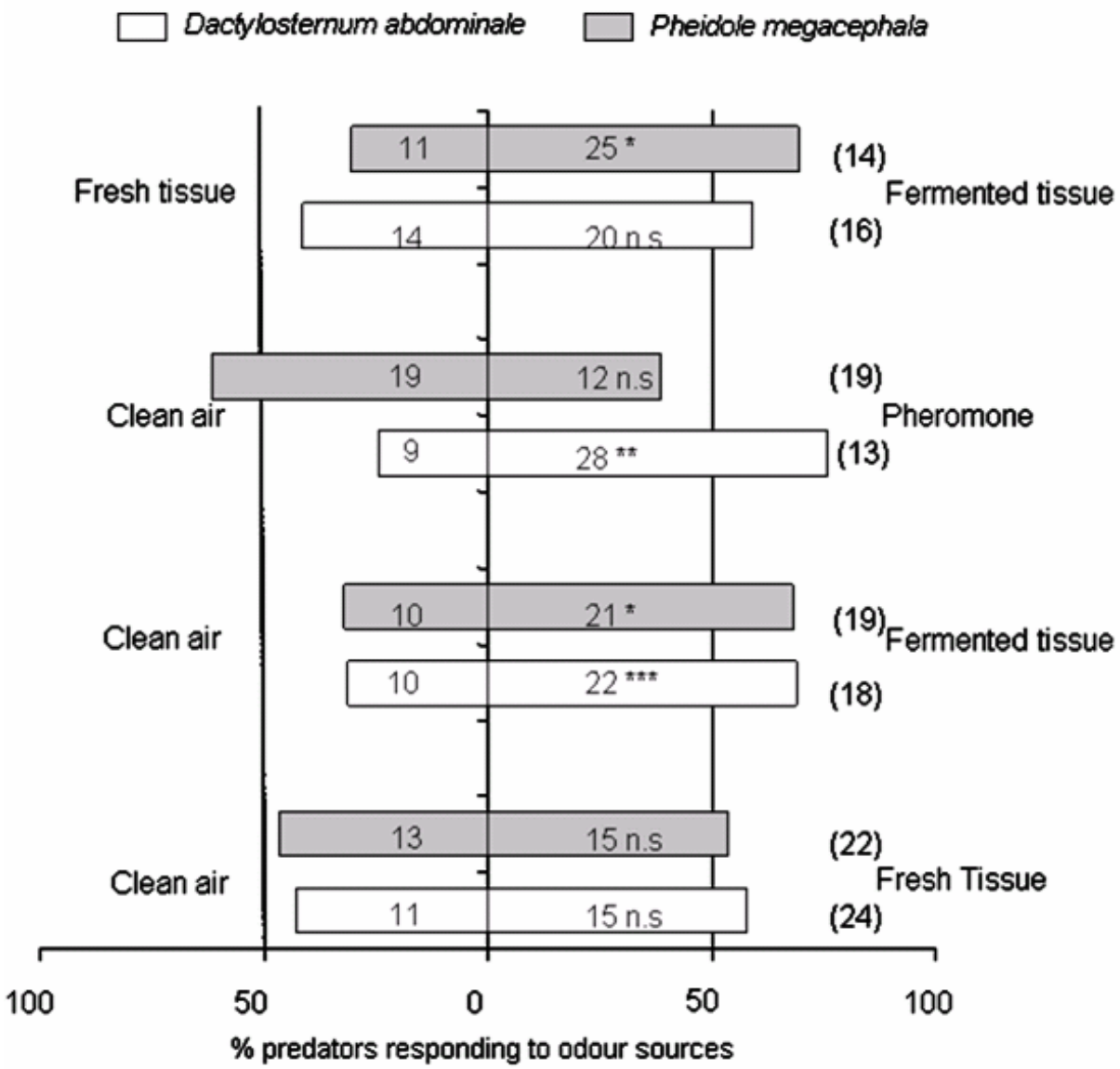


Figure 2: Response of the banana weevil predators *D. abominale* and *P. megacephala* to volatiles from banana pseudostem tissue and the pheromone in an olfactometer: percentage of responding individuals of each predator per comparison set, choosing one odour source or the other. Significantly different values are indicated with * $P<0.05$, ** $P<0.01$ and *** $P<0.001$; n.s = non significant at $P>0.05$, $\chi$-test, N=50 (total number of individuals tested per odour set). The non-responding predators are indicated in brackets at the right of bars.

### *3.3 Predator response to different dosages of fermented pseudostem tissue*

The response of both *D. abdominale* and *P. megacephala* to fermented pseudostem tissue was dose-dependent. At all doses the number of *D. abdominale* and *P. megacephala* choosing the side of the olfactometer with the fermented banana tissue was higher than the number choosing the side with clean air (Table 2), even when small amounts were used (down to 1 g). However, only the

following responses were significant: more *D. abdominale* chose for fermented pseudostem tissue when 5, 25 and 125 g were used than for clean air, and more *P. megacephala* chose for the pseudostem tissue when 125 g was used compared to clean air. Both *D. abdominale* and *P. megacephala* also preferred 50 g of fermented tissue over clean air (experiment 2, Figure 2).

Table 2: Dose related response of *Dactylosternum abdominale* and *Pheidole megacephala* to fermented banana pseudostem tissue in an olfactometer.

| Amount of tissue (g) offered versus clean air | *Dactylosternum abdominale* | | | *Pheidole megacephala* | | |
|---|---|---|---|---|---|---|
| | Fermented pseudostem | Clean air | No response | Fermented pseudostem | Clean air | No response |
| 1 | 18 | 14 ns | 18 | 14 | 12 ns | 24 |
| 5 | 21 | 10 * | 19 | 16 | 12 ns | 22 |
| 25 | 25 | 9 *** | 16 | 20 | 14 ns | 16 |
| 125 | 32 | 8 *** | 10 | 26 | 10 ** | 14 |

*P<0.05, **P< 0.01, ***P<0.001 = significant differences between fermented pseudostem and clean air ($\chi$2-test, n =50 (individuals tested per odour set); ns = non significant difference (P>0.05).

*3.4 Predator response to pseudostem tissue fed upon by weevils*

The presence of the larvae did not influence the predators' responses to pseudostem tissue. More *D. abdominale* chose for fermented pseudostem tissue with or without feeding larvae present than for clean air (P<0.05) (Figure 3).There was no significant (P>0.05) difference between the numbers of beetles choosing: 1. for the larvae versus clean air; 2. for fermented pseudostem with larvae versus larvae only; and for fermented pseudostem tissue with either larvae present or absent.

More *P. megacephala* ants chose the side of the olfactometer with fermented pseudostem with larvae present than for the side with clean air only (P<0.05). There was no significant difference in the responses between the other odour sources tested.

*3.5 Predator response to weevil-damaged pseudostem tissue in the presence of pheromone*

More *D. abdominale* beetles chose for damaged pseudostem tissue from which the feeding had been removed (F-LR) than for clean air (P<0.05), but when pheromone was added to the banana tissue (F-LR+PH) the effect was no longer significant (Figure 4). When the effect of the pheromone was tested versus clean air or in the presence of fermented pseudostem tissue (without larvae), there was no significant effect (P>0.05). The ant *P. megacephala* preferred the odour from fermented pseudostem tissue in absence of the pheromone but not when the pheromone was present.

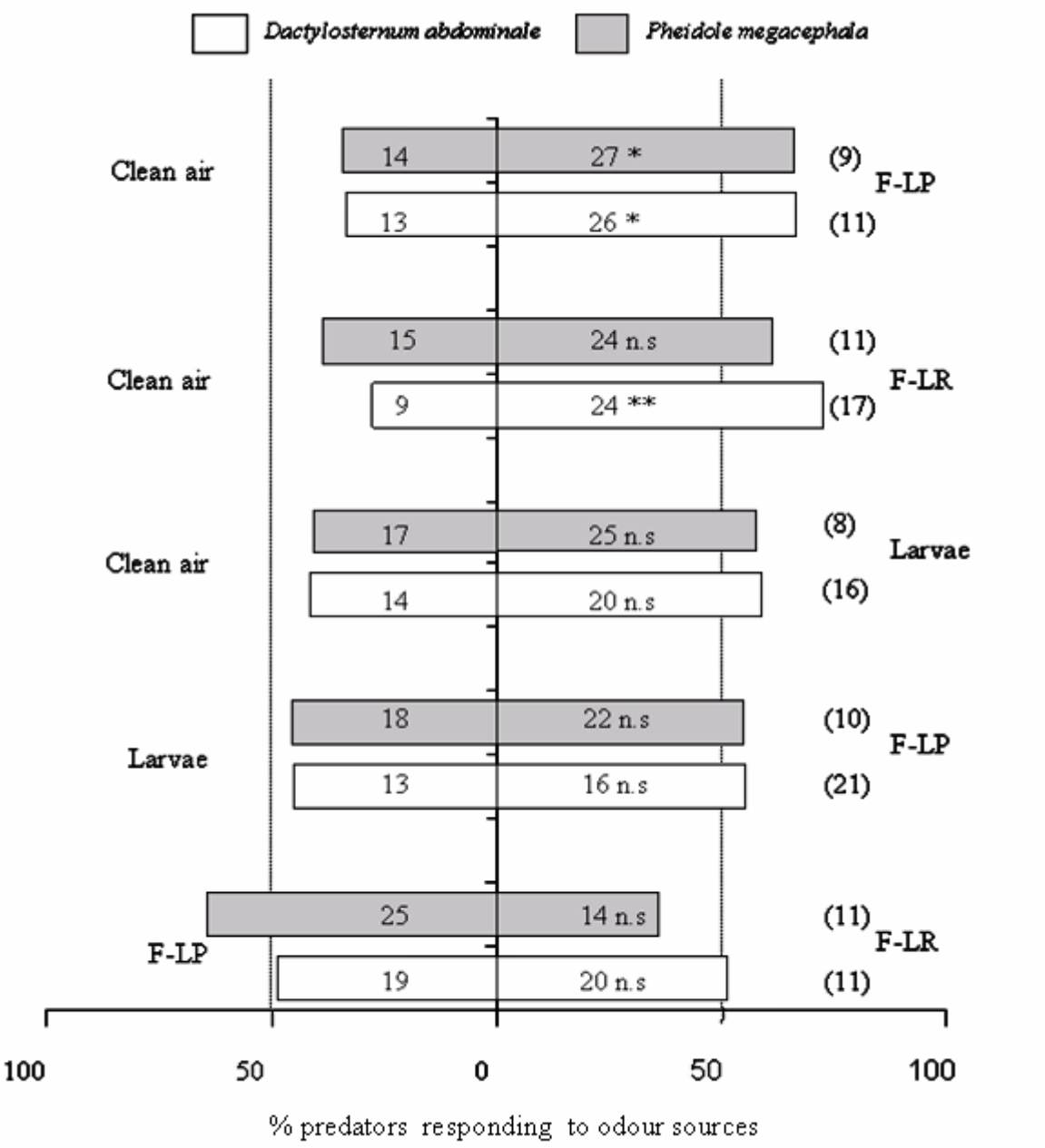


Figure 3: Response of predators in an olfactometer to fermented tissue with (F-LP) or without (F-LR) feeding weevil larvae: percentage of responding individuals of each predator per comparison set, choosing one odour source or the other. Significantly different values are indicated with *P<0.05 and **P<0.01; n.s = non significant at P>0.05,$\chi$2-test, N= 50 (total number of individuals tested per odour set). Non-responding predators are indicated in brackets at the right of bars.

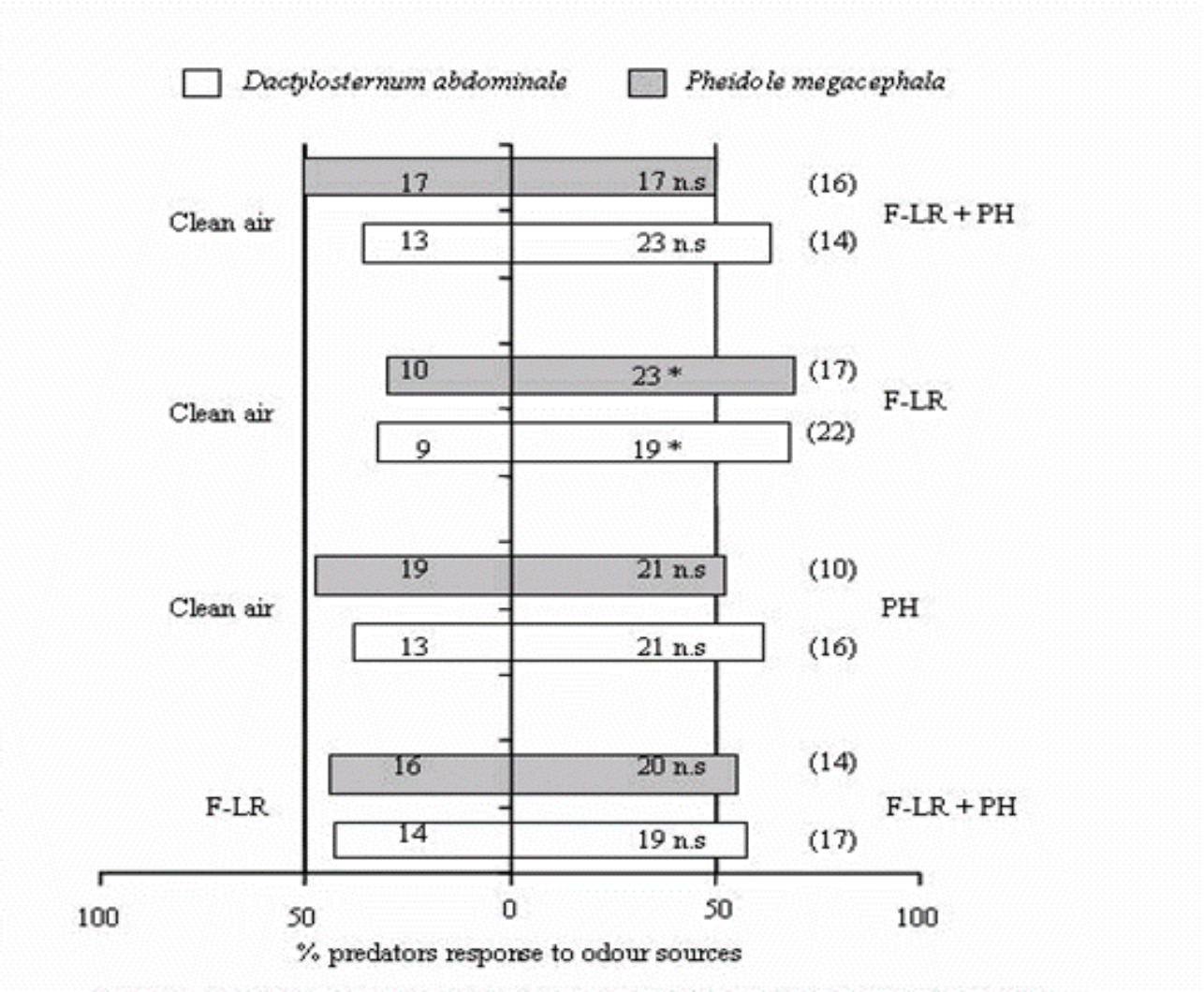


Figure 4: Response of predators in an olfactometer to fermented tissue with (F-LP) or without feeding weevil larvae (F-LR) combine with the pheromone (PH): percentage of responding individuals of each predator per comparison set, choosing one odour source or the other. Significantly different values are indicated with *P<0.05; n.s = non significant at P>0.05, $\chi$2-test, N= 50 (total number of individuals tested per odour set). Non-responding predators are indicated in brackets at the right of bars.

*3.6 Field distribution of predators around the pheromone-baited trap*

The presence of pheromone traps had no effect on predator distribution in the field. The number of *D. abdominale* and *P. megacephala* that were recovered on the mats where pheromone traps were present compared to those that were recovered from mats less or more than 5 m from the trap were similar (Table 3). Numbers of weevil adults were significantly higher at the pheromone trap mat than on mats less 5 m and in the range of 5.1-10 m from the trap. The number of weevil larvae recovered at all distances from the pheromone trap was similar.

The ants *P. megacephala* were the only predators that were captured in the pheromone-baited

traps in the field. The mean number of weevils and *P. megacephala* captured in pheromone traps was 2.4 (± 0.4 S.E) and 4.2 (± 1.2 S.E) per plot per three days respectively. There was no significant relationship between the numbers of *P. megacephala* and the number of weevils caught in pheromone-baited traps ($r^2$=0.04, P=0.61).

Table 3. Mean number (±se) of predators and *C. sordidus* recovered from pseudostem pieces placed at different distances from the pheromone-baited trap in banana plots at KARI, Uganda.

| Predators and *C. sordidus* | Number of insects recovered from different distances (m) | | |
|---|---|---|---|
| | 0 | 0.1-5 | 5.1-10 m |
| *Labia* spp (Dermaptera: Labiidae) | 2.0± 0.9a | 0.8±0.3a | 1.3±0.6a |
| *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) | 4.8±1.0a | 3.2±0.9a | 3.3±1.0a |
| *Pheidole megacephala* (Hymenoptera: Formicidae) | 2.0 ±1.2a | 0.3±0.3a | 0.6±0.3a |
| Banana weevil larvae, *C. sordidus* | 3.8 ±2.0a | 1.1±0.4a | 1.1±0.7a |
| Adult banana weevils, *C. sordidus* | 21.5± 6.3a | 7.1±2.3b | 6.5±1.2b |

In each of the six plots, there were 10 pseudostem traps at each of the points per distance range. Means for each predator and *C. sordidus* followed by similar letter in a row are not significantly different (P > 0.05, SNK).

## 4 DISCUSSION

Predators of the banana weevil are often observed in decomposing banana tissue such as harvested stumps and then often in tunnels where banana weevil eggs, larvae and pupae are normally found (Koppenhofer, 1993). The results of our olfactometer experiments demonstrate that the predators *D. abdominale* and *P. megacephala* respond to volatiles from banana fermented pseudostem tissue. Attraction to the food of its host was similarly reported for several predator species such as *Anthocoris nemorum* (Heteroptera: Anthocoridae) (Dwumfour *et al*., 1992), *Orius tristicolor* (Hemiptera: Anthocoridae) (Van Laerhoven *et al*., 2000) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) (Reddy *et al*., 2002).

Many predator species are known to respond more to volatiles from herbivore-damaged than undamaged plants (Geervliet *et al*., 1994; Dicke, 1999b). Damaged plants become more attractive soon after the herbivores start feeding on them (Dicke *et al*., 1990, Turlings *et al*., 1990, Dicke & Vet, 1992; Geervliet *et al*., 1994). In our study, the predators did not discriminate between volatiles from damaged pseudostem tissue with or without the larvae. In addition, neither predator species discriminated between volatiles from weevil larvae and clean air. Similar data have been reported for other tritrophic systems as well (e.g. Turlings *et al*., 1990; Dicke & Vet, 1992; Geervliet *et al*., 1994), including a system consisting of fermenting substrates, a fungivore and its parasitoid (Dicke

*et al*., 1984). Fermented pseudostems that had not been damaged by weevil larvae were also attractive to the predators. Stimuli originating from the host habitat may influence host habitat location although volatile stimuli originating from the host are more reliable (Vet & Dicke, 1992, Wiskerke *et al*., 1993). Our results indicate that the predators depend on volatiles from fermented pseudostem tissue and that prey odour does not play a role in prey and habitat location.

Several species of natural enemies have been reported to use the aggregation pheromones of their hosts during host searching and location (Vet & Papaj, 1992; Wiskerke *et al*., 1993; Hedlund *et al*., 1996; Reddy *et al*., 2002; Wertheim *et al*., 2003). In our study, the predatory beetle *D. abdominale* was observed to respond significantly to the pheromone compared to clean air in the laboratory. There was, however, no evidence that the pheromone enhances predator response to volatiles from weevil-damaged pseudostem tissue in the laboratory. The ant *P. megacephala* was not attracted to the banana weevil's aggregation pheromone in the laboratory. In the field the number of predators recovered in pseudostem material placed near the pheromone trap and those placed more than 5 m away in the field was similar. Our data indicate that banana weevil aggregation pheromone has no effect on the predator distribution around the trap. The environmental conditions in the banana field are heterogeneous with natural background volatiles. Although volatiles from the herbivore itself would provide reliable information to the predator, the distribution of both predator species in the field was not related to the number of adult weevils captured in pheromone traps indicating that the aggregation pheromone released by male *C. sordidus* is not used by the predators in the field. In addition to the lack of response to the aggregation pheromone, generalist predators such as formicine ants have not been reported to use prey derived chemicals as kairomones during foraging (Cosens & Toussaint, 1985). In contrast, several other predator species have been reported to use pheromones of their prey during prey searching and location (Dwumfour, 1992; Hedlund *et al*., 1996; Haberkern & Raffa, 2003; Steidle & van Loon, 2003).

Our study demonstrates that the predators discriminate between fermented banana pseudostem tissue and clean air. The banana weevil aggregation pheromone did not show either synergistic or additive effects to the banana tissue in terms of attraction of the banana weevil predators.

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Chapter **9**

# Use of pheromone-baited traps enhances dissemination of entomopathogenic fungi to control the banana weevil, *Cosmopolites sordidus*


## Abstract

Previously, candidate strains of the fungal pathogen, *Beauveria bassiana* have been identified for use in integrated pest management of the banana weevil *Cosmopolites sordidus*. However, the lack of an economic and effective delivery system to maximize field effects has been an important limiting factor to their application. Integration of pheromone trapping and application of *B. bassiana* may provide a cost-effective strategy for the control of the pest. We conducted field studies to determine the potential for pheromone-baited traps to aggregate the banana weevil around the trap. Field transmission and use of different delivery systems of *B. bassiana* using pheromones were investigated. There were significantly more weevils captured in pseudostem traps at the banana mat where the pheromone was present than on adjacent mats. We further observed that infected weevils could transmit the fungal pathogen to healthy individuals. Most of the dead weevils due to *B. bassiana* infection were found at the base of the plant in the leaf sheath and from soil near the mat. There were significantly more weevils that died after incubation due to pathogen infection from plots where pheromones were used in combination with *B. bassiana* applied on the mats where the pheromone trap was placed and four adjacent mats than when the pathogen was applied without the pheromones. Our data demonstrate that the banana weevil aggregation pheromone Cosmolure+ could be used to enhance the dissemination of *B. bassiana* for the control of *C. sordidus.*



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# 1 INTRODUCTION

The banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important insect pest of bananas (*Musa* spp., AAA-EA genome group) and plantains (AAB) in Africa (Gold *et al*., 2001). Weevil oviposition takes place at the base of the plant (Abera *et al*., 2000). After hatching, the larvae bore in the corm, causing damage resulting in reduced nutrient uptake of the plant. Attack in newly established banana stands may lead to crop failure. Heavy infestation in established fields may lead to plant loss, reduced bunch weights, mat disappearance and shortened life span of the plants (Rukazambunga *et al*., 1998; Gold *et al*., 2004a).

Adult banana weevils are soil dwelling nocturnal and relatively sedentary insects (Uzakah, 1995; Gold *et al*., 2001). They are widely known to favour crop residues and moist environments, including places in or under newly cut or rotting pseudostems, decaying stalks, cut or damaged corms and moist trash (Gold *et al*., 2004b). Females tend to be more active than males (Gold *et al*., 1999b). In six months of release, some weevils were found to move 60 m while many moved less than 5 m (Whalley, 1957).

Control of the banana weevil is difficult. Cultural control is labour intensive while chemical control is too expensive and can only be afforded by wealthier farmers in Uganda. Use of microbial control provides a plausible method for the management of this pest (Nankinga, 1999; Nankinga & Moore, 2000; Godonou, 2000). Candidate strains (e.g isolate G41) of *Beauveria bassiana* (Bal) Vuillemin have been identified for the management of the banana weevil (Nankinga, 1999). Weevils infected with the pathogen show signs of mycosis within two weeks. Mortalities up to 100% due to *B. bassiana* infection have been reported in the laboratory (Nankinga, 1994). Dissemination of *B. bassiana* using pseudostem traps was tested but found ineffective (Nankinga, 1999). An economic and effective delivery system to maximize field effects still needs to be developed. Using pheromones for the delivery of entomopathogens to control the banana weevil has been previously suggested (Budenberg *et al*., 1993b; Gold *et al*., 2001; Tinzaara *et al*., 2002a, 2004a) but no research has been done to investigate whether this is possible.

Entomopathogenic fungi such as *B. bassiana* have the potential to grow, multiply and persist on the insect which they eventually kill (Roy & Pell, 2000). In addition, infected individuals can move away from the infected point, thus carrying the pathogen throughout the insect's habitat. The transmission of the fungal pathogen by the banana weevil to conspecifics has been demonstrated in the laboratory (Nankinga, 1994; Schoeman & Schoeman, 1999; Godonou *et al.*, 2000). Detailed information on the extent that *B. bassiana* can be transmitted from infested individuals to non-

infested conspecifics is necessary to develop an effective delivery system for the pathogen. As the movement of infected hosts determines the way in which a pathogen is transmitted and spread within a field (Fuxa & Tanada, 1987), the behaviour of infected banana weevils in the field should be studied. However, the question remains whether the transmission of the fungus from the infected individuals to healthy ones can be enhanced by the application of pheromones under field conditions in Uganda. A gallon ramp trap that was used in Costa Rica (Alpizar *et al*., 1999) and was tested for efficacy in Ugandan conditions (Tinzaara *et al*., 2000) could be used in this regard. This trap is designed to allow both entry and exit of weevils. The use of pheromone as lure for the dissemination of *B. bassiana* has been reported to be effective for other beetles (Klein & Lacey, 1999; Yasuda, 1999; Vega *et al*., 2000).

The objectives of this study were to: (1) determine the potential for the pheromone-baited traps to aggregate banana weevils around a pheromone trap; (2) evaluate the field transmission of *B. bassiana* from infected to uninfected banana weevils (3) determine the effect of *B. bassiana* infection on weevil behaviour (movement and location) in the field, and (4) evaluate the different delivery systems of *B. bassiana* using pheromone for the control of the weevil.

# 2 MATERIALS AND METHODS

## 2.1 Study site

The studies were conducted at the Sendusu Farm of the International Institute of Tropical Agriculture (IITA) ($0^{o}$32'N $32^{o}$35'E and 1260 meters above sea level (m.a.s.l.)), located 28 km north east of Kampala, Uganda and at Kawanda Agricultural Research Institute (KARI) ($0^{o}$25'N, $32^{o}$51'E, 1190 m.a.s.l.) located 12 km north of Kampala. Both sites have two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and a daily mean temperature of $21^{o}$C.

## 2.2 Source of pheromones and *B. bassiana*

Cosmolure+ (Chemtica International, San Jose, Costa Rica) was used as a pheromone lure in all experiments. It is comprised of a polythene pack of pure sordidin (1S,3R,5R,7S)-(+)-1-ethyl-3,5,7-trimethyl-2,8-dioxabicyclo[3.2.1]octane) (Mori *et al*., 1996) that is released at 3 mg/day (A.C. Oehlschlager, pers. comm.). For storage, packs were tightly sealed and kept in a dark cupboard at 21-$25^{o}$C. Pheromone packs from opened containers were stored in the freezer at –5 $^{o}$C.

*Beauveria bassiana* (isolate G41) (3 x $10^9$ conidia/g) was obtained from the Laboratory of Pathology at KARI in a crushed maize formulation as described by Nankinga and Moore (2000).

### 2.3 Aggregation of *C. sordidus* around traps (experiment 1)

To determine the degree to which banana weevils aggregate near pheromone-baited traps, trials were conducted at Sendusu and KARI. The experiment at Sendusu was conducted in 8-year old banana plots (25 x 25 m, 40 plants/ plot, separated by 10-m wide alleys) planted with cultivar Atwalira (*Musa spp*. AAA-EA type). The plots were weeded and self-mulching. The banana field at KARI was also planted with the highland cultivar Atwalira. The field consisted of experimental plots (36 mats, 3 x 3 m arrangement) in which the weevil population had increased over the three years of the plantation age. Five-m grass alleys separated plots. The plots were mulched with elephant grass (*Pennisetum purpureum*). Prior to the placement of pheromone-baited pitfall traps, pseudostem traps were placed in the field for three days to give an indication of banana weevil abundance. The banana weevils captured in pseudostem traps were released on the mat of capture after counting and recording their numbers.

Pitfall traps baited with pheromone were placed in the selected plots. Each trap was prepared out of a 10-litre bucket (for details see Tinzaara *et al*., 2000). The pheromone pack was hung from the top of the bucket. One trap per plot was placed at the central mat. There were 12 pheromone-baited pitfall traps (one per plot) placed in each of the fields for 30 days. Traps were checked every three days to make sure that they flushed well with soil level. A liquid detergent solution (1 litre) was placed in the trap as a collection agent and was changed every five days. After the pheromone trapping period of 30 days, two pseudostem traps (Mitchell, 1978) were then placed at the base of each mat in the study plots. The number of weevils found in each pseudostem trap was recorded after three days and at three different distances from the pheromone-baited trap, (i) at mats where pheromone was present (ii) mats less than 5 m from the pheromone-treated mats, and (iii) mats more than 5 m from the pheromone-treated mats.

### 2.4 Field transmission of *B. bassiana* (experiment 2)

Two trials to evaluate field transmission of *B. bassiana* from infected to uninfected *C. sordidus* were carried out at Sendusu banana plots. The first trial was conducted in a plot planted with banana cultivar Nabusa (*Musa* spp, AAA-EA group). The second trial was conducted in another plot planted with the same cultivar located at 200 m from the plot used for first trial. The plot size for

both trials consisted of 42 mats, at a spacing of 3 x 3 m (i.e 3 m between the mats). The five year old banana plots used were unmulched and well-weeded at the start of the trials.

Before release of weevils, pseudostem trapping was conducted to determine if *B. bassiana* was present in plots. Two split pseudostem pieces were placed per mat and checked after three days. For each mat, three of the captured living weevils were placed in petri dishes and taken to the laboratory for incubation, while the number of dead weevils showing mycosis (whitish fungal mycelial growth) was recorded. Weevils brought to the laboratory were individually placed in a petri dish with moist tissue paper at 25-27 $^{o}$C and 80-90% r.h. Observations for mycosis were taken every three days for a period of 21 days.

The weevils used were collected from the Masaka district, Uganda (140 km south of Kampala), marked according to sex, mat of release, and whether they were inoculated with the fungus. Ten infected weevils (5 males and 5 females) were released per mat one week after the release of 16 uninfected weevils (8 males and 8 females) per mat in the same plot. Weevils were released in the plots (on marked mats) in the evening (7.00-8.00 p.m) when they were active and can avoid predation.

To infect weevils in the laboratory, healthy ones were placed in 3-cm diameter petri dishes containing 2 g of maize-formulated *B. bassiana* (approx. 3 x $10^9$ conidia/g) for 6 h. After 6 h, infected weevils were placed in petri dishes (9 cm diameter) with moist tissue paper and maintained in the laboratory (25-27$^{o}$C, 80-90 % r.h.) for three days before releasing them in the field.

In both trials, pseudostem trapping was conducted 7, 14, 21, 35 and 42 days after release of infected weevils. Two split pseudostem pieces were placed per mat and inspected after three days. For every sampling occasion, weevils captured were placed in vials according to mat of capture and type of release (released infected or uninfected), and taken to the laboratory for incubation. In the laboratory at KARI, weevils were placed according to recapture mat in petri dishes (9-cm diameter) lined with moist filter paper. For each sampling date, we monitored the number of dead weevils showing signs of mycosis from each plot after three days for a period of 21 days .The data of the five sampling dates were pooled before analysis.

Hand searching in the plots was carried out every two weeks after release of infected weevils to determine the proportion of dead weevils in the field. Searching was thoroughly done in the different locations in the plot (i.e. in leaf sheath, soil near the mat, residues off mat and in alleys). Both infected and uninfected weevils found were recorded.

### 2.6 Relative dispersal of *C. sordidus* in the field (experiment 3)

This experiment was conducted to investigate whether infection of weevils with *B. bassiana* influenced their dispersal in the field. Infected and uninfected weevils were released in the plots as described in experiment 2. Pseudostem trapping was conducted 7 and 14 days after release of infected weevils. Two pseudostem pieces were placed per mat and inspected after one day. Data on numbers of weevils per mat was recorded. Weevils were placed back on the mat of capture. The distance moved by the recaptured weevils was calculated.

### 2.7 Locations of *C. sordidus* in the field (experiment 4)

Trials to determine locations of dead banana weevils due to *B. bassiana* infection were conducted in cages and in the field plots.

*Caged trial*: An open cage trial was conducted at Sendusu to determine locations of weevils killed by *B. bassiana* infection. The cages were prepared out of polythene sheets by sewing to form a cuboid (120 x 90 x 60 cm) in which soil was placed. Stakes were put on sides of the cuboid to make the polythene sheets straight. Sword suckers (3 by 4 plants per cage) were planted at a spacing of 30 x 30 cm in the open cages so that the soil level reached the collar of the plant. The sides of the cages were 30 cm higher than the soil level to reduce weevil escape. After planting, some grass mulch was placed evenly in the cages to simulate natural field conditions. There were six replicate cages.

Weevils were inoculated in the laboratory with maize-formulated *B. bassiana*. Weevils were kept in petri dishes (3-cm-diameter) containing 2 g of the pathogen for six hours. Then they were placed in petri dishes (9-cm diameter) lined with moist tissue paper. After three days they were released into the cages. Ten infected weevils (5 males and 5 females) were released per plant in the evening (7:00 – 8:00 p.m.). Weevils had been collected from the Masaka district (Uganda), and marked according to sex and mat of release.

Sampling for dead weevils with mycosis was carried out two weeks after releasing of the weevils. The plots were searched and plants then uprooted. The numbers of dead and alive weevils were recorded for each of the following locations: (i) mulch, (ii) soil near mat, (iii) soil in alleys, (iv) plant base in leaf sheath, (v) pseudostem, (vi) corm surface, and (vii) inner corm.

*Field trial*: Two trials were conducted to determine the locations preferred by weevils infected by the pathogen. The trials were conducted in the same plots as used for experiment 2 in which details of weevil release are described. Searching and sampling by hand in the field was conducted every

two weeks after release of infected weevils. We searched in the following locations: (i) trash, (ii) residues (iii) soil near mat, and (iv) plant base in leaf sheath. Dead weevils showing mycosis were recorded according to the locations of recovery. The percentage of weevils of the total recovered was calculated for each location. The locations searched in the field are different from those in the cage trial because it was not possible to uproot plants in the field.

### 2.8 *Pheromone*-baited delivery systems of *B. bassiana* (experiment 5)

The experiment to evaluate the rate of transmission of *B. bassiana* to the banana weevil using different delivery systems was conducted at Sendusu. The treatments (delivery systems) applied to plots were: (i) control, (ii) pathogen applied to central mat (Bb), (iii) pheromone + pathogen inside trap (Ph + Bb-IT), (iv) pheromone + pathogen around the trap mat (a trap mat is where pheromone baited trap was placed) (Ph + Bb-AT), and (v) pheromone + pathogen on trap mat and four adjacent mats (Ph + Bb-AT + 4 mats). Each treatment was replicated three times. The experiment was conducted in six-year-old banana plots at Sendusu planted with cultivar Atwalira. Each plot consisted of 5 by 5 mats, spaced at 3 x 3m. Fifteen plots were selected and those that had gaps (i.e. missing mats) were re-planted. The experiment was first conducted in July 2003 and repeated using the same plots six months later, i.e. in January 2004. Crushed-maize formulated pathogen (200 g) was applied by spreading the formulation at the base of each mat or placing it inside a pheromone. Mulch was applied over the fungus to prolong its viability (Nankinga, 1999).

In both replicates, weevils that had been collected from Masaka and kept in the laboratory for a week were used. Ten weevils (5 females and 5 males) marked according to their sex and mat of release, were released at each of the mats. Weevils were released in the evening (7:00 – 8:00 p.m.).

A gallon trap with a ramp that allows easy entry and exit of the weevils was used (Figure 1). The trap was made out of a 5-litre jerry can. A window was cut in each side of the jerry can and the flap folded down to make a walk-in ramp. The gallon traps were placed at the centre of selected plots two days after weevil release.

Pseudostem trapping was conducted after 14, 28 and 42 days. The recaptured weevils were inspected for pathogen infection, and the distance from the site of recapture to the fungus source or pheromone source was recorded. The weevils were then placed in vials (in groups) according to distance of recapture (trap mat – 0 m, less than 5 m, and more than 5 m from the pheromone-baited mat) and taken to the laboratory for incubation and then assessing percentage mortality. In the laboratory, weevils were individually placed in petri dishes lined with a moist tissue paper according to recapture distance. This was done within less than two h after collection from the field.

The number of dead weevils showing signs of mycosis from each plot or treatment at different distances was recorded every three days for a total of 21 days.

On the same day the pheromone traps were checked, inspection for dead weevils was also done at the following locations in the field: (i) trash, (ii) corm or pseudostem residues, (iii) soil by mat, and (iv) plant base in leaf sheath. Weevils with mycosis were recorded.

When the pheromone lure sachets in the pheromone traps were observed to be empty, they were replaced with new ones.

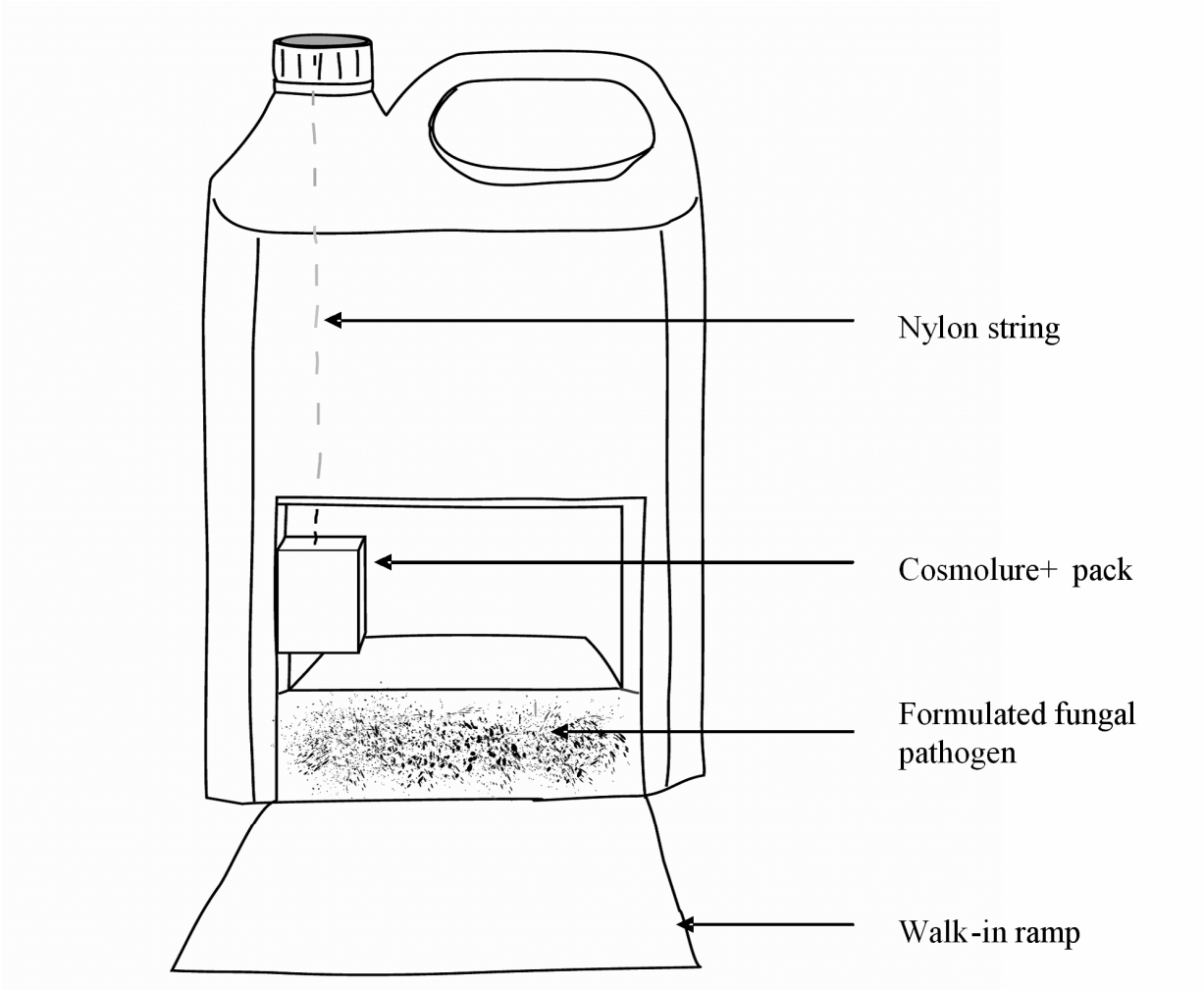


Figure 1: A gallon trap baited with the aggregation pheromone Cosmolure+ and the formulated fungal pathogen, *Beauveria bassiana* (Tinzaara *et al*., 2000)

### 2.9 Statistical analysis

The data on weevil aggregation around the pheromone-baited trap were square-root-transformed and analyzed using ANOVA with the SAS software (SAS® Institute Inc., 1999). Means were separated using the Student-Newman-Keuls test (SNK). Distances moved per week for infected and non-infected weevils were compared using a t-test. The association between the number of weevils and distance range moved was analysed using the $\chi^2$-test. The percentage of weevils recovered by pseudostem trapping from plots with different pathogen delivery systems that showed mycosis after incubation were analysed using contingency table test on numbers (pooled for two trials), followed by Bonferroni correction, $\alpha = 0.05/6 = 0.008$, for multiple comparisons for those that showed significant differences.

## 3 RESULTS

### 3.1 Aggregation of *C. sordidus* around traps (experiment 1)

Prior to the experiment, pseudostem trap captures averaged two weevils in the Sendusu stands and six weevils per three days in the KARI plots. The mean numbers of weevils captured in pheromone traps in the trapping month were 18.2 (± 3.7 s.e) per trap per three days and 14.8 (± 2.1 s.e) per trap per three days for Sendusa and KARI plots respectively. After the pheromone trapping period, significantly ($P<0.05$) more (at least twice as many) weevils were captured in pseudostem traps at the base of the trap mat than at mats < 5 m or > 5 m from the trap mat in the two locations (Figure 2). The number of weevils captured on mats < 5m and >5 m away from the pheromone- baited trap were statistically similar ($P>0.05$).

### 3.2 Field transmission of *B. bassiana* (experiment 2)

None of the 120 weevils that were sampled in the field before the experiment showed any signs of infection after incubation for 21 days in the laboratory. In contrast, after the release of *B. bassiana* infected weevils, infected weevils were recovered. Of the weevils recovered by searching, about 5-10% of the weevils that were in the field (unmarked) and those that were released uninfected died due to pathogen infection (Table 1). Four to seven percent of those that were recovered by pseudostem trapping died due to pathogen infection after incubation in the laboratory for 21 days.

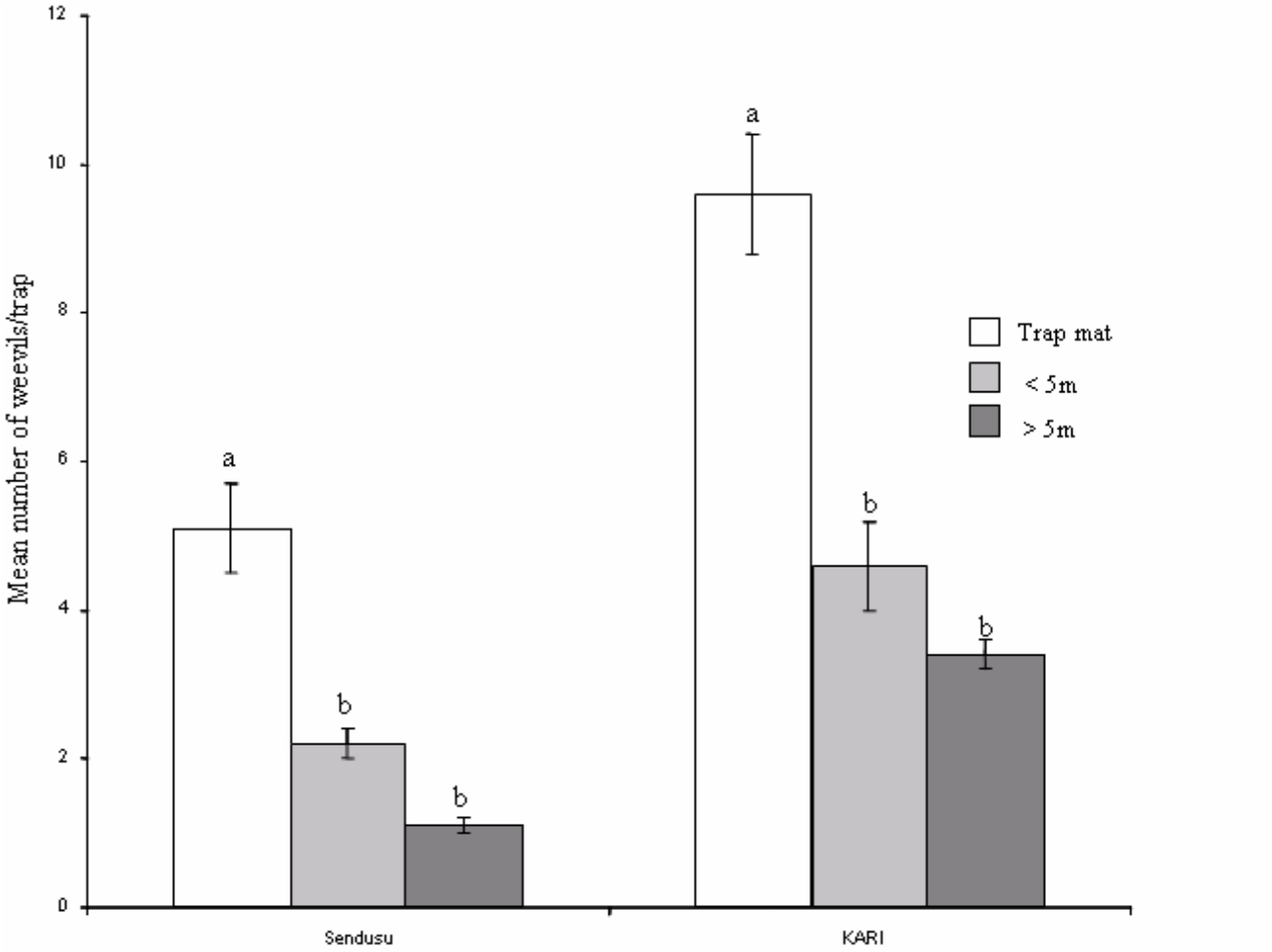


Figure 2: Mean (S.E) banana weevils captured with pseudostem traps on banana mats at different distances (m) from the pheromone-baited trap: trap mat = mat where pheromone had been placed prior to the pseudostem trapping period, < 5 m = mats less than 5 m, and > 5m= mats greater than 5 m from the pheromone trap. Mean of bars with similar letters are not significantly different (P>0.05, Student-Newman-Keuls test). There were 12 pheromone-baited traps per location.

### 3.3 Relative dispersal of *C. sordidus* in the field (experiment 3)

There was an association between the level of infection and the distance moved by weevils ($\chi 2$ = 27.8, d.f. = 4, P = 0.00004). Among weevils that had been released uninfected, a higher percentage was found on the same mat of release or on a mat less than 3 m from the release point, as compared to weevils released infected (Table 2). Still, over 20% of the infected weevils released moved for distances ranging from 3 to 12 m from the release point. The mean distances (m) moved per week by infected (0.96±0.15) and uninfected (1.17±0.07) weevils were not significantly different (P>0.05, t-test).

Table 1: Percentage banana weevil mortality to *B. bassiana* for uninfected and laboratory infected adults released into banana fields at Sendusu, Uganda and recovered by searching and pseudostem trapping.

*a) Searching*

| Treatment | Weevils recovered showing mycosis | | |
|---|---|---|---|
| | Number recovered | Number dead | % mortality[1] |
| Released infected | 113 | 107 | 94.7 |
| Released uninfected | 147 | 14 | 9.5 |
| Unmarked (field weevils) | 199 | 11 | 5.5 |

[1]Pooled mortality of weevils for two trials with each having three searching occasions

*b) Pseudostem trapping followed by incubation in the laboratory*

| Treatment | Weevils recovered showing mycosis | | |
|---|---|---|---|
| | Number recovered | Number dead | % mortality[1] |
| Released infected | 56 | 37 | 66.1 |
| Released uninfected | 595 | 39 | 6.6 |
| Unmarked (field weevils) | 876 | 37 | 4.2 |

[1]Pooled mortality of weevils for two trials with each having five sampling occasions

Table 2. Percentage of weevils that were released infected and uninfected that were recovered by pseudostem traps after moving different distances in the field.

| Distance moved (m) | Infected weevils | | Uninfected weevils | |
|---|---|---|---|---|
| | Number recaptured | % recaptured of total | Number recaptured | % recaptured of total |
| 0- 3 | 135 | 71.4 | 300 | 52. 3 |
| 3.1-6 | 30 | 15.9 | 121 | 21.1 |
| 6.1-9 | 11 | 5.8 | 86 | 15.5 |
| 9.1-12 | 4 | 2.1 | 38 | 6.6 |
| >12 | 9 | 4.8 | 29 | 5.1 |

There was an association between the distance ranges moved and the level of infection of the banana weevil ($\chi 2 = 27.8$, d.f. = 4, P = 0.00004, 2 x 5 contingency table test).

### 3.4 Locations of *C. sordidus* in the field (experiment 4)

*Caged trial:* Out of the total number of weevils (720) that were released infected with *B. bassiana* in six cages, 81.9% were recaptured. The majority of weevils dead due to *B. bassiana* infection were recovered on the corm surface (70.7%) (Table 3). Very few dead weevils due to *B. bassiana* infection were found in alleys and in the pseudostem.

*Field trial*: Most of the dead weevils (>70%) infected with the pathogen were recovered in the leaf sheath at the plant base and soil near the mat (Table 4). The number of dead weevils recovered from the leaf sheath was higher than those recovered from other locations combined. There were equal numbers of dead weevils due to pathogen infection that were recovered from soil near the mat, residues and trash. Equal numbers of dead males and females were recovered within the different locations in the banana field.

Table 3. Percentage of weevils infected with *B. bassiana* recovered with mycosis in different locations in an open caged trial at Sendusu, Uganda.

| Location | No. of weevils recaptured in each location with mycosis | | % of total recovered with mycosis |
|---|---|---|---|
| | Male | Female | |
| Corm surface | 191 | 226 | 70.7 |
| Soil near mat | 26 | 28 | 9.1 |
| Leaf sheath | 35 | 16 | 8.6 |
| Inside corm | 20 | 14 | 5.8 |
| Soil in alleys | 14 | 5 | 3.3 |
| Mulch | 5 | 6 | 1.8 |
| Pseudostem | 2 | 2 | 0.7 |
| Total | 293 | 297 | 100 |

The total number of weevils recovered with mycosis was 590 out of the 720 weevils that were released. Five weevils were recovered alive.

Table 4. Percentage of weevils killed by *B. bassiana* infection of the total that were recovered by hand searching in different locations in the banana field.

| Location of recapture | Number of recaptured weevils | | | % weevils showing signs of mycosis[1] |
|---|---|---|---|---|
| | Males | Females | Total | |
| Plant base- leaf sheath | 48 | 52 | 100 | 51.8 |
| Soil near mat | 19 | 17 | 36 | 18.7 |
| Residues | 16 | 16 | 32 | 16.6 |
| Trash | 15 | 10 | 25 | 12.9 |

[1]Pooled percentage of dead weevils recovered in different locations for two replications (three searching occasions per replication). Equal numbers of males and females were recovered in each of the locations ($\chi$2- test, P>0.05).

### 3.5 Pheromone-baited delivery systems of *B. bassiana* (experiment 5)

More weevils died due pathogen infection from plots where pheromones were used in combination with *B. bassiana* applied around the trap and on four adjacent mats compared to all other treatments (Figure 3). This was true for all distances from the pathogen release point. The number of weevils recovered from plots where *B. bassiana* was used alone without pheromone traps that died due to pathogen infection was generally lower but similar to those from plots where *B. bassiana* was applied inside and around the trap. The data show that the weevils can pick the pathogen from the aggregation point and disperse it: weevils showing signs of mycosis were recovered beyond 5 m from the pathogen release point. Overall, more weevils died due to *B. bassiana* infection in plots where *B. bassiana* was applied on the trap and four adjacent mats (12.5%) than where the pathogen was applied in the pheromone trap or around the pheromone trap (5.1%). Equal numbers of dead weevils were observed when the pathogen was applied inside and around the trap, although the percentage mortality was significantly higher than when the pathogen was applied alone without the pheromone trap.

Significantly more weevils infected with the pathogen were recovered by searching in plots where the pathogen was applied around the trap plus four adjacent mats compared to all other treatments (Table 5). The number of dead weevils showing mycosis recovered by searching was similar for plots where *B. bassiana* was applied inside the trap, around the trap mat and without pheromone traps.

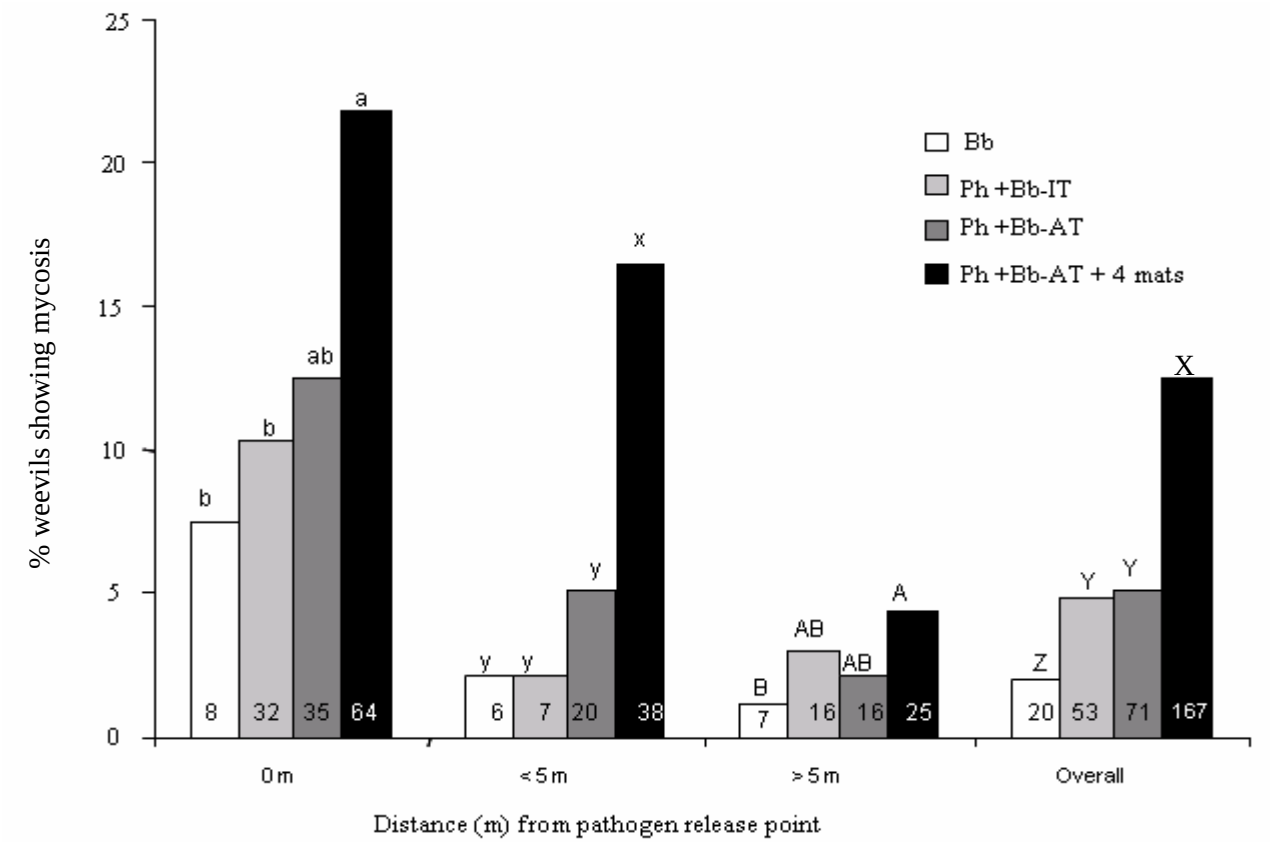


Figure 3: Percentage of weevils that died and showed signs of mycosis after incubation for 21 days and recaptured with pseudostem traps at different distances from the pathogen release point (central/ pheromone trap mat) in banana plots using different ways of applying *B. bassiana*. Bb=*B. bassiana* applied alone, Ph+Bb-IT= *B. bassiana* placed inside pheromone (Ph) trap; Ph+ Bb-AT =*B. bassiana* placed around pheromone trap mat; and Ph+Bb-AT + 4 mats = *B. bassiana* placed around trap mat and four adjacent mats. The percentages in a column followed by similar letters are not significantly different (tested per distance from pathogen release point): 4x2 contingency table test followed by Bonferroni correction α=0.05/6=0.008. The total number of dead weevils is indicated in the bars. Analysis was done on numbers and the four weevils dead from the control plots were not included in the analysis.

Table 5. Number of weevils recaptured by searching at different distances from the pheromone trap or fungus release points in plots with different delivery systems that were found dead due to *B. bassiana* infection.

| Treatment | Number of weevils recovered by searching | | | |
|---|---|---|---|---|
| | 0 m | < 5 m | > 5m | Overall |
| Bb | 3 | 2 | 0 | 5b |
| Ph + Bb-IT | 6 | 5 | 2 | 13b |
| Ph + Bb-AT | 7 | 4 | 2 | 13b |
| Ph + Bb-AT + 4 mats | 9 | 14 | 10 | 38a |

Bb= *B. bassiana* applied alone, Ph+ Bb-IT= *B. bassiana* placed inside pheromone (Ph) trap; Ph+ Bb-AT =*B. bassiana* placed around pheromone trap mat; and Ph + Bb-AT + 4 mats = *B. bassiana* placed around trap mat and four adjacent mats. The data presented in the table is pooled for two trials. There were no weevils recovered in control plots by searching. Numbers of weevils in column "Overall" followed by similar letters are not significantly different: chi square test followed by Bonferroni correction α=0.05/6=0.008.

# 4 DISCUSSION

Pheromone lures have been used successfully to spread pathogens to suppress populations of insect pests (Vega *et al*., 1995; Klein & Lacey, 1999). Possibilities of using pheromone for dissemination of fungal pathogens for the control of *C. sordidus* have been previously suggested (Budenberg *et al*., 1993b; Gold *et al*., 2001; Tinzaara *et al*., 2002a). Aggregation pheromone may be used to attract *C. sordidus* at sites where the entomopathogen *B. bassiana* is delivered. Successful use of pheromone lures in the dissemination of *B. bassiana* would require (1) that weevils can enter a trap, be exposed to the fungus and leave the trap; and (2) that the fungus can be transmitted from infected to uninfected weevils. In our study the potential of the pheromone to aggregate weevils around the pheromone-baited traps was demonstrated. More weevils were observed on the mat where the pheromone trap was placed compared to positions on mats 5 m away. The aggregation effect is likely to be influenced by the efficacy of the pheromone trap. If the pheromone attracts a higher proportion of the population to the pathogen delivery site, chances of transmission would be increased. Pheromone efficacy will need to be further increased to attract more weevils to the trap that would lead to higher percentage transmission of *B. bassiana.* Theoretically, the strategy of using the pheromone to aggregate weevils on the pathogen delivery sites would have the advantage of reducing the amount of fungal pathogen applied per unit area compared to the technique of treating individual mats (plants), and would be less labour intensive.

The effective control of the banana weevil using pheromone in a delivery system for *B. bassiana* assumes that infected individuals can disperse from the infection point carrying the pathogen throughout the pest's habitat. We have observed in our study that weevils that were released uninfected into an area where infected weevils had been introduced prior were later found dead with the fungus. It is important to note that previous assessment of pathogen-infection had shown that the pathogen was not present in the plots before the trials started. Although percentage mortality was low, it is possible that under suitable conditions (e.g. more moisture) such a level of infection would increase the overall inoculum level. This fungus-carry-over-effect through the application of pheromone has been previously reported for sweet potato weevils, *Cylas formicarius* (Fabricius), as an effective way of spreading the fungal pathogens throughout a specific habitat (Yasuda, 1999).

Observations on field locations preferred by infected weevils indicated that most cadavers were found within the vicinity of the banana mat (i.e on the corm, leaf sheath at the base of the mat and in the soil near the mat). These results have an important implication for the use of this pathogen in the control of *C. sordidus:* after being infected with *B. bassiana* the weevils move to locations where healthy individuals oviposit and mate (Gold *et al*., 2004a). This has the potential to increase chances of pathogen transmission during mating or individuals may be contaminated with the conidia from the cadaver. In

laboratory studies, Nankinga (1994) observed that transmission was higher from dead banana weevils than from live infected ones.

We know that the capacity to disperse, in addition to host factors, pathogen virulence, infectivity and persistence is a key factor in the ability of entomopathogens to develop epizootics (Roy & Pell, 2000). The use of an attractant in the system of introducing a deleterious agent into a pest population requires that the lured individuals can sufficiently disperse after visiting the self-contaminating site (Vega *et al*., 1995; Klein & Lacey, 1999; Roy & Pell, 2000). In our study using different delivery systems, it was observed that a number of weevils that died due to *B. bassiana* infection were recaptured at a distance of 10 m from the pathogen source. This suggests that these weevils were contaminated with the pathogen from the gallon trap baited with the pheromone and dispersed after infection. Placement of the pathogen in the pheromone trap and on a few adjacent mats was found to be more effective in disseminating the pathogen compared to other delivery systems that were tested. The observed generally low mortality or transmission of the fungal pathogen could be because the pheromone was not effective in attracting a large proportion of the weevils. It is also possible that the amount of the pathogen (200 g) was not sufficient. The amount of pathogen placed in the dissemination trap was reported to influence the effectiveness of the delivery system (Roy & Pell, 2000).

Our study generally demonstrated that weevils can aggregate around pheromone-baited traps. Application of *B. bassiana* in combination with pheromone-baited traps enhanced transmission of the pathogen from infected to uninfected individuals. Studies are currently in progress to evaluate the effect of integrating pheromones with *B. bassiana* on weevil populations and damage. If the strategy is found promising, the data will be used in exploiting opportunities for integration of pheromones into a broader IPM programme to control the banana weevil. Further studies will be required to investigate the amount of pathogen that is required in the pheromone trap for effective control of the pest. Additional field-testing will be required to further validate the delivery system of *B. bassiana* using pheromone-baited traps.

**Acknowledgement**

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Chapter **10**

# General discussion

## Infochemicals and banana weevil control

## 1 INTRODUCTION

Many organisms depend on chemical information to communicate with each other, to find suitable food or to avoid their enemies (Vinson, 1976; Roitberg & Isman, 1992; Vet & Dicke, 1992; Steidle & van Loon, 2003; Carde & Millar, 2004). Insects and other arthropods appear to be especially dependent on chemical stimuli that allow making 'decisions' that affect survival and reproduction (Schoonhoven *et al*., 1998). Chemicals that influence behaviour, which are termed infochemicals (Dicke & Sabelis, 1988), can be exploited by man to develop environmentally benign pest management options (Lewis & Nordlund, 1985; Dicke *et al*., 1990; Vite & Baader, 1990; Dicke, 1997; Foster & Harris, 1997). Infochemicals, especially pheromones can be used in pest monitoring and as a control measure through mating disruption, mass trapping and as a means of aggregating herbivores at delivery sites for biological control agents (Phillips, 1997; Giblin-Davis *et al*., 1996a; Howse *et al*., 1998; Hardie & Minks, 1999).

Control of the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), a pest of East African highland banana and plantains in most banana growing regions of the world is difficult. Cultural control is labour intensive while chemical control is too expensive and can only be afforded by wealthier farmers. Current research results suggest that no single control strategy will provide complete control of the banana weevil. An IPM strategy involving several methods (e.g cultural control, biological control, host plant resistance, and judicial use of chemical control) might offer the best chance for success in controlling this pest (Gold *et al*., 2001). The aim of this research project was to investigate whether infochemicals (pheromones and kairomones) can be used in an IPM approach for the banana weevil (Alpizar *et al*., 1999, Tinzaara *et al*., 2002a).

The banana weevil produces an aggregation pheromone that attracts both male and female conspecifics (Budenberg *et al.*, 1993b). The most abundant pheromone component, sordidin was

identified, synthesized and tested (Mori *et al.*, 1996; Fletcher *et al.*, 1997; Jayamaran *et al.*, 1997). The pheromone, synthesized in Costa Rica by Chemtica International and commercially available under the trade name Cosmolure+, has been recommended as an effective method of trapping and controlling the banana weevil (Alpizar *et al*., 1999; Tinzaara *et al*., 1999a). Use of pheromone lures may provide a weevil control option that is more effective and less labour-intensive than the use of pseudostem traps.

The overall objective of this research project was to evaluate the potential of using the weevil's aggregation pheromone to control the banana weevil under Ugandan conditions. Therefore, the chemical ecology of the banana weevil needed to be investigated. The research focus was first to elucidate the effects of the weevil aggregation pheromone and host-plant kairomone on weevil trapping as related to weevil behaviour and environmental conditions. Second, we investigated the impact of adult removal on weevil population dynamics and damage to the host plant. Finally, the potential was investigated of combining infochemical use with biological control using predators and entomopathogens for the management of the banana weevil. Specific experiments were conducted to determine: 1) the additive effects of host plant odours to the pheromone; 2) the factors that influence pheromone trap effectiveness; 3) the effect of pheromone trap density on weevil population and damage; 4) whether the predators of the banana weevil respond to host plant odours and the aggregation pheromone; and 5) whether the aggregation pheromone can be used to enhance dissemination of the entomopathogenic fungus *B. bassiana* for the control of the banana weevil.

In this chapter, an overview is given on the use of the aggregation pheromone and host-plant kairomone as components of an integrated management approach of the banana weevil, viz. the use of the aggregation pheromone in mass trapping, enhancement of the pheromone with host plant volatiles (kairomone), and integration of the pheromone with predators and entomopathogenic fungus, for the control of the banana weevil (Figure 1).

## 2 PHEROMONES AND BANANA WEEVIL CONTROL

Control of insect pests can be achieved by mass trapping using pheromone-baited traps that lure insects to their death (Jansson *et al*., 1993; Giblin-Davis *et al*., 1994a; Howse *et al*., 1998; Hardie & Minks, 1999). Successful suppression of the pest population by mass trapping using pheromones has been previously reported for other weevils, such as the boll weevil *Anthonomus grandis grandis* (Hardee, 1982), the sweet potato weevil *Cylas formicarius* (Alvarez, 1996) and the palm weevil *Rhynchophorus palmarum* (Oehlschlager *et al*., 1995). Therefore, the potential for using an

aggregation pheromone to control coleopteran populations by mass trapping exists. The advantage is that, with few exceptions, coleopterans use aggregation pheromones, attracting both sexes. By trapping males and females the reproductive capacity of the population will be negatively affected (Trematerra, 1997; Hardie & Minks, 1999). The banana weevil pheromone Cosmolure+ attracts both males and females (Alpizar *et al*., 1999; Tinzaara *et al*., 2000), suggesting that the banana weevil is a good candidate for mass trapping. Mass trapping is most promising for insect pests (such as the banana weevil) with low fecundity, slow population build up, limited dispersal and long life span (Giblin-Davis *et al.,* 1996a).

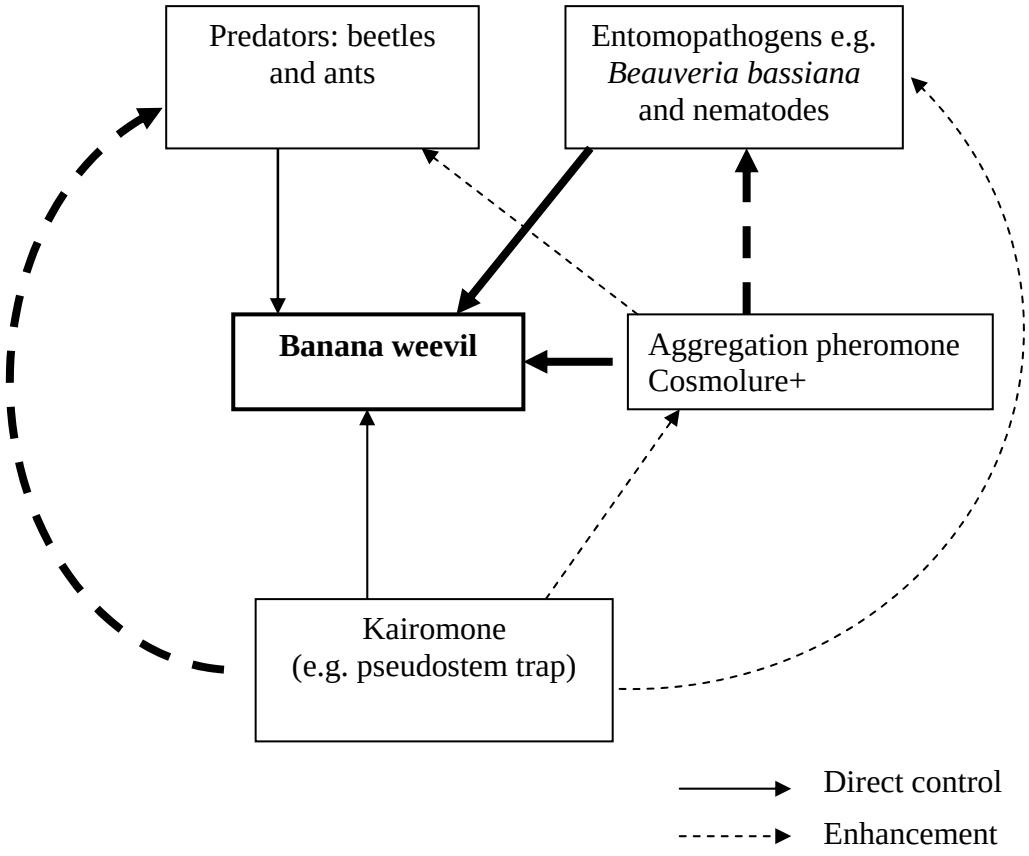



Figure 1: The aggregation pheromone Cosmolure+ as a component in the integrated management of the banana weevil *Cosmopolites sordidus*. The thickness of the lines represents the relative importance of the indicated effect.

Use of the aggregation pheromone Cosmolure+ for trapping the banana weevil was reported in Costa Rica as a promising option (Alpizar *et al.,* 1999). The studies in Costa Rica indicated that the pitfall trap baited with the pheromone captured up to 10 times more weevils than the pseudostem traps (Alpizar *et al*., 1999). In preliminary studies conducted in Uganda, the pitfall trap baited with the pheromone captured up to 18 times more weevils than pseudostem traps (Tinzaara *et al.*, 2000).

The pitfall pheromone-baited trap captured more weevils than the gallon-with-a-ramp trap (Figure 2). The numbers of weevils captured in my subsequent studies were lower than in the initial tests and more were captured in on-station studies than in farmers' fields (chapters 4-7). Possible reasons for the low trap efficacy in my studies are discussed in the next section.

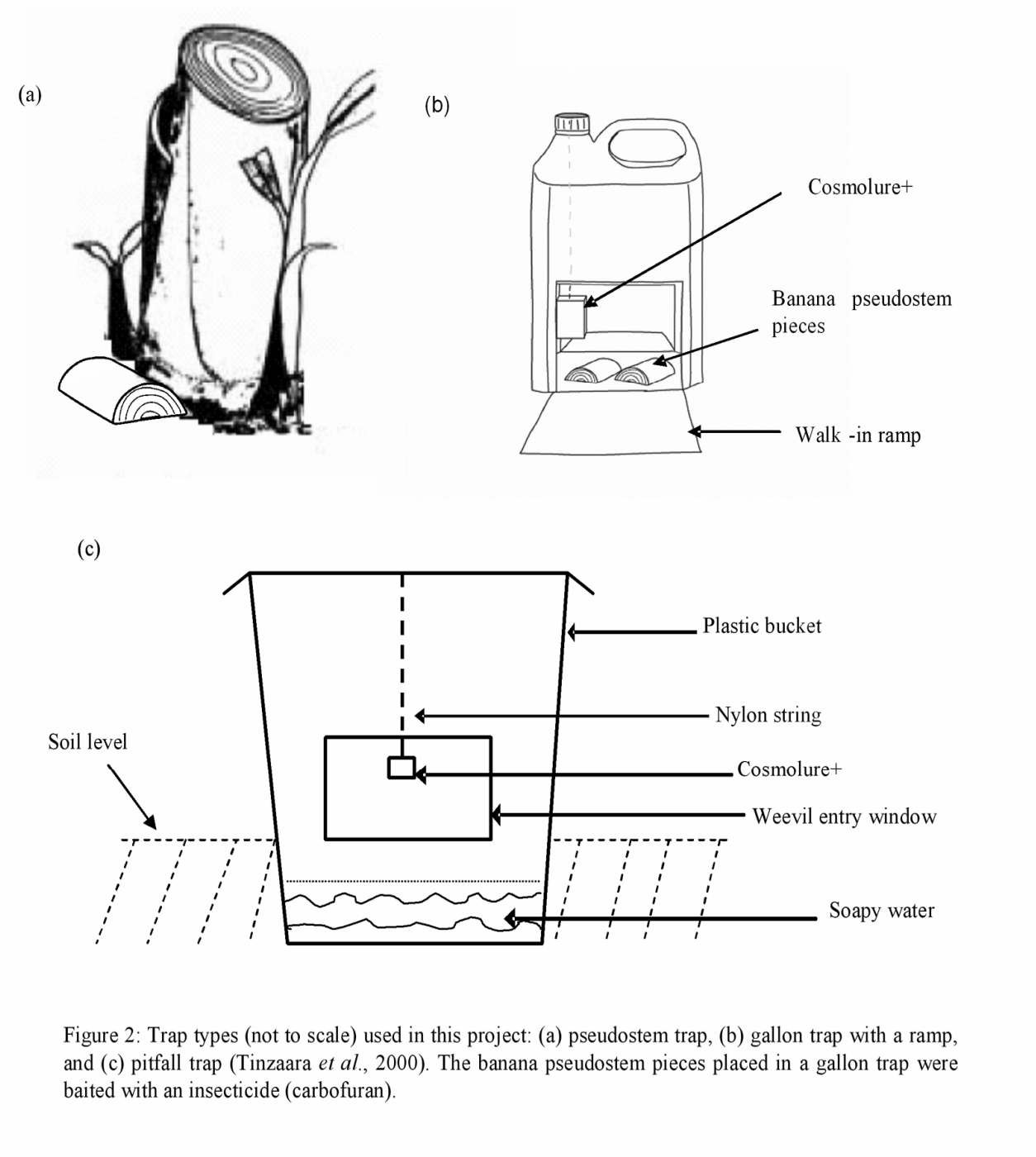



Figure 2: Trap types (not to scale) used in this project: (a) pseudostem trap, (b) gallon trap with a ramp, and (c) pitfall trap (Tinzaara *et al.*, 2000). The banana pseudostem pieces placed in a gallon trap were baited with an insecticide (carbofuran).

To optimise trap placement, it is critical to know the trap density that is required to adequately sample the pest population (David & Birch, 1986; Howse *et al.,* 1998). The effective trap radius has direct implications on the trap density used and related costs. Studies in Costa Rica estimated the radius of effective attraction of banana weevil by the pheromone trap to range from 5-15 m (A.C. Oehlschlager, pers. comm.). The effective trap radius was estimated to be 10 m. My studies confirmed the observation from Costa Rica (chapter 5). In mass trapping strategies, it is important to know the proportion of the population that is captured. The proportion of weevils recaptured in a month within a range of 10 m from the trap or even in the close vicinity (< 0.5 m) of the trap was, however, generally low (up to 14%). One possible explanation for such low recapture rates is that weevils may be attracted to the trap but remain only within the vicinity without entering it (Tinzaara *et al.*, 2002b). An alternative explanation may be that a large proportion of the released weevils dispersed from the trap.

Farmer adoption of the pheromone trapping system will be more likely when yields are increased by a significant reduction of the weevil population and, consequently, its damage. In Costa Rica, mass trapping trials in plantations of banana and plantains with pheromone traps at a density of four per ha were effective in reducing weevil damage to banana corm by over 60% and bunch weight was increased by 20% (Alpizar *et al.*, 1999). Studies were conducted to investigate whether these results could be replicated under Ugandan conditions (chapter 7). Banana fields with weevil populations of up to 12,000 weevils per ha in Masaka district, Uganda were used. In pheromone-treated fields (at trap density of 4 or 8 traps per ha), adult banana weevil population or corm damage was not significantly reduced when compared to the control. The proportion of adult banana weevils captured in pheromone traps was so low that the trapping system probably had only limited impact on the insect's pest status on study farms. In my studies, because of the low weevil catches in pheromone traps, doubling the number of traps from four to eight per hectare increased the number of weevils captured, but not the rate of reduction of weevil damage. Theoretically, the effectiveness of mass trapping could be improved by increasing the density of traps (Knipling, 1979), but densities higher than eight traps per ha are neither economical nor practical. My data, which are in agreement with studies conducted in Cameroon (Messiaen, 2001), suggest that the pheromone trapping system by itself was not effective in the Ugandan banana cropping system.

# 3 EFFECTIVENESS OF PHEROMONE TRAPPING

Several factors (such as the pheromone efficacy, trap parameters, cropping system and environmental factors) may influence the pheromone trap effectiveness. An understanding of how these factors influence catches of the banana weevil in pheromone-baited traps is critical in the effective deployment of the trap.

## 3.1 Pheromone efficacy

The success of suppressing pest populations depends on the efficacy of the trap used. Pheromone dose and release rate may influence how traps perform relative to the natural pheromone sources and thus determine how well they attract insects (Mason & Jansson, 1991; De Groot & De Barr, 1998). Cosmolure+ is packed in plastic containers of 90 mg releasing at a rate of 3 mg per day (Oehlschlager pers. comm.). The number of weevils attracted by Cosmolure+ may be enhanced by increasing the release rate of the pheromone or by using two lures per trap. Increasing the number of lures per trap may increase the number of weevils captured but the efficiency (number captured per lure) may be decreased (chapter 3). Increasing the number of lures has the disadvantage of making the trapping system costly. There are also cases where reduced pheromone trap catches due to higher release rates of the pheromone were observed (Howse *et al.,* 1998; Hardie & Minks, 1999). In this case the insect may reach a response threshold before reaching the pheromone trap.

The efficacy of the lure may also depend on how well it is stored and on the length of the storage period. The low pheromone efficacy (due to transportation and storage) may have contributed to the low weevil catches in traps on-station and on-farm (chapter 7).

## 3.2 Trap parameters

Trap placement should be appropriate to have a large proportion of the weevil population enter the trap. The location of the trap relative to the host plant has been observed to influence trap catches (McNeil, 1991). Weevils are normally found at the base of the banana mat (Bakyalire, 1992; Gold *et al*., 2004b). The pheromone-baited traps placed at the base of the banana mat were expected to capture more weevils than those placed in alleys. However, in my studies weevil catches did not depend on the location relative to the banana mat (chapter 5).

Pheromone trap maintenance is another major factor that influences catches in traps. Trap catches in on-station fields where the trap was regularly checked were normally higher than in on-farm studies (pers. observ.). Low trap catches were observed in pheromone traps where the soapy

water was not regularly replaced. The traps also need to keep contact with the soil as this facilitates the entry of the weevils. Covering the soil around the trap with banana leaves may maintain the moisture around the trap and this keeps the soil in contact with the trap (Murad, 2001). In my studies pheromone-baited traps with banana leaves covering the soil around the trap captured higher numbers of weevils compared to uncovered traps (chapter 5).

Trap size, colour and shape are some of the other trap parameters that may influence pheromone trap catches. These parameters were not investigated in my studies since previous studies with other members of the Rhyncophorinae subfamily (e.g *Metamasius hemipterus*) showed that these factors did not have a substantial effect on pheromone trap catches (Oehlschlager *et al.*, 1993; Giblin-Davis *et al*., 1996a).

### 3.3 Cropping system

The pheromone trapping efficiency may be influenced by cropping pattern (monocropping or mixed cropping), whether particular crops have one or several planting seasons, or whether planting is consolidated into blocks of single crops or comprises of small fields of various crops (Hebblethwaite, 1989). For example, mass trapping with Cosmolure+ pheromone was a success in Costa Rica where the experiments were conducted on large and well-managed cropping systems (Alpizar *et al*., 1999), while in Uganda bananas are produced in complex mixtures of crops with low management levels. The pheromone may compete with natural volatiles in the system that reduces its effect. Non-host plant volatiles have been reported to have inhibitory effects that interfere with insect responses to pheromones (Dickens *et al*., 1992; Byers *et al*., 1998; Reddy & Guerrero, 2004).

The role of mulching on weevil catches in pheromone traps was not known and yet mulching is a practice that is widely recommended to farmers in Uganda for conserving soil moisture and reducing soil erosion. Weevils are reported to have greater activity and movement in mulched than unmulched areas (Gold *et al*., 1999b). I hypothesised that mulching results in lower catches in pheromone-baited traps when the mulch odour would mask the pheromone perception by the weevils. However, mulching may also increase weevil activity leading to higher catches in pheromone traps. My experiments to test these hypotheses showed that similar numbers of banana weevils were captured in pheromone-baited traps in mulched and unmulched plots (chapter 6). Mulching is therefore compatible with the use of pheromone-baited traps in the control of the banana weevil.

Pheromone trapping may not result in the suppression of the pest populations especially when host plant residues provide a basis for pest multiplication (Weslein, 1992; James *et al*., 1996). In banana fields that are poorly managed, the pheromone may have to compete with volatiles from residues and stumps. This may imply that residue management in banana fields in Ugandan cropping systems will need to be improved before employing the pheromone traps. However, although residue management may to contribute substantially to pheromone trap catches, I was not able to investigate the role of this factor on weevil catches in pheromone traps during my research period. Studies on the role of residue management on pheromone trap catches are, however, currently in progress in Uganda.

### 3.4 Environmental factors

Climatic factors such as rainfall, relative humidity, temperature, wind speed and direction have been reported to influence insect catches in pheromone-baited traps (Jansson *et al.*, 1989; Laboke, *et al*., 2000; Sappington & Spurgeon, 2000). In my field studies, there was no relationship between temperature or wind speed and catches of the banana weevil in pheromone traps (chapter 5). The wind speed at the soil level in banana fields is negligible and was expected to have limited effect on weevil trap catches. Relative humidity but not rainfall showed a significant positive relationship with weevil catches in pheromone-baited traps. In all my studies, more weevils were caught in the wet season than in the dry season although the effect was not statistically significant. A reason could be that during the dry season weevils had difficulties in entering the trap as shrinking soil produced a gap between the trap and the soil surface (chapter 7). Pheromone-baited pitfall traps need to be placed tightly against the soil surface to allow the banana weevil to enter. In a related study (chapter 5), it was demonstrated that covering the soil around the pheromone trap with banana leaves kept the trap tight to the soil as it conserved soil moisture around the trap. This led to increased trap catches in our studies and in those conducted in the Canary Islands (A. Carnero, pers. comm.) and Australia (Murad, 2001). The effect of covering the soil around the trap is probably only effective during the dry season and may not be necessary during the wet season.

# 4 ENHANCEMENT OF THE PHEROMONE WITH HOST PLANT ODOURS

There are a number of laboratory and field studies that demonstrated that the effect of male-produced aggregation pheromones of several insect orders can be enhanced by host plant volatiles (Oehlschlager *et al.*, 1993; Giblin-Davis *et al.*, 1996a; Hallet *et al*., 1999; Reddy & Guerrero, 2004). Synergism in which responses to the mixture of pheromone and plant volatiles is greater than the combined responses to the individual components has been reported for several weevils in the subfamily Rhynchophoridae, such as *R. palmarum* and *M. hemipterus* (Giblin-Davis *et al*., 1994a; Rochat *et al.*, 2000). However, laboratory studies in which several different bioassay set-ups were used for evaluating banana weevil orientation responses showed that host plant volatiles have an additive rather than a synergistic effect to the synthetic pheromone with respect to banana weevil attraction (Tinzaara *et al*., 2003, chapter 2). Detailed studies were then conducted on relative attractivity of the host plant tissue and the aggregation pheromone Cosmolure+ to the banana weevil in the laboratory and in the field (chapter 3). In the laboratory, I used a double pitfall olfactometer because this set-up was easy to fabricate. Budenberg *et al* (1993b) and Treverrow (1994) used a similar apparatus for evaluating the banana weevil response to banana kairomones. I further observed that volatiles from pseudostem tissue had an additive effect on attraction of weevils to the pheromone in the laboratory but the effect was limited in the field.

A weak dose-dependent additive effect was observed of pseudostem tissue to the pheromone in the field. A dose dependence response to the pheromone combined with different ratios of the host plant volatiles was reported for the palm weevil, *R. palmarum* (Oehlschlager *et al*., 1993) and the West Indian sugar cane borer, *M. hemipterus* (Giblin-Davis *et al.,* 1994a). The catches of the banana weevil would theoretically be improved by increasing the amounts of pseudostem tissue added to the pheromone trap but this would make the trapping system labour intensive and costly. Enhancement of the aggregation pheromone with pseudostem tissue was not influenced by the banana cultivar (W. Tinzaara & C. Gold, unpubl.). My studies generally suggest that for mass trapping of the banana weevil, the pheromone should be used alone rather than combining it with either fresh or fermented pseudostem tissue.

# 5 INTEGRATION OF INFOCHEMICALS WITH BIOLOGICAL CONTROL

Mass trapping should be considered as one component of a set of management practices. An IPM strategy involving several methods might offer the best chance for success in controlling this pest (Gold *et al*., 2001). The pheromone and kairomones may play a critical role in enhancement of predators and entomopathogens for the control of the banana weevil (Figure 1).

## 5.1 Infochemicals and predators

Infochemicals serve as cues mediating interactions among plants, herbivorous insects and their natural enemies (Vet & Dicke, 1992; Dicke, 2000; Turlings *et al*., 1995; Steidle & van Loon, 2003). They play an essential role in almost all stages of prey searching and prey selection (Dicke *et al*., 1999a). Understanding the role of these chemicals can be important for effective deployment of predators as biological control agents. Predator behaviour can be manipulated in the field by the use of infochemicals (Sabelis & Dicke, 1985; Dicke *et al*., 1990; Foster & Harris, 1997; Steidle & van Loon, 2002).

Predators of the banana weevil are often observed in decomposing banana tissue such as harvested stumps, pseudostem traps and often in tunnels where banana weevil eggs, larvae and pupae are normally found (Koppenhofer *et al*., 1992; Koppenhofer, 2003). The beetle *Dactylosternum abdominale* and the ant *Pheidole megacephala* are the most abundant predators in environments preferred by weevils in Uganda (Tinzaara *et al*., 1999b; Gold *et al*., 2001; Abera, 2004). These predators are generalists that feed on micro-fauna and –flora in decomposing plant tissues, and on eggs and small larvae of insects. Banana pseudostem tissue in a decomposing condition is more attractive to these predators than when fresh (Koppenhoefer, 1993). Use of infochemicals by generalist predators during prey location has been documented (Dwumfour, 1992; Scutareanu *et al*., 1997; Haberkern & Raffa, 2003; Steidle & van Loon, 2003; McGregor & Gillespie, 2004). Information on how volatiles from decomposing banana pseudostem tissue influence these predators in their host searching and location was lacking prior to the research described in this thesis (chapter 8). We assessed whether *D. abdominale* and *P. megacephala* predators under laboratory and field conditions used volatile infochemicals associated with banana weevils. Both predator species discriminated between volatiles from fermented pseudostem tissue and clean air. Neither of the predators discriminated between volatiles from weevil larvae and clean air. Although volatiles from prey may be reliable cues (Vet & Dicke, 1992; Wiskerke *et al*., 1993), fermented plant volatiles appear to be more important to the predators of the banana weevil than volatiles from the prey itself during habitat and prey location. This suggests that the decomposing pseudostem traps (kairomones) and residues normally found in the banana fields may be manipulated to enhance natural enemy population build up (Masanza, 2003).

The banana weevil aggregation pheromone had neither synergist nor additive effects to volatiles from the banana tissue with respect to attraction of the banana weevil predators. My field studies did not show that the pheromone influences predator distribution around the pheromone trap. In contrast, several other predator species have been reported to use pheromones of their prey during

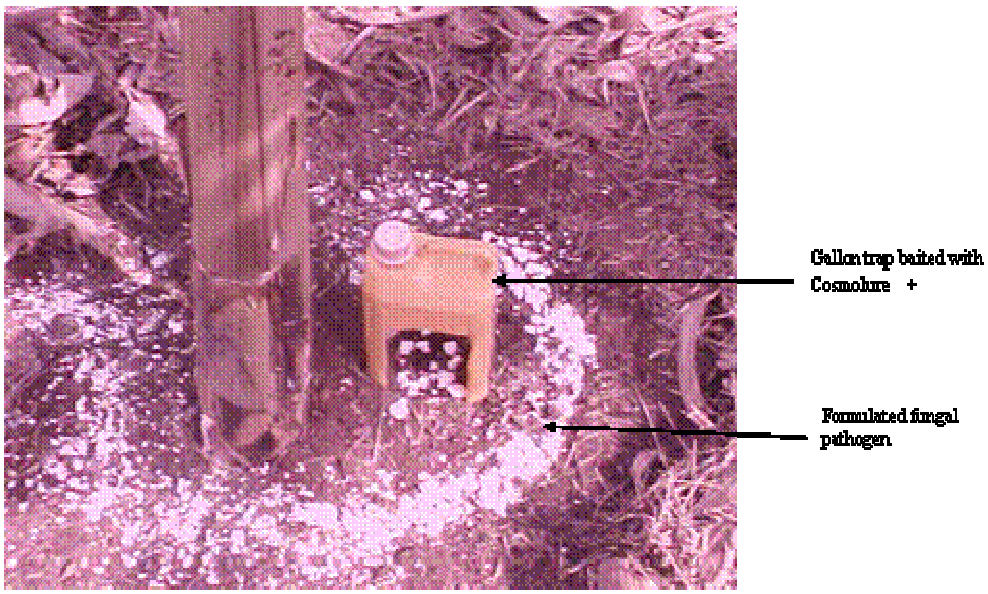
prey searching and location (Dwumfour, 1992; Hedlund *et al*., 1996; Haberkern & Raffa, 2003; Steidle & van Loon, 2003).

## 5.2 The aggregation pheromone for delivery of *Beauveria bassiana*

Entomopathogens such as the fungus *Beauveria bassiana* have the potential to control the banana weevil but their application is limited by the lack of a viable delivery system (Nankinga, 1999; Nankinga & Moore, 2000; Godonou, 2000). Pseudostem traps can be used to deliver fungal pathogens (Figure 1) but were found ineffective (Nankinga, 1999). The aggregation pheromone Cosmulre+ has been suggested as an option that can be used to aggregate the banana weevil at delivery sites of entomopathogens (Budenberg *et al*., 1993b; Gold *et al*., 2001; Tinzaara *et al*., 2002a, b). Successful use of pheromones for delivery of fungal pathogens has been reported for several insect pests (Klein & Lacey, 1999; Yasuda, 1999; Vega *et al*., 2000). The development of a pheromone trapping system for dissemination of biological control agents would require pheromone-baited traps that allow both entry and exit of the banana weevils (Tinzaara *et al*., 2000). Successful use of pheromone lures to enhance dissemination of *B. bassiana* would also require that large numbers of the pest are attracted to the trap, where they pick up the pathogen and infect healthy individuals outside the trap. This delivery system would have the advantage of reduced costs as only a limited amount of the pathogen is needed in traps and field applications would not be necessary.

Pheromone-baited traps were observed to aggregate the banana weevil on trap mats and adjacent mats in my field studies (Tinzaara *et al.*, 2004a, chapter 9). These studies demonstrated that weevils can get contaminated with the pathogen from the gallon trap baited with the pheromone and that they disperse after infection (Tinzaara *et al*., 2004a, chapter 9). Infected weevils were observed to effectively transmit the fungus to uninfected individuals in the field. Infected weevils were mainly found within the vicinity of the banana mat (i.e leaf sheath at the base of the mat and in the soil near the mat) (Figure 3). These results have an important implication for the transmission of the pathogen: after being infected with *B. bassiana* the weevils move to locations where healthy individuals oviposit and mate (Gold *et al*., 2004b). Placement of the pathogen in the pheromone trap and on the mat of the pheromone trap (Figure 3) and on a few adjacent mats was found to be more effective in delivering the pathogen compared to placing the pathogen only inside the trap. Although the percentage of weevils infected in the field was 5-10% after 42 days, it is likely that greater numbers could become infected over time.

(a)




(b)

Figure 3. Maize formulated fungal pathogen, *Beauveria bassiana* applied around banana mat and in a gallon trap baited with the aggregation pheromone (a). White mycelium growing out of dead weevils found at the base of the banana plant in the leaf sheath (b).

The percentage transmission is also likely to increase when more pathogen is placed in the trap. My data are promising for incorporating this method in a strategy to control of the banana weevil. Further studies should be conducted to investigate the factors that can improve the percentage transmission for the control of the banana weevil.

# 6 FUTURE RESEARCH ASPECTS

The aggregation pheromone is species specific and the negative impact on non-targets is limited. The pheromone has a low potential for development of pest resistance. A major restriction to use of the banana weevil aggregation pheromone is the availability and the price of the lures. The pheromone lures are commercially available in Costa Rica at about 2 US dollars per lure. In addition to the costs of the lure, the costs of transportation, distribution and storage will be prohibitive to subsistence farmers in Uganda. For any future use, a study should be conducted about the feasibility of locally producing an effective pheromone that can be easily accessed.

Mass trapping by using the aggregation pheromone alone does not seem to be an effective method for the control of the banana weevil under Ugandan conditions, even at an increased trap density. The trapping system did not substantially reduce weevil populations and damage to banana plants. Whatever the cause of the low trap catches, there appears to be little practical scope for further optimizing the trapping system with pheromone only under Ugandan conditions. The next strategy for use of pheromones is to exploit the potential of integrating it with the use of *B. bassiana* and entomopathogenic nematodes (EPNs).

Studies to evaluate the effect of integrating pheromone trapping with the use of *B. bassiana* on weevil populations and its damage to banana plants are being conducted in Uganda. Should the integration strategy prove effective in reducing weevil population densities and damage in the field, this method would have the advantage of reducing the amount of fungal pathogen applied per unit area compared to the technique of treating individual mats (plants). Moreover, it would be less labour intensive. Subsequently, studies would be required to investigate the amount of pathogen required in the pheromone trap for effective control of the pest. Additional field-testing will be necessary to validate further the delivery system of *B. bassiana* using pheromone-baited traps on farmers' fields.

The potential of integrating pheromone trapping with entomopathogenic nematodes (EPNs) has been reported to be promising for control of the banana weevil in the French West Indies (M. Guillon; pers. comm.). The potential of using EPNs (e.g *Steinernema carpocapsae*) to control the

banana weevil has been reported by Schmitt (1993) and Treverrow and Bedding (1993). EPNs are widespread and are more effective against banana weevil larvae than against adults (Pena & Duncan, 1991). Their widespread use will be limited by lack of a viable delivery system. The EPNs need to be evaluated in Uganda and opportunities for integration with pheromones should be explored for the management of the banana weevil.

# References


Abera, K.A.M., Gold, C.S., Kyamanya, S. & Karamura, E.B. 2000. Banana Weevil *Cosmopolites sordidus* Germar Ovipositional preferences, timing of attack and larval survival survivorship in a mixed cultivar trial in Uganda. *Acta Horticulturae*, 540: 487-496.

Abera, K.A.M. 2004. Patterns of predation by natural enemies of the banana weevil (Coleoptera: Curculionidae) in Indonesia and Uganda. Ph.D Thesis, University of Massachusetts, Amherst, USA.

Agelopoulos, N., Birkett, M.A., Hick, J.A., Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E, Smuiley, D.W.M., Wadhams, I.J. & Woodcook, C.M. 1999. Exploiting semiochemicals in Insect Control. *Pesticide Science*, 55: 225-235.

Aldrich, J.R. 1999. Predators. In: Hardie, J. & Minks, A.K. (eds), *Pheromones of non-lepidopteran Insects Associated with Agricultural plants.* CAB International, UK, pp. 357-381.

Alpizar, D., Fallas, M., Oehlschlager, A.C., Gonzalez, L.M. & Jayaraman, S. 1999. Pheromone-based mass trapping of the banana weevil, *Cosmopolites sordidus* (Germar) and the West Indian sugarcane weevil *Metamasius hemipterus* L. (Coleoptera: Curculionidae) in banana and plantain. *Memorias XIII Reunion ACORBAT*, 23-27 November 1998. Guayaquil, pp. 515-538.

Alvarez, P., Escarraman, V., Gomez, E., Villar, A., Jimenez, R., Ortiz, O., Alcazar, J. & Palacois, M. 1996. Economic impact of managing sweet potato weevil (*Cylas formicarius*) with sex pheromones in the Dominican Republic. In: Walker, T. & Chrissman, C. (eds), *Case studies of the economic impact of CIP related technology.* International Potato Centre, Lima, Peru, pp. 83-94.

Arleu, R.J. 1982. Dinamica populacional e controle do *Cosmopolites sordidus* (Germar, 1824) e *Metamasius hemipterus* L., 1764 (Col.: Curculionidae), em bananais da cv. Prata, no Espirito Santo. Brasil, Piracicaba. ESALQ. 66 pp.

Aukema, B.H., Dahlsten, D.I. & Raffa, K.F. 2000. Improved population monitoring of bark beetles and predators by incorporating disparate behavioural responses to semiochemicals. *Environmental Entomology*, 29: 618-629.

Bakke, A.1981. Utilisation of aggregation pheromones for the control of spruce bark beetle. In: Leonhardt, B.A. & Beroza, M. (eds), *Insect pheromone technology*: *chemistry and applications.* American Chemical Society, Washington, pp. 210-229.

Bakyalire, R. & Ogenga-Latigo, M.W. 1994. Aspects of the life cycle and behaviour of *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). *East Africa Agricultural and Forestry Journal*, 59: 337-344.

Bakyalire, R. 1992. A study of the life cycle and behaviour of the banana weevil *Cosmopolites sordidus* Germar, in Uganda. Unpublished M.Sc. thesis. Makerere University, 118 pp.

Bartelt, R.J. 1999. Weevils. In: Hardie, J. & Minks, A.K. (eds), *Pheromones of non-lepidopteran insects associated with agricultural plants.* CAB International, UK, pp. 91-112.

Beauhaire, J., Ducrot, D.G., Malosse, C., Rochat, D., Ndiege, I.O. & Otieno, D. O. 1995. Identification and synthesis of sordidin, a male pheromone emitted by *Cosmopolites sordidus*. *Tetrahedron letters*, 36: 1043-1046.

Bell, W.J. & Carde, R.T. (Eds), 1984. Chemical Ecology of Insects. Chapman and Hall, London, 554 pp.

Bendicho, L.A. 1987 Poder de perception de la hormiga *Tetramorium guineense* para el control biolologico del picudo negro del platano. *Ciencias de la Agricultura*, 30: 13-15.

Bernasconi, M.L., Turlings, T.C.J., Edwards, P.J., Fritschze-Hoballah, M.E., Ambrosetti, L., Bassetti, P. & Dorn, S. 2001. Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). *Agricultural and Forest Entomology*, 3:201-209.

Birch, M.C. & Haynes, K.F. 1982. *Insect pheromones*. Arnold Publishers, London, 247pp.

Blum M. S. 1974 Pheromone basis of social manifestations in insects. In: Birch, M.C. (ed) *Pheromone.* Amsterdam, North Holland Publishing Company, pp. 135-199.

Bolter, C.J., Dicke, M., van Loon, J.J.A., Visser, J.H. & Posthumus, M.A. 1997. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, 23: 1003-1023.

Borden, J.H. 1977. Behavioural responses of Coleoptera to pheromones, allomones and kairomones. In: Shorey, H.H. & Mckelvey, J.J. (eds), *Chemical control of insect behaviour, theory and application.* John Wiley and Sons, London, pp. 169-198.

Borden, J.H. 1993. Strategies and tactics for the use of semiochemicals against forest insect pests in North America. In: Lumsden, R.D. & Vaughin, J.L. (eds), *Pest management biologically based technology*. American Chemical Society Conference Proceedings Services. Washington, D.C., pp: 265-279.

Borden, J.H., Lindergren, B.S. & Chong, L. 1980. Ethanol and alpha pinene as synergists for the aggregation pheromones of two *Gnathotrichus* species. *Canadian Journal of Forestry Research*, 10: 290-292.

Bottrell, D.G., Barbosa, P. & Gould, F. 1998. Manipulating natural enemies by plant variety selection and modification: A realistic strategy? *Annual Review of Entomology*, 43: 347-367.

Bowers, W.S., Nault, l., Webb, R.E. & Dutky, S.R. 1972. Aphid alarm pheromone: isolation, identification and synthesis. *Science,* 177: 121-1122.

Braimah, H. & Van Emden, H.F. 1999. Evidence for the presence of chemicals attractive to the banana weevil, *Cosmopolites sordidus* (Colepoptera: Curculionidae) in dead banana leaves. *Bulletinof Entomological Research,* 89: 485-491

Budenberg, W.J., Ndiege, I.O. & Karago, F.W. 1993b. Evidence for volatile male-produced pheromone in banana weevil *Cosmopolites sordidus. Journal of Chemical Ecology*, 19: 1905-1916.

Budenberg, W.J., Ndiege, I.O., Karago F.W., & Hansson, B.S. 1993a. Behavioural and electrophysiological responses of the banana weevil, *Cosmopolites sordidus* to host plant volatiles. *Journal of Chemical Ecology*, 19: 267-272.

Burkholder W.E. & Bousch, G.M. 1974. Pheromones in stored product insect trapping and pathogen dissemination. *Bulletin Organisation-Europeenne-et-Mediterraneenne-pour-la-Protection-des-Plantet,* 4: 455-461.

Burkholder, W.E. & Ma, M. 1985. Pheromones for monitoring and control of stored product insects. *Annual Review of Entomology,* 40: 559-586.

Burkholder, W.E. 1990. Practical use of pheromones and other attractants for stored product insects. In: Ridgway, R.L., Silverstein, R.M. & Inscoe, M.N. (eds), *Behaviour modifying chemicals for insect management, applications of pheromones and other attractants,* Marcel Dekker, New York, pp. 497-516.

Butenandt, A., Beckmann, R., Stamm, D. & Hecker, E. 1959 Uber densexual-lockstoff des seidenspinners *Bombyx mori*. Reinderstellung und Konstitution. *Naturforsch*. B14, 283-284.

Byers, J.A. 1999. Effects of attraction radius and flight paths on catch of scolytid beetles dispersing outward through rings of pheromone traps. *Journal of Chemical Ecology*, 25: 985-1005.

Byers, J.A., Zhang, Q.H., Schlyter, F. & Birgersson, G. 1998. Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles. *Naturwissenschaften,* 85:557-561.

Byers, J.A. 1992. Attraction of bark beetles, *Tomicus piniperda, Hylurgops palliatus and Trypodendron domesticum* and other insects to short chain alcohols and monoterpenes. *Journal of Chemical Ecology,* 18: 2385-2402.

Campion, D.G., Critchley, B.R. & McVeigh, L.J. 1989. Mating disruption. In: Jutsum, A.R. & Gordon, R.F.S. (eds), *Insect pheromones in plant protection.* John Wiley and Sons, Chichester, pp. 89-119.

Carde, R.T. & Bell, W.J. (eds), 1995. *Chemical ecology of Insects.* Volume 2. Chapman and Hall, New York, 562 pp.

Carde, R.T. & Minks, A.K. 1995. Control of moth pests by mating disruption: Successes and constraints. *Annual Review of Entomology,* 40: 559-585.

Carde, R.T. & Millar, J.G. 2004. Advances in insect chemical ecology. Cambridge University press, London, 346 p.

Cardé, R.T. & Minks, A.K. (eds.). 1997. Insect Pheromone research: new directions. New York, Chapman & Hall, pp. 333-445.

Cerda, H., Lopez, A., Sanoja, O., Sanchez P., & Jaffe, K. 1996. Atracccion olfactiva de *Cosmopolites sordidus* Germar (1824) estmulado por volatiles originados en Musaceas de distintas edades y variedades genomicas. *Agronomia Tropical*, 46:413-429.

Cerda, H., Fernandez, G., Lopez, A. & Varga, J. 1999. Olfactory attraction of the sugar cane weevil (Coleoptera: Curculionidae) to host plant odors, and its aggregation pheromone. *Florida Entomologist,* 82: 103-112.

Cerda, H., Lopez, A., Sanoja, O., Sanchez, P. & Jaffe, K. 1996. Atracccion olfactiva de *Cosmopolites sordidus* Germar (1824) estmulado por volatiles originados en Musaceas de distintas edades y variedades genomicas. *Agronomia Tropica,* 46: 413-429.

Coffelt, J.A. & Burkholder, W.E. 1972. Reproductive biology of the cigarette beetle, *Lasiderma serricorne*. I. Quantitative laboratory bioassay of the female sex pheromone from females of different ages. *Annals of Entomological Society of America,* 65: 447-450.

Collins, P.J., Treverrow, N.L. & Lambkin, T.M. 1991. Organophosphorus insecticide resistance and its management in the banana weevil borer, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), in Australia. *Crop Protection,* 10: 215-221.

Cordova-Yamauchi, L., Gianoli, E., Quiroz, A. & Niemeyer, H.M. 1998. The argentine ant, *Linepithema humile* (Hymonptera: Formicidae: Dolichoderinae) is sensitive to semiochemicals involved in the spacing behaviour in the bird cheryy-oat aphid *Rhopalosiphum padi* (Sternorrhyncha: Aphididae). *European Journal of Entomology,* 95: 501-508.

Cork, A., De Souza, K., Krishnaiah, K., Kumar, D.V., Ashok Reddy, A. & Casagrande, E. 1996. Control of the yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) by mating disruption in rice in India: effect of unnatural pheromone blends and application time on efficacy. *Bulletin of Entomological Research,* 86: 515-524.

Cork, A., Hall, D.R., Hodges, R.J. & Pickett, J.A. 1991. Identification of major component of male produced aggregation pheromone of larger grain borer, *Prostephanus trunctus* (Horn) (Coleoptera: Cucujidae). *Journal of Stored Products Research,* 26: 169-174.

Cosens, D. & Toussaint, N. 1985. An experimental study of the foraging strategy of the wood ant *Formica aquilonia*. *Animal Behaviour*, 33: 541-552.

Cournoyer, M. & Boivin, G. 2004. Infochemical mediated preference behaviour of the parasitoid *Microctonus hyperodae* when searching for adult weevil hosts. *Entomologia Experimentalis et Applicata,* 112: 117-124

Cuille, J. 1950. Recherches sur le charancon du bananier (*Cosmopolites sordidus* Germar). *Institut de Fruits et Agrumes Coloniaux series Technicale*, (4). 225 pp.

Cuthbert, R.A., Peacock, J.W. & Cannon, W.N. 1977. An estimate of the effectiveness of pheromone- baited traps for the suppression of *Scolytus multistiatus*. *Journal of Chemical Ecology*, 3: 527-537.

David, C.T. & Birch, M.C. 1986. Pheromone and Insect Behaviour. In: Jutsum, A.R. & Gordon, R.F.S. (eds). *Insect Pheromones in plant protection*. John Wiley and Sons, New York, pp. 240-267.

De Groot, P. & Debarr, G.L. 1998. Factors affecting capture of the white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Coleoptera: Scolytidae) in pheromone traps. *Journal of Applied Entomology*, 122: 281-286.

Delattre, P. 1980. Recherche d'une methode d'estimation des populations du charancon du bananier, *Cosmopolites sordidus* Germar (Col., Curculionidae). *Acta Oecologica: Oecologia Applicata*, 1:83-92.

Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematic and Ecology*, 28: 601-617.

Dicke, M. & Vet, L.E.M. 1999. Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and Carnivore. In: Olff, H., Brown, V.K. & Drent, R.H. (eds), *Herbivore*: *between plants and predator*. Blackwell and Scientific, Oxford. UK, pp 483-520.

Dicke, M. 1999a Direct and indirect effects of plants on performance of beneficial organisms. In: Ruberson, J.R. (ed). *Handbook of pest management*, pp.105-153. Marcel Dekker, inc, New York.

Dicke, M. 1999b. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthoropods? *Entomologia Experimentalis et Applicata*, 92:131-142.

Dicke, M. 1999c Evolution of induced indirect defence of plants. In Tollrian, R. & Harvell, C.D *(eds), The Ecology and Evolution of Inducible Defenses.* Princeton University Press, Princeton N.J., pp. 62-88.

Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J. & Posthumus, M.A. 1990. Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *Journal of Chemical Ecology*, 16: 3091-3118.

Dicke, M., Takabayashi, J., Posthumus, M.A., Schutte, C. & Krips O.E. 1998. Plant-phytoseiid interactions mediated by herbivore induced plant volatiles: variation in production of cues and in response of predatory mites. *Experiment of Applied Acarology*, 22: 311-333.

Dicke, M., van Lenteren, J.C., Boskamp, G.J.F. & van Dongen-van Leeuwen, E. 1984. Chemical stimuli in host-habitat location by *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae), a parasite of *Drosophila*. *Journal of Chemical Ecology*, 10: 695-712.

Dicke, M. 1997. Crop plants influence the performance of biocontrol agents: implications for integration of host plant resistance and biological control. Proceedings of a CTA/IAR/IIBC seminar on Integrating biological control and host plant resistance. Addis Ababa, Ethiopia, 9-14 October 1995, pp. 46-56.

Dicke, M. & Sabelis, M.W. 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds. *Functional Ecology,* 2: 131-139.

Dickens, J.C. 1984. Olfaction in the boll weevil, *Anthonomus grandis* (Boh.) (Coleoptera: Curculionidae): electroantennogram studies. *Journal of Chemical Ecology,* 10: 1759-1785.

Dickens, J.C. 1989. Green leaf volatiles enhance aggregation pheromone of the boll weevil, *Anthonomus grandis*. *Entomologia Experimentalis et Applicata,* 52: 191-203.

Dickens, J.C., Billings, R.F. & Payne, T.L. 1992. Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines. *Experientia,* 48:523-524.

Downham, M.C.A., Smit, N.E.J.M., Laboke, P.O., Hall, D.R. & Odongo, B. 2001. Reduction of preharvest infestations of Africam sweat potato weevils *Cylas brunneus* and *C. puncticollis* (Coleoptera: Apionidae) using a pheromone mating disruption technique. *Crop Protection,* 20: 163-166.

Drukker, B., Scutareanu, P. & Sabelis M.W. 1995. Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? *Entomologia Experimentalis et Applicata,* 77:1993-203.

Dwumfour, E.F. 1992. Volatile substances evoking orientation in the predatory flowerbug *Anthocoris nemorum* (Heteroptera: Anthocoridae). *Bulletin of Entomological Research,* 82: 465-469.

Eller, F.J. & Bartelt, R.J. 1996. Grandsoic acid, a male produced aggregation pheromone from the plum curculio, *Conotrachelus nenuphar*. *Journal of Natural Products,* 59: 451-453.

Evenden, M.L., Judd, G.J.R. & Borden, J.H. 2000. Investigation of the mechanisms of pheromone communication Disruption of *Choristoneura rosaceana* (Harris) in a wind tunnel. *Journal of Insect Behaviour,* 13: 499-510.

Fletcher, T.M., Moore, J.C. & Kitching, W. 1997. Absolute configuration of Sordidin and 7-Episordidn emitted by the banana weevil, *Cosmopolites sordidus. Tetrehedron letters,* 38: 3475-3476.

Fletcher, T.M., Moore, J.C. & Kitching, W. 1997. Absolute configuration of Sordidin and 7-Episordidn emitted by the banana weevil, *Cosmopolites sordidus*. *Tetrehedron Letters,* 38: 3475-3476

Foster, S.P. & Harris, M.O. 1997 Behavioural manipulation methods for insect pest management. *Annual Review of Entomology,* 42: 123-146

Fuxa, J.R. & Tanada, Y. 1987. Epizootiology of Insect Diseases. Wiley Interscience, New York, 555p.

Geervliet, J.B.F., Vet, L.E.M. & Dicke, M. 1994. Volatiles from damaged plants as major cues in long-range host searching by the specialist parasitoid *Cotesia rubecula. Entomologia Experimentalis et Applicata,* 73:289-297.

Giblin-Davis, R.M., Gries, R., Gies, G., Pena-Rojas, E., Pinzon, I., Pena, J.E., Perez, A.L., Pierce, H.D. & Oehlschlager, A.C. 1997 Aggregation pheromone of palm weevil, *Dynamis borassi*. *Journal of Chemical Ecology,* 23: 2287-2297

Giblin-Davis, R.M., Pena, J.E., Oehlschlager A.C. & A.L. Perez, A.C. 1996b. Optimisation of semiochemical-based trapping of *Metamasius hemipterus sericeus* Oliver (Coleoptera: Curculionidae). *Journal of Chemical Ecology, 2*2:1389-1410.

Giblin-Davis, R.M., Oehlschlager, A.C., Perez, A., Gries, G., Gries, R., Weissling T.J., Chinchilla, C.M., Pena, J.E., Hallet, R.H., Pierce, H.D. & Gonzalez, L.M. 1996a. Chemical and behavioural ecology of palm weevils (Curculionidae: Rhynchophorinae). *Florida Entomologist,* 79:153-170.

Giblin-Davis, R.M., Pena, E.J., & Duncan, R.T. 1994b. Lethal pitfall trap for evaluation of semiochemical-mediated attraction of *Metamasius hemipterus sericeus* (Olivier) (Coleoptera: Curculionidae). *Florida Entomologist,* 77:247-255

Giblin-Davis, R.M., Weissling, T.J., Oehlschlager, A.C. & Gonzalez, L. 1994a. Field responses of *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) to its aggregation pheromone and fermenting plant volatiles. *Florida Entomologist,* 77: 196-111.

Godonou, I., Green, K.R., Oduro, K.A., Lomer, C.J. & Afre-Nuamah, K., 2000. Field evaluation of selected formulation of *Beauveria bassiana* for the management of the banana weevil (*Cosmopolites sordidus*) in plantain (*Musa* spp., AAB group). *Biocontrol Science Technology,* 10: 779-788.

Gold, C.S., Night, G., Ragama, P.E., Kagezi, G.H. & Tinzaara, W. 2004a. Field distribution for banana weevil adults in cooking banana stands in Uganda. *Insect Science and its Application,* 24:242-248.

Gold, C.S., Kagezi, G.H., Night, G. & Ragama, P.E. 2004b. The effects of banana weevil, *Cosmopolites sordidus* (Germar) damage on highland banana growth, yield and stand duration in Uganda. *Annals of Applied Biology* (in press).

Gold, C.S., Okech, S. H. & Nokoe, S. 2002. Evaluation of the pseudostem trapping as a control measure against banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae) in Uganda. *Bulletin of Entomological Research,* 92:35-44

Gold, C.S, Pena, J.E, & Karamura, E.B. 2001. Biology and integrated pest management for the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Integrated Pest Management Reviews,* 6: 79-155.

Gold, C.S., Bagabe, M.I. & Sendege, R. 1999a. Banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae): test for suspected resistance to Carbofuran and Dieldrin in Masaka District, Uganda. *African Entomology,* 7: 189-196.

Gold, C.S., Rukazambuga, N.D.M., Karamura, E.B., Nemeye, P. & Night, G. 1999b. Recent advances in banana weevil biology, population dynamics and pest status with emphasis on East Africa. In: Frison, E.A., Gold, C.S., Karamura, E.B. & Sikora, R.A. (eds), *Mobilizing IPM for sustainable banana production in East Africa:* Proceedings of the Workshop on banana IPM held in Nelspruit, South Africa. 23-28 November, 1998, pp. 35-50.

Gold, C.S., Karamura, E.B., Kiggundu, A., Bagamba, F. & Abera, A.M. 1999c. Geographic shifts in highland banana (*Musa* spp., group AAA-EA) production in Uganda. *The International Journal of Sustainable Development and World Ecology,* 6: 45-56.

Gold, C.S. 1998. Banana weevil: Ecology, Pest status and Prospectus of Integrated Control with Emphasis on East Africa. In Siani, R.K. (ed), *Proceedings of the Third International Conference on Tropical Entomology.* 30 Oct - 4 Nov 1994, Nairobi, International Centre for Insect Physiology and Ecology, Nairobi, pp.49-74.

Gold, C.S. & Bagabe, M.I. 1997. Banana weevil, *Cosmopolites sordidus* Germar (Coleoptera, Curculionidae), infestation of cooking and beer bananas in adjacent stands in Uganda. *African Entomology,* 5: 103-108.

Gold, C.S., Speijer, P.R., Karamura, E B. & Rukazambuga, N.D.T. M. 1994. Assessment of banana weevils in East African highland banana systems and strategies for control. In: Valmayor, R.R., Davide, R.G., Stanton, J.M., Treverrow, N.L.& Roa, V.N. (eds), *Proceedings of Banana Nematode/Borer Weevil Conference,* pp. 170-190., 18-22 April 1994, Kuala Lumpur, Malaysia.

Gold, C.S., Ogenga-Latigo, M.W., Tushemereirwe, W., Kashaija, I. & Nankinga, C.M. 1993. Farmer perceptions of banana pest constraints in Uganda: Results from a rapid rural appraisal. In: Gold, C.S. & Gemmil, B. (eds), *Biological and Integrated Control of Highland Banana and plantain, Proceedings of Research Co-ordination Meeting,* pp. 3-25. Ibadan, IITA.

Gowen, S.R. 1995. Pests, In: Gowen, S. (ed.), *Bananas and Plantains.* Chapman and Hall, London, pp. 382-402.

Gries, G., Gries, R., Perez, A.L., Oehlschlager, A.C., Gonzales, L.M., Pierce, H.D., Kouda-Bonafos, M., Zebeyou, M. & Nanou, N. 1993. Aggregation pheromone of African palm weevil, *Rhynchophorus phoenicis* F. *Naturwissenschaften,* 80: 90-91.

Griffiths, D.C., Maniar, S.P., Merrit, L.A., Mudd, A. & Pickett, J.A. 1991 Laboratory evaluation of pest management strategies combining antifeedants with insect growth regulator insecticides. *Crop Protection,* 10: 145-152.

Gross, H.R. 1981. Employment of kairomones in the management of parasitoids. In: Nordlund, D.A., Jones, R.L. & Lewis, W.J. (eds), *Semiochemicals-Their role in pest control,* John Wiley and Sons, New Yok, pp. 137-150.

Grostal, P. & Dicke, M. 1999. Direct and indirect cues of predation risk influence behaviour and reproduction prey: a case for acarine interactions. *Behaviour Ecology,* 10: 422-427.

Haberkern, K.E. & Raffa, K.F. 2003. Phloephagous andpredaceous insects responding to synthetic pheromones of bark beetles inhabiting white spruce stands in the Great Lakes region. *Journal of Chemical Ecology,* 29: 1651-1663.

Hagen, K.S., Wall, E.F. & Tassan, R.L. 1971. The use of food sprays to increase effectiveness of entomophagous insects. *Proceedings of Tall Timbers Conference on Ecological Animal Control by Habitat management,* 2:59-81.

Hall, D.R., Chamberlain, D.J., Cork, A., Desouza, J.L., McVeigh, L.J., Ahmad, Z., Krishnaiah, K., Brown N.J., Casagrade, E. & Jones, O.T. 1994. The use of pheromones for mating disruption of cotton bollworms and rice stem borer in developing countries. *Brighton Crop Protection. conference*: *pests and diseases.* The British Crop Protection Council, Farnham, Surrey, pp. 1231-1238.

Hallet, R.H., Oehlschlager, A.C. & Borden, J.H. 1999. Pheromone trapping protocols fort he Asian palm weevil, *Rhynchophorus ferugineus* (Coleoptera: Curculionidae). *International Journal of Pest management,* 45: 231-237.

Hallett, R.H., Gries, G., Gries, R., Borden, J.H., Czyzewska, E., Oehlschlager, A.C., Pierce, H.D., Angerilli, N.P.D. & Rauf, A. 1993. Aggregation pheromone of two Asian palm weevils, *Rhynchophorus ferrugnineus* and *R. vulneratus*. *Naturwissenschaften,* 80: 328-331.

Harari, A.R. & Landolt, P.J. 1997. Orientation of sugar rootstalk borer weevil, *Diaprepes abbreviatus*, to weevil frass and food odors. *Journal of Chemical Ecology,* 23: 857-868.

Hardee, D.D., McKibben, G.H., Gueldner, R.C., Mitchell, E.B., Tumlinson, J.H. & Cross, W.H. 1972. Boll weevils in nature respond to grandlure, a synthetic pheromone. *Journal of Economic Entomology,* 65: 97-100.

Hardee, D.D. 1982. Mass trapping and trap cropping of the boll weevil *Anthonomus grandis* Oheman. In: Kydonieus, A. F. & Beroza, M. (eds), *Insect suppression with controlled released pheromone system.* Vol 2, CRC. Boca Raton, FL. pp. 65-71.

Hardee, D.D., Cross, W.H., Mitchell, E.B., Huddleston, P.M., Mitchell, H.C., Merkl, M.E. & Davich, T.B. 1969. Biological factors influencing responses of the female boll weevil to the

male sex pheromone in field and large cage tests. *Journal of Economic Entomology,* 62: 161-165.

Hardee, D.D., Mckibben, G.H., Rummel, D.R., Huddleston, P.M. & Coppedge, J.R. 1974. Response of boll weevils to component ratios and doses of the pheromone, grandlure. *Environmental Entomology,* 3: 135-138.

Hardee, D.D., Mitchell, E.B., Huddleston, P.M. & Davich, T.B. 1966. A laboratory technique for bioassay of plant attractants for the boll weevil. *Journal of Economic Entomology,* 59: 240-241.

Hardie, J. & Minks, A.K. (eds). 1999. Pheromones of non-lepidopteran insects associated with agricultural plants. Wallingford, CABI publishing, 466 p.

Heard, T.A. 1995. Oviposition and feeding preferences of a flower-feeding weevil, *Coelocephalapion aculeatum,* in relation to conspecific damage to its host-plant. *Entomologia Experimentalis et Applicata,* 76:203-209

Hebblethwaite, M. 1989. Adoption of Pheromones in Pest Control. In: Jutsum, A.R. & Gordon, R.F.S. (eds), *Insect pheromones in plant protection.* John Wiley and Sons, New York, pp. 303-322.

Hedlund, K., Vet, L.E.M. & Dicke, M. 1996. Generalist and Specialist parasitoid strategies of using odours of adult drosophilid flies when searching for larval hosts. *Oikos,* 77:390-398.

Hord, H.Y. & Flippin, R.S. 1956. Studies of banana borer in the Honduras. *Journal of Economic Entomology,* 49: 296-300.

Howse, P., Stevens, I. & Jones, O. 1998. *Insect pheromones and their use in pest management*. Chapman and Hall, London, pp.105-130.

Jaffe, K., Sanchez, P., Cerda, H., Hernandez, J.V., Urdaneta, N., Guerra, G., Martinez, R., & Miras, B. 1993. Chemical ecology of the palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): Attraction to host plants and to a male-produced aggregation pheromone. *Journal of Chemical Ecology,* 19: 1703-1720.

James, D.G., Bartlet, R.J. & Moore, J.C. 1996. Mass trapping of *Carpophilus* spp. (Coleoptera: Nititulidae) in stone fruit orchards using synthetic aggregation pheromones and a coattractant: Development of a strategy for population suppression. *Journal of Chemical Ecology,* 22: 1541-1556.

Jansson, R. K., Mason, L.J., Heath, R.R., Lecrone, S.H. & Forey, D.E. 1993. Pheromone trap monitoring system for sweetpotato weevil (Coleoptera; Apionidae) in the Southern United States: Effects of lure type, age, and duration in storage. *Journal of Economic Entomology,* 86: 1109-115.

Jansson, R.K., Heath, R.R. & Coffelf, J.A. 1989. Temporal and spatial patterns of sweet potato weevil (Coleoptera: Curculionidae) counts in pheromone-baited traps in sweet potato fields in southern Florida. *Environmental Entomology*, 18: 691-697.

Jansson, R.K., Manson, L.J. & Heath, R.R. 1991. Use of sex pheromones for monitoring and managing *Cylas formicarius*. In: Jansson, R.K. & Raman, K.V. (eds), Sweet potato pest management, a global perspective. Westview Press, Oxford, pp. 97-138.

Jayaraman, S., Ndiege, I.O., Oehlschelager, C.A., Gonzales, L.M., Alpizar, D., Fallas, M. Budenburg, W.J. & Ahuya, P. 1997. Synthesis, Analysis, and field activity of Sordidin, a male-produced aggregation pheromone of the banana weevil, *Cosmopolites sordidus. Journal of Chemical Ecology*, 23: 1145-1161.

Kagezi, G.H., Tinzaara, W., Gold, C.S., Nankinga, C., Tushemereirwe, W. & Ragama, P. 2002. Field evaluation of pheromone-enhanced traps for management of the banana weevil, *Cosmopolites sordidus*, in Uganda. In: Tenywa, J.S., Nampala, M.P., Kyamanywa, S. & Osiru, M. (eds). Integrated Pest Management Conference Proceedings. Kampala, Uganda, 8-12 September 2002, pp: 94-100.

Kaya, G.P., Seshu-Reddy, K.V., Kokwaro, E.D. & Munyinyi, D.M. 1993. Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Serratia marcescens* to the Banana Weevil *Cosmopolites sordidus*. *Biocontrol Science Technology*, 3: 177-187.

Kehat, M., Gothilf, S., Dunkelblum, E. & Greeberg, S. 1980. Field evaluation of female sex pheromone components of the cotton bollworm, *Heliothis armigera*. *Entomologia Experimentalis et Applicata*, 27: 188-193.

Kiggundu, A. 2000. Host plant reactions and resistance mechanisms to banana weevil, *Cosmopolites sordidus* (Germar) in Ugandan *Musa* germplasm. M.Sc Thesis. Orange Free State University, South Africa, 98 pp.

Klein, M.G. & Lacey, L.A. 1999. An attractant trap for auto-dissemination of Entomopathogenic fungi into populations of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae). *Biocontrol Science Technology*, 9: 151-158.

Knipling, E.F. 1979. The basic principles of insect population suppression and management. In: Agriculture handbook, Volume 512, United States Department of agriculture, Washington.

Koppenhofer, A.M., Seshu Reddy, K.V. &. Sikora, R.A. 1994. Reduction of banana weevil populations with pseudostem traps. *International Journal of Pest Management*, 40: 300-304.

Koppenhofer, A.M. 1993. Egg predators of the banana weevil, *Cosmopolites sordidus* (Germar) (Col., Curculionidae) in Western Kenya. *Journal of Applied Entomology*, 116: 352-357.

Koppenhofer, A.M., Seshu-Reddy, K.V., Madel, G. & Lubega, M.C. 1992. Predators of the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) in Western Kenya. *Journal of Applied Entomology*, 114: 530-533.

Laboke, P.O., Ogenga-Latigo, M.W., Smit, N.E.J., Downham, M.C.A., Odongo, B., Hall, D.R. & Farman, D.I. 2000. Environmental factors affecting catches of sweat potato weevils, *Cylas brunneus* (Fabricius) and *C. puncticollis* (Boheman) in pheromone traps. *African Potato Association conference Proceedings*, 5: 217-227.

Lacey, L.A., Martins, A. & Ribeiro, C. 1994. The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *European Journal of Entomology*, 91: 313-319.

Ladd, T.L. & Klein, M.G. 1982. Trapping Japanese beetles with synthetic female sex pheromone and food type lures. In: Kydonieus, A.F & Beroza, M. (eds), *Insect suppression with controlled release pheromone systems.* Volume 2, CRC Press, Florida, pp. 57-64.

Landolt, P.J. & Phillips, T.W. 1997. Host plant influences on sex pheromone behaviour of phytophagous insects. *Annual Review of Entomology*, 42: 371-391.

Landolt, P.J. 1997. Sex attractant and Aggregation Pheromones of Male phytophagous Insects. *American Entomologist*, Spring 1997, 12-22.

Legget, J.E. & Cross, W.H. 1978. Boll weevils: the relative importance of colour and pheromone in orientation and attraction to traps. *Environmental Entomology*, 7: 4-6.

Legget, J.E., Dickerson, W.A. & Lloyd, E.P. 1989. Suppressing low-level boll weevil populations with traps: Influence of trap placement, grandlure concentration and population level. *Southwestern Entomologist*, 13: 205-216.

Lewis, W.J. & Martin, W.R. 1990. Semiochemicals for use with parasitoids: status and future. *Journal of Chemical Ecology*, 16: 3067-3089.

Lewis, W.J. & Nordlund, D.A. 1985. Behaviour-modifying chemicals to enhance natural enemy effectiveness. In: Hoy, M.A. & Herzog, D.C. (eds), *Biological control in agricultural IPM systems*Academic press, New York, pp 89-101.

Lewis, W.J., Nordlund, D.A., Gross, H.R., Jones, R.L. & Jones, S.L. 1977. Kairomones and their use for management of entomophagous insects: Moth scales as a stimulus for predation of *Heliothis zea* (Boddie) eggs by *Chrysopa carnea* Stephens larvae. *Journal of Chemical Ecology*, 3: 483-487.

Lewis, W.J., Nordlund, D.A., Gueldner, R.C., Teal, P.E.A. & Tumlinson, J.H. 1982. Kairomones and their use for management of entomophagous insects: XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretions and synthetic pheromone blend of *Heliothis zea* (Boddie) moths. *Journal of Chemical Ecology*, 8: 1323-1331.

Liu, B. & Sengonca, C. 1994 Development of 8-armed olfactometers for measuring olfactory responses of insect predators. *Anzeiger fuer Schaedlingskunde Pflanzenchutz Umweltschutz,* 67: 30-34.

Lloyd, E.P., Mickibben, G.H., Knipling, E.F., Legget, J.E., Witz, J.A., Hartstack, D.W. & Lockwood, D.F. 1981. Mass trapping for detection, suppression and integration with other suppression measures against the boll weevil. In: Mitchell, E.R. (ed.), *Management of insect pests with semiochemicals*. *Proceedings of an International Colloquium on management of insects with semiochemicals.* March 23-28, 1980, Ganesville, Florida, pp.162-185.

Lofgren, C.S., Glancey, B.M., Glover, A., Rocca, J. & Tumlinson, F. 1983. Behaviuor of workers of *Solenopsis invicta* (Hymenoptera: Formicidae) to the Queen recognition pheromone: Laboratory studies with an Olfactometer and surrogate Queens. *Annals of the Entomological Society of America,* 76: 44-50.

Longoria, A. 1968. Diferencias sexuales en la morfologia externa de *Cosmopolites sordidus* Germar (Coleoptera, Curculionidae). *Ciencias Biol.l, La Habana,* 1: 1-11.

Mafra-Neto, A. & Habid, M. 1996. Evidence that mass trapping suppresses pink bollworm populations in cotton fields. *Entomologia Experimentalis et Applicata,* 81: 315-323.

Masanza, M. 1995. Integrating pseudostem trapping, chemical and biological control for the management of the banana weevil (*Cosmopolites sordidus* Germar). Unpubl. M.Sc. thesis. Makerere University. 93 pp.

Masanza, M. 2003. Effect of crop sanaitation on banana weevil Cosmopolites sordidus (Germar) populations and associated damage. Ph.D Thesis, Wagenigen University, 164 pp.

Mason, L.J. & Jansson, R.K. 1991. Disruption of sex pheromones communication in *Cylas formicarius* (Coleoptera: Apionidae) as a potential means of control. *Florida Entomologist,* 74: 469-472.

Mason, L.J., Jansson, R.K. & Heath, R.R. 1990. Sampling range of male sweet potato weevils (*Cylas formicarius elegantulus*) (Summers) (Coleoptera: Curculionidae) to pheromone traps: Influence of pheromone dosage and lure age. *Journal of Chemical ecology*, 16: 2493-2502.

McGregor, R.R. & Gillespie, D.R. 2004. Olfactory responses of the omnivorous generalist predator *Dicyphus hesperus* to plant and prey odours. *Entomologia Experimentalis et Applicata*, 112: 201-205.

McIntyre, B.D., Gold, C.S., Kashaija, I.N., Ssali, H., Night, G. & Bwamiki, D.P. 2001. Biomass, nutrients, soil water and pest incidence in banana-legume intercrops. *Biology and Fertility of Soils*, 34: 342-348.

Mclaughlin, R.E., Cleveland, T.C., Daum, R.J. & Bell, M.R. 1969. Development of the bait principle for boll weevil control. Field tests with bait containing a feeding stimulant and sporozoans *Glugea gasti* and *Mattesia grandis*. *Journal of Invertebrate Pathology*, 13: 429-441.

McNeil, J.N. 1991. Behavioural Ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Annual Review of Entomology,* 36: 407-430.

McVeigh, L.J., Campion, D.G. & Critchley, B.R. 1990. The use of pheromones for the control of cotton bollworms and *Spodoptera* spp. in Africa and Asia. In: Ridgway, R.L, Silerstein, R.M. & Inscoe, M.N. (eds), *Behaviour modifying chemicals for insect management.* Marcel Dekker, New York, pp. 407-415.

Messiaen, S. 2001. Evaluation of pheromone baited trapping system for *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in Cameroon. *INIBAP*. 8 pp.

Metcalf, R.L & Metcalf, E.R. 1992. *Plant Kairomones in Insect ecology and control*. Chapman and Hall, London, 254pp.

Mgenzi, S.R.B. 1999. The effect of non-host plant odour on olfactory orientation of the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). M.Sc Thesis, Wageningen University, 36pp.

Miller, C.A. & McDougall, G.A. 1973. Spruce budworm moth trapping using virgin females. *Canadian Journal of Zoology,* 51: 853-858.

Minitab. 1995. Minitab for Windows Release 10.5, Minitab Inc. 3081 Enterprise Drive, State College, USA

Mitchell, G. 1978. The estimation of banana borer population and resistance levels. *Technical Bulletin* 2, WINBAN, St. Lucia. 34 pp.

Mori, K., Kakayama, T. & Takikawa, H. 1996. Synthesis and absolute configuration of sordidin, the male-produced aggregation pheromone of the banana weevil *Cosmopolites sordidus*. *Tetrahedron Letters,* 37: 3741-3744.

Muirhead-Thompson, R.C. 1991. Trap responses by flying insects. The influence of trap design on capture efficiency. Academic press, London. 287 pp.

Murad, Z. 2001. Using pheromones to trap banana weevil borers. Banana Topics, Issue 30/2001. DPI South Johnstone, Australia, 6 pp.

Nankinga, C.M. & Moore, D. 2000. Reduction of banana weevil populations using different formulations of the Entomopathogenic fungus *Beauveria bassiana*. *Biocontrol Science and Technology,* 10: 645-657.

Nankinga, M.C. & Ogenge-Latigo, W.M. 1996. Effect of method of application on the effectiveness of *Beauveria bassiana* against the banana weevil, *Cosmopolites sordidus*. *African Journal of Plant Protection,* 6: 12-21.

Nankinga, M.C. 1994. Potential of indigenous fungal pathogens for the biological control of the banana weevil, *Cosmopolites sordidus* (Germar). MSc Thesis, Makerere University, Kampala, Uganda, 99 pp.

Nankinga, M.C. 1999. Characterisation of entomopathogenic fungi and evaluation of delivery systems of *Beauveria bassiana* for the biological control of the banana weevil, *Cosmopolites sordidus*. Ph.D Thesis, University of Reading, UK, 277 pp.

Nankinga, M.C., Ogenga-Latigo, W.M. & Allard, G.B. 1996. Pathogenicity of indigenous isolates of *B. basssiana* and *Metarhizium anisopliae* to the banana weevil *Cosmopolites sordidus* (Germar). *African Journal of Plant Protection,* 6: 1-11

Ndege, L.J., Ndile, M.R. & Bosch, C.H. 1995. Farmers' assessment of a weevil trapping trial in Ntoija village, Bukoba district. Lake Zone Farming Systems project, ARI Maruku. *Progress Report* No. 9. 10 pp.

Ndiege, I.O., Jayaraman, S., Oehlschlager, A.C., Gonzalez, L.M., Alpizar, D. & Fallas, M. 1996. Convenient synthesis and field activity of a male-produced aggregation pheromone for *Cosmopolites sordidus*. *Naturwissenschaften,* 83: 280-282.

Ngode, L. 1998. Management of banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) for improved banana yield in western Kenya. Ph.D. thesis. Kenyatta University, 180 pp.

Noldus, J.J. 1989. Semiochemicals, for foraging behaviour and quality of entomophagous insects for biological control. *Journal of Applied Entomology*, 108: 425-452.

Obeng-Ofori, D. & Coaker, T.H. 1990. Some factors affecting responses of four stored product beetles (Coleoptera: Tenebrionidae & Bostrichidae) to pheromones. *Bulletin of Entomological Research*, 80: 433-441.

Ochieng, V.O. 2001. Genetic biodiversity in banana weevil *Cosmopolites sordidus*, populations in banana growing regions of the world. Ph.D. Thesis, University of Nairobi. Kenya, 139 pp.

Oehlschlager, A.C., Chinchilla, C.M. & Gonzalez, L.M. 1992. Management of the American palm weevil (*Rhynchophorus palmarum*) and the red ring disease in oil palm by pheromone-based trapping. In: *International Seminar on Coconut Research and Development for Latin America and the Caribeean*. Kingston, Jamaica, 16 pp.

Oehlschlager, A.C., Chinchilla, C.M. Gonzalez, L.M., Jiron, L.F., Mexzon, R. & Morgan, B. 1993. Development of a pheromone based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, 86: 1381-1392.

Oehlschlager, A.C., McDonald, R.S., Chinchilla, C.M. & Patschke, S.N. 1995. Influence of a pheromone-based mass-trapping system on the distribution of *Rhynchophorus palmarum* (Coleoptera: Curculionidae) in oil palm. *Environmental Entomology*, 24: 1005-1012.

Oehlschlager, C.O., Alpizar, D., Fallas, M., Gonzalez, L.M. & Jayaraman, S. 2000. Pheromone-based mass trapping of the banana weevil, *Cosmopolites sordidus* and the West Indian sugarcane weevil, *Metamasius hemipterus* in banana and plantain. *Abstracts of the XXI International Congress of* Entomology, Iguassu Falls, Brazil, August 20-26, 2000, pp. 155.

Ogenga-Latigo, M.W. & Bakyalire, R. 1993. Use of pseudostem traps and cooefficient of infestation (PCI) for assessing banana infestation and damage by *Cosmopolites sordidus* Germar. *Africa Crop Science Journal,* 1: 31-38.

Okech, S.H.O., Karamura, E.B. & Gold, C.S. 1999. Banana IPM in Uganda. In: Frison, E.A., Gold, C.S., Karamura, E.B. & Sikora, R.A. (eds), *Mobilizing IPM for sustainable banana production in East Africa*. *Proceedings of the Workshop on banana IPM*. Nelspruit, South Africa, 23-28 November, 1998, pp: 225-236.

Ortiz, R., Vuylsteke, D., Dumpe, B. & Ferris, R.S.B. 1995. Banana weevil resistance and corm hardness in *Musa* germplasm. *Euphytica,* 86: 95-102.

Pell, J.K., Macaulay, E.D.M. & Wilding, N. 1993. A pheromone trap for dispersal of the pathogen *Zoophthora radicans* Brefeld. (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Biocontrol Science and Technology*, 3: 315-320.

Pena, J.E. & Duncan, R. 1991. Preliminary results on biological control of *Cosmopolites sordidus* in Florida. *Tropical Fruit News*. August 91: 8-10.

Pena, J.E., Giblin-Davis, R.M. & Duncan, R. 1995. Impact of indigenous *Beauveria bassiana* (Balsamo) Vuillemin on banana weevil and rotten sugarcane weevil (Coleoptera: Curculionidae) populations in banana in Florida. *Journal of Agricultural Entomology,* 12: 163-167.

Phillips, J.K. & Burkholder, W.E. 1981. Evidence for a male-produced aggregation pheromone in the rice weevil. *Journal of Economic Entomology,* 74: 539-542

Phillips, T.W. 1997. Semiochemicals of stored product Insects: Research and Applications. *Journal Stored Products Research,* 33:17-30.

Phillips, T.W., West, J.R., Foltz, J.L., Silverstein, R.M. & Lanier, G.N. 1984. Aggregation pheromone of the deodar weevil, *Pissodes nemorensis* (Coleoptera: Curculionidae): Isolation and activity of grandisol and grandisal. *Journal of Chemical Ecology,* 10:1417-1423.

Pickett, J.A. 1988. Integrating use of beneficial organisms with chemical Crop Protection. *Phill. Transactions Royal Society of London,* 318:203-211.

Pierce, A.M., Borden, J.H. & Oehslchlager, A.C. 1983. Effect of age and population density on response to beetle and food volatiles by *Orycaephilus surinamensis* and *O. mercator* (Coleoptera: Cucujidae). *Environmental Entomology*, 12:1367-1374.

Price, N.S. 1993. Preliminary weevil trapping studies in Cameroon. In: Gold, C.S. & Gemmill, B. (eds), *Proceedings of a Research Coordination Meeting for Biological and Integrated Control of Highland Banana Pests and Disease.* 12-14 November 1991, Cotonou, Benin, pp. 51-57.

Prokopy, R.J. 1981. Epideictic pheromones that influence spacing patterns of phytophagous insects. In: Nordlund, D.A., Jones R.L. & Lewis, W.J. (eds), *Semiochemicals*: *Their role in pest control.* Wiley, New York, pp. 181-213.

Propopy, R.J., Cooley, S.S. & Phelan, P.L. 1995. Bioassay approaches to assessing behavioural responses of plum curculio adults (Coleoptera: Curculionidae) to host odour. *Journal of Chemical Ecology*, 21:1073-1084.

Ramirez-Lucas, P., Malosse, C., Ducrot, P.H., Lettere, M. & Zagatti, P. 1996. Chemical Identification, Electrophysiological and Behavioral Activities of the Pheromone of *Metamasius hemipterus* (Coleoptera: Curculionidae). *Bioorganic and Medical Biochemistry*, 4: 323-330.

Reddy, G.V.P. & Guerraro, A. 2004. Interactions of insect pheromones and plant semiochemicals. *Trends in plant Sciences*, 9:253-261.

Reddy, G.V.P., Holopainen, J.K. & Guerrero, A. 2002. olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larva frass and green leaf cabbage volatiles. *Journal of Chemical Ecology*, 28: 131-143.

Renwick, J.A.A. 1992. New directions in semiochemical research. *Journal of Applied Entomology*, 114: 431-438.

Ridgway, R.L., Inscoe, M.N. & Dickerson, W.A. 1990. Role of the Boll weevil pheromone in pest mangement. In: Ridgway, R.L., Silverstein, R.M. & Inscoe, M.N. (eds), *Behaviour modifying chemicals for insect management: Applications of pheromones and their attractants.* Marcel Dekker Inc., New York, pp.437-471.

Rochat, D., Gonzalez, V., Mariau, D., Villanueva, G. & Zaggatti, P. 1991. Evidence for a male-produced aggregation pheromone of the American palm weevil, *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *Journal of Chemical Ecology*, 17: 1221-1230.

Rochat, D., Meillour, P.N., Rafael, J.E., Molasse, C., Perthuis, B., Morin, J. & Descoins, C. 2000. Identification of pheromone synergists in American Palm weevil, *Rhynchophorus palmarum* and attraction of related *Dynamis borass*. *Journal of Chemical Ecology*, 26: 155-187.

Roche, R. & Abreu, S. 1983. Control del picudo negro del platano (*Cosmopolites sordidus*) por la hormiga *Teteramorium guineense*. *Ciencias De la agricultura*, 17: 41-49.

Roitberg, D.B. & Isman, M.B. 1992. Insect chemical ecology: an evolutionary approach. Chapman and Hall New York, 359 pp.

Roth, L. & Willis, E. 1963. The humidity behaviour of *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Annual Entomological Society of America*, 56:41-42.

Roy, H.E. & Pell, J.K. 2000 Interactions between entomopathogenic fungi and other natural enemies: Implications for biological. *Biocontrol Science Technology*, 10: 737-752.

Rukazambuga, N.D.T.M., Gold, C.S. & Gowen, S.R. 1998. Yield loss in East African highland banana (*Musa* spp., AAA-EA group) caused by the banana weevil, *Cosmopolites sordidus* Germar. *Crop Protection,* 17:581-589.

Rukazambuga, N.D.T.M. 1996. The effects of banana weevil (*Cosmopolites Sordidus* Germar) on the growth and productivity of bananas (*Musa* spp, AAA-EA) and the influence of host vigour on attack. Ph.D Thesis, University of Reading, United Kingdom, 249 pp.

Rukazambuga, N.D.T.M., Gold, C.S. & Gowen, S.R. 2002. The influence of crop management on banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae) populations and yield of highland cooking banana (cv Atwalira) in Uganda. *Bulletin of Entomological Research,* 92:413-421.

Sabelis, M.W. & Dicke, M. 1985. Long range dispersal and searching behaviour. In: Helle, W. & Sabelis, M.W. (eds), *Spider mites, Their biology, Natural enemies and control. World crop pests.* Volume 1, Elsevier, Amsterdam, pp. 141-160

Sappington, T.W. & Spurgeon, D.W. 2000. Variation in boll weevil (Coleoptera: curculionidae) captures in pheromone traps arising from wind speed moderation by brush lines. *Environmental Entomology,* 29: 63-68.

SAS, 1990. SAS Institute Inc., SAS/STAT Users' Guide Version 6, Fourth Edition, V.II.

Schlyter, F. 1992. Sampling range, attraction range and effective attraction radius: Estimates of trap efficiency and communication distance in coleopteran pheromone and host attractant systems. *Journal Applied Entomology*, 114: 439-454.

Schmitt, A.T. 1993. Biological control of the banana weevil (*Cosmopolites sordidus* (Germar)) with entomopathogenic nematodes. Unpublished Ph.D. thesis. University of Reading, 210 pp.

Schoeman, P.S. & Schoeman, M.H. 1999. Transmission of *Beauveria bassiana* from infected adults of the banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae). *African Plant Protection*, 5: 53-54.

Schoonhoven, L.M. 1990. Host-marking pheromones in Lepidoptera, with sepecial reference to two *Pieris* spp. *Journal of Chemical Ecology,* 16: 3043-3052.

Schoonhoven, L.M., Jermy, T. & Van Loon, J.J.A. 1998. *Insect-Plant Biology from physiology to evolution.* Chapman & Hall, UK.

Schwalbe, C.P., Burkholder, W.E. & Boush, G.M. 1974. *Mattesia trogodermae* infection rates as influenced by mode of transmission, dosage and host species. *Journal of Stored Products Research*, 10: 161-166

Scutareanu, P., Drukker, B., Bruin. J., Posthumus, M.A. & Sabelis, M.W. 1997. Volatiles from Psylla-infected pear trees and their possible involvement in attraction of anthocorid predators. *Journal of Chemical ecology*, 23:2241-2260.

Sengooba, T. 1986. Survey of banana pest problem complex in Rakai and Masaka districts, August 1986: Preliminary trip report. Ministry of Agriculture, Namulonge Research Station, Uganda. Unpublished, 10 pp.

Seshu Reddy, K.V., Gold, C.S. & Ngode, L. 1999. Cultural control strategies for banana weevil, *Cosmopolites sordidus* Germar, In: Frison, E., Gold, C.S., Karamura, E.B. & Sikora, R.A. (eds), *Mobilizing IPM for sustainable banana production in Africa. Proceedings of a workshop on banana IPM.* 23-28 November 1998. Nelspruit, South Africa. INIBAP. Montpellier, pp. 51-57.

Seshu Reddy, K.V., Koppenhoefer, A.M. & Uronu, B. 1993. Cultural practices for the banana weevil control. In: Gold, C.S. & Gemmill, B. (eds), *Biological and integrated control of highland banana and plantain pests and diseases. Proceedings of a research Co-ordination meeting.* Cotonou, 12-14 November, 1991. Ibadan, IITA, pp. 140-146.

Seshu Reddy, K.V., Prasad, J.S., Ngode, L. & Sikora, R.A. 1995. Influence of trapping of the banana weevil, *Cosmopolites sordidus* (Germar 1824) on root-lesion nematode, *Pratylenchus goodeyi* (Sher and Allen 1953) population densities and subsequent banana yield. *Acta Oecologia,* 16: 593-598.

Shapas, T.J., Burkholder, W.E. & Boush, G.M. 1977. Population suppression of *Trogoderma glabrum* by using pheromone luring for protozoan pathogen dissemination. *Journal of Economic Entomology,* 70: 469-474.

Shimoda, T., Takabayashi, J., Ashira, W. Takafuji, A. 1997. Response of predory insect Scolothrips takahashi towards herbivore induced plant volatiles under laboratory and field conditions. *Journal of Chemical Ecology,* 23:2033-2048.

Shonouda, M.L., Bombosch, S., Shalaby, A.M. & Osman, S.I. 1998. Biological and chemical characterization of kairomone excreted by the bean aphids, *Aphis fabae* Scop. (Homoptera: Aphididae) and its effects on predator *Metasyrphus corollae* Fabr. I. Isolation, identification and bioassay of aphid-kairomone. *Journal of Applied Entomology,* 122:15-23.

Sikora, R.A., Bafokuzara, N.D., Mbwana, A.S.S., Oloo, G.W., Uronu, B. & Seshu Reddy, K.V. 1989. Interrelationship between banana weevil, root lesion nematode and agronomic practices and their importance for banana decline in the united Republic of Tanzania. *FAO Plant Protection bulletin,* 37, 151-157.

Silverstein, R.M. 1981. Pheromones: Background and potential for use in insect control. *Science,* 213: 1326-1332

Smit, N.E.J.M., Downham, M.C.A., Odongo, B., Hall, D.R. & Laboke, P.O. 1997. Development of pheromone traps for control and monitoring of sweet potato weevils, *Cylas puncticollis* and *C. brunneus,* in Uganda. *Entomologia Experimentalis et Applicata,* 85: 95-104.

Southwood, T.R.E. 1978. Ecological Methods. Chapman and Hall, London, 2nd edition, 524 pp.

Srivastava, C.P., Pimbert, M.P. & Reed, W. 1992. Monitoring of *Helicoverpa* (*Helionthis*) *armigera* (Hubner) moths with light and its pheromone trap in India. *Insect Science and its* A*pplication,* 13: 205-210.

Ssennyonga, J.W., Bagamba, F., Gold, C.S., Tushemereirwe, W.K., Karamura, E.B. & Katungi, E. 1999. Understanding current banana production with reference to integrated pest management in Southwestern Uganda, In: Frison, E., Gold, C.S., Karamura, E.B. & Sikora, R.A. (eds), *Mobilizing IPM for sustainable banana production in Africa. Proceedings of a workshop on banana IPM.* 23-28 November 1998. Nelspruit, South Africa. INIBAP. Montpellier, pp. 291-318.

Stapel, J.O., Cortesero, A.M., Demoraes, C.M., Tumlinson, J.H. & Lewis, W.J. 1997. Effects of extrafloral nectar, honeydew and sucrose on searching behaviour and efficiency of *Microplitis croeipes* (Hymnoptera: Braconidae) in cotton. *Environmental Entomology,* 26: 617-623.

Staten, R.T., El-Lissy, O. & Antilla, L. 1997. Successful area-wide program to control the bollworm by mating disruption. In: Carde, R.T. & Minks, A.K. (eds), *Insect Pheromones*: *New Research Directions.* Chapman and Hall, New York, pp. 383-396.

Steidle, J.L.M. & van Loon, J.J.A. 2002. Chemoecology of parasitoids and predator oviposition behaviour. In: Hilker, M. & Meiners, T. (eds), *Chemecology of insect eggs and egg deposition*. Blackwell, London. First edition, pp. 291-311.

Steidle, J.L.M. & Van Loon, J.J.A. 2003. Dietary specialisation and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata*, 108:133-148

Steinberg, S., Dicke, M., Vet, L.E.M. & Wanningen, R. 1992. Response of the braconid parasitoid *Cotesia (Apanteles) glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomologia Experimentalis et Applicata,* 63: 163-175.

Stover, R.H. & Simmonds, N.W. 1987. *Bananas*. Tropical Agricultural series, Scientific and technical, U.K, pp. 467.

Suckling, D.M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. *Crop Protection,* 19: 677-683.

Tadas, P.L., Sarnaik, D.N., Kene, H.K. & Satpute, U.S. 1994. Effect of weather parameters on monitoring of cotton bollworms with pheromones. *PKV Research Journal,* 18: 87-90.

Tamhankar, A.J., Gahukar, R.T. & Rajendran, T.P. 2000. Pheromones in the management of major lepidopterous and coleopterous pests of cotton. *Integrated Pest Management Review,* 5:11-23.

Thiery, D. & J.H. Visser, 1986. Masking of host plant odour in olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata,* 41:165-172.

Tinzaara, W., Gold, C.S., Nankinga, C., Dicke, M., van Huis, A., Ragama, P.E. &. Kagezi, G.H. 2004a. Integration of pheromones and the entomopathogenic fungus for the management of the banana weevils. *Uganda Journal of Agricultural Sciences,* 9:621-629.

Tinzaara, W., Gold, C.S., Kagezi, G.H., Dicke, M., Van Huis., A., Nankinga, C.M., Tushemereirwe, W. & Ragama, P.E. 2004b. Effects of two pheromone trap densities against banana weevil, *Cosmopolites sordidus,* populations and their impact on plant damage in Uganda. *Journal of Applied Entomology* (submitted).

Tinzaara, W., Gold, C.S., Dicke, M., van Huis, A. & Ragama, P.E. 2004c. Factors influencing pheromone trap effectiveness in attracting the banana weevil, *Cosmopolites sordidus. International Jouranl of Pest Management (*submitted).

Tinzaara, W., Dicke, M., Van Huis, A., Joop, J.J.A & Gold, C.S. 2003. Different bioassays for investigating orientation responses of the banana weevil, *Cosmopolites sordidus* (Germar) show additive effects of host plant volatiles and the synthetic pheromone. *Entomologia Experimentalis et Applicata,* 106: 169-175.

Tinzaara, W., Dicke, M., van Huis, A. & Gold, C.S. 2002a. Use of infochemicals in pest management with special reference to the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae): a review. *Insect Science and its Application,* 22: 241-261.

Tinzaara, W., Gold, C.S., Dicke, M., Van Huis, A. & Nankinga, C. 2002b. Use of infochemicals for the management of the banana weevil in Uganda. In: Tenywa, J.S., Nampala, M.P., Kyamanywa, S. & Osiru, M. (eds), Integrated Pest Management Conference Proceedings, Kampala, Uganda, 8-12 September 2002, pp: 244-255.

Tinzaara, W., Kashaija, I., Tushemereirwe, W. & Nankinga, C. 2002c. Comparative studies on chemical, hot water and cold water treatments of banana suckers to control the banana weevil, C*osmopolites sordidus G*ermar and the effect of paring suckers on banana nematodes in Uganda. *Uganda Journal of Agricultural Science*s, 7: 43-47.

Tinzaara, W., Tushemereirwe, W. & Kashaija, I. 2000. Efficiency of pheromones and trap types in the capture of the banana weevil C*osmopolites sordidus* Germar in Uganda. *Uganda Journal of Agricultural Science*s, 5: 91-97.

Tinzaara, W., Tushemereirwe, W. & Kashaija, I. 1999a. The potential of using pheromone traps for the control of *Cosmopolites sordidus* Germar in Uganda. In: Frison, E., Gold, C.S., Karamura, E.B. & Sikora, R.A. (eds), *Mobilizing IPM for sustainable banana production in Africa. Proceedings of a workshop on banana IPM,* 23-28 November 1998. Nelspruit, South Africa. INIBAP. Montpellier, pp. 327-332.

Tinzaara, W., Karamura, E. & Tushemereirwe, W. 1999b. Preliminary observations on natural enemies associated with the banana weevil *Cosmopolites sordidus* Germar in Uganda. *INFOMUSA*, 8: 28-29.

Trematerra, P. 1997. Integrated Pest management of stored product insects: Practical utilisation of pheromones. International Conference on pests in Agriculture. 6-8 January 1997, Montpellier, France Vol. 3, pp: 847-855.

Treverrow, N.L. & Bedding, R.A. 1993. Development of a system for the control of the banana weevil borer, *Cosmopolites sordidus* with entompathogenic nematodes In: Bedding, R., Akhurst, R. &. Kaya, H. (eds), Nematodes *and the Biological Control of Insect Pests*. CSIRO. Melbourne, pp 41-47.

Treverrow, N.L. 1994. Identification of components of banana corm attractive to banana weevil borer, *Cosmopolites sordidus.* Final Report HDRC Project Number Fr/227/RO, Wollongbar Agricultural Institute, New South Wales, Australia.

Tumlinson, J.H., Hardee, D.D., Gueldner, R.C., Thompson, A.C., Hedin, P.A. & Minyard, J.P. 1969. Sex pheromone produced by male boll weevil: isolation, identification and synthesis. *Science*, 166: 1010-1012.

Tumlinson, J.H., Klein, M.G., Doolittle, R.E., Ladd, T.L. & Proveaux, A.T. 1977. Identification of the female Japanese beetle sex pheromone: Inhibition of male response by an enantiomer. *Science*, 197: 789-792.

Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Rose, U.S.R., Lewis, W.J., & Tumlinson, J.H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proceedings of the National Academy of Sciences of the United States of America, 92: 4169-4174.

Turlings, T.C.J., Scheepmaker, J.W.A., Vet, L.E.M., Tumlinson, J.H. & Lewis, W.J. 1990. How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Journal of Chemical Ecology*, 16: 1577-1589.

Uzakah, R.P. 1995. The reproductive biology, behaviour and pheromones of the banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). Ph.D. dissertation. University of Ibadan, 177 pp.

Van Laerhoven, S., Gillespie, D.R. & McGregor, R.R. 2000. Leaf damage and prey type determine search effort in *Orius tristicolor*. *Entomologia Experimentalis et Applicata,* 97:167-174

Van Loon, J.J.A., De Vos, E.W. & Dicke, M. 2000. Orientation behaviour of the predatory hemipteran *Perillus bioculatus* to plant and prey odours. *Entomologia Experimentalis et Applicata,* 96: 51-58.

Vega, F.E., Dowd, F.P. & Bartelt, R. 1995. Dissemination of microbial agents using an auto-inoculating device and several insect species as vectors. *Biological control,* 5: 545-552

Vega, F.E., Dowd, P.F., Lacey L.A., Pell, J.K., Jackson, D.M. & Klein, M.G. 2000. Dissemination of Beneficial Microbial Agents by Insects. In: Lacey, A.L. & Kaya, H.K. (eds), *Field manual of techniques in invertebrate pathology*. *Application and evaluation of pathogens for control of insects and other invertebrate pests.* Kluwer Academic Publishers, Dordrebt, pp. 153-177.

Vet, L.E.M. & Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritophic context. *Annual Review of Entomology*, 32: 141-172.

Vinson, S.B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology*, 21:109-134.

Visser, J.H. 1986. Host odour perception in phytophagous insects. *Annual Review of Entomology*, 31: 121-144.

Vite, J.P. & Baader, E. 1990. Present and future use of semiochemicals in pest management of Bark beetles. *Journal of Chemical Ecology,* 16: 3031-3041.

Walgenbach, C.A. & Burkholder, W.E. 1986. Factors affecting the response of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), to its aggregation pheromone. *Environmental Entomology*, 15:733-738.

Walgenbach, C.A., Burkholder, W.E., Curtis, M.J. & Khan, Z.A. 1987. Laboratory trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, 80: 763-767.

Walgenbach, C.A., Phillips, J.K., Faustini, D.L. & Burkholder, W.E. 1983. Male-produced aggregation pheromone of the maize weevil, *Sitophilus zeamais*, and interspecific attaction between three *Sitophilus zeamais* species. *Journal of Chemical Ecology*, 9: 931-841.

Wall, C. 1989. Monitoring and spraying time. In: Jutsum, A.R. & Gordon, R.F.S. (eds), *Insect pheromones in plant protection.* John Wiley and Sons, Chichester, pp. 39-66.

Weissling, T.J., Giblin-Davis, R.M. & Scheffrahn, R.H. 1993. Laboratory and field evidence of male produced aggregation pheromone in *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *Journal of Chemical Ecology,* 19: 1195-1203.

Weissling, T.J., Giblin-Davis, R.M., Scheffrahn, R.H. & Marban-Mendoza, N.M. 1992. Trap for capturing and retaining *Rhynchophorus creutatus* (Coleoptera: Curculionidae) adults using Sabal palmatto as bait. *Florida Entomologist*, 75: 212-221.

Weissling, T.J., Giblin-Davis, R.M., Gries, G., Gries, R., Perez, A.L., Pierce H.D. & Oehlschlager, A.C. 1994. Aggregation pheromone of palmetto weevil, *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *Journal of Chemical Ecology*, 20: 505-515.

Wertheim, B., Vet, L.E.M. & Dicke, M. 2003. Increased risk of parasitism as ecological costs of using aggregation pheromones: laboratory and field study of *Drosophila-Leptopilina* interaction. *Oikos,* 100: 269-282.

Weslein, J. 1992. Effects of mass trapping on *Ips typographus* (L.) populations. *Journal of Applied Entomology,* 114: 228-232.

Whalley, P. 1957. The banana weevil and its control. *East Africa Agricultural Journal*, 23:110-112

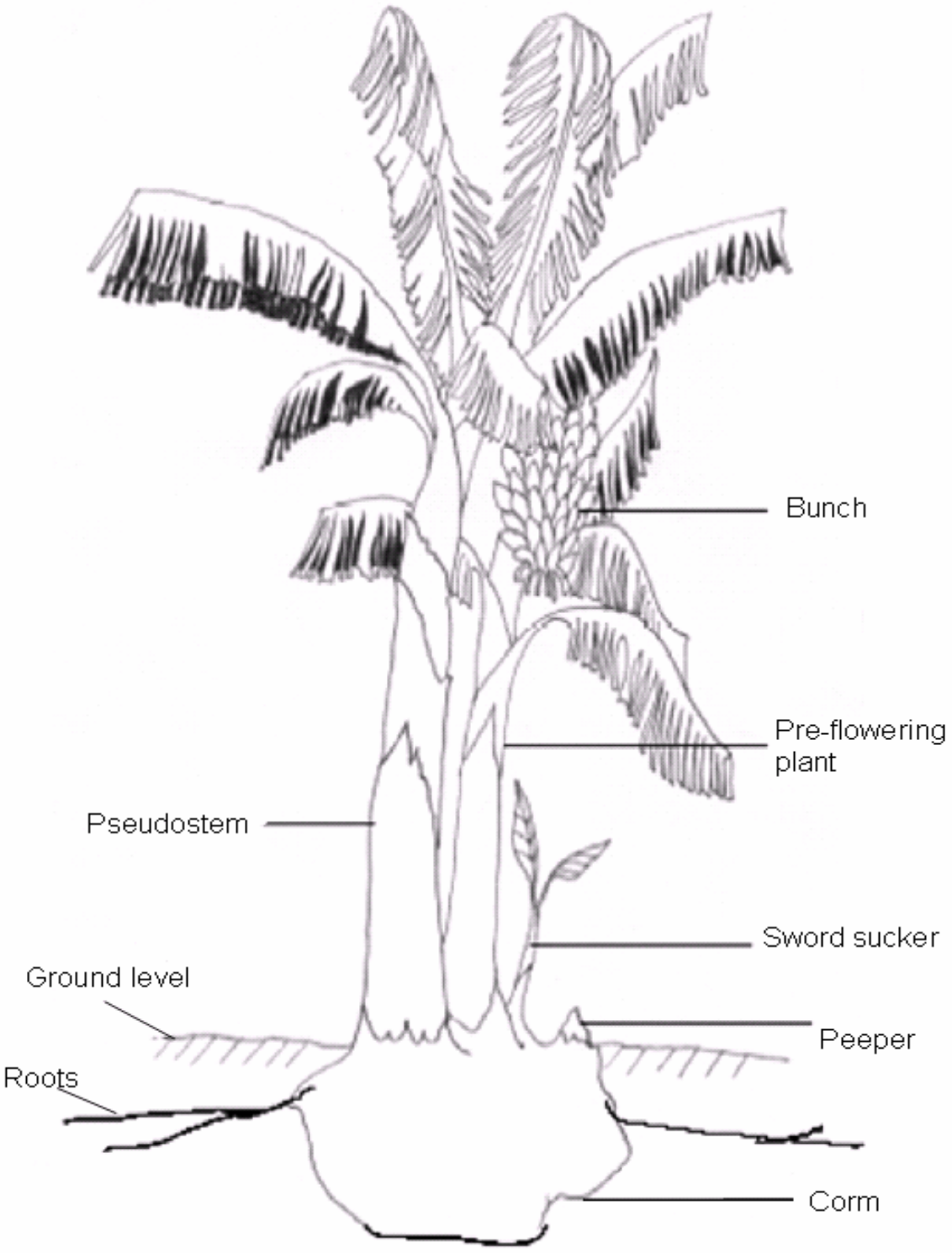
Whittaker, R.H. & Feeny, P.P. 1971. Allelochemicals; chemical interactions between species. *Science,* 171: 757-770.

Wiskerke, J.S.C., Dicke, M. & Vet, L.E.M. 1993. Larval parasitoid uses aggregation pheromone of adult hosts in foraging behaviour: a solution to the reliability-detectability problem. *Oecologia*, 93: 145-148.

Wolf, W.W., Kishaba, A.N. & Toba, H.H. 1971. Proposed method for determining density of traps required to reduce an Insect population. *Journal Economic Entomology,* 64: 872-877.

Yasuda, K. 1999. Auto-ifection system for the sweet potato weevil, *Cylas formicarius* (Fabricus) Colleoptera: Curculionidae) with Entomopathogenic fungi, *Beauveria bassiana* using a modified sex pheromone trap in the field. *Applied Entomology and Zoology*, 34: 501-505.

Zimmermann, G. 1992. Use of the fungus, *Beauveria brongniartii*, for the control of European Cockchafers, *Melolontha* spp. in Europe. In: Jackson, T.A & Glare, T.R. (eds), *Use of pathogens in Scarab Pest Management*. Intercept, Andover, pp. 199-208.




A schematic representation of a banana (AAA-AE genome group) mat (Mansaza, 2003)

# Samenvatting

Chemische signaalstoffen spelen een belangrijke rol in de biologie van veel insectensoorten. Kennis van hun rol in de interacties tussen planten, herbivoren en carnivoren kan belangrijk zijn voor het ontwikkelen van milieuvriendelijke methoden van plaagbestrijding. Chemische signaalstoffen, vooral feromonen, kunnen niet alleen worden gebruikt voor het monitoren van insecten maar ook voor directe bestrijding van plaaginsecten. Met behulp van signaalstoffen kunnen paringen tussen insecten worden verstoord, insecten massaal worden gevangen, en insecten naar plekken worden gelokt waar natuurlijke vijanden aanwezig zijn. Feromonen and kairomonen zijn signaalstoffen die zouden kunnen worden gebruikt voor de bestrijding van de bananenkever *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Cosmopolites sordidus* is een serieuze plaag in hooggelegen banenenplantages in Oost Afrika en bananenplantages in andere delen van de wereld. *Cosmopolites sordidus* produceert een aggregatieferomoon dat zowel mannetjes als vrouwtjes aantrekt. Het attractieve isomeer sordidine is inmiddels als zodanig geïndentificeerd en gesynthetiseerd, en ook commercieel verkrijgbaar. Het doel van het onderzoek dat is beschreven in dit proefschrift was het onderzoeken of een op-signaalstoffen-gebaseerde vangstmethode kan worden gebruikt ter bestrijding van de bananenkever in Uganda.

In verschillende biotoetsen werd de respons van de bananenkever op de geur van de bananenplant en synthetisch feromoon (cosmolure+) met elkaar vergeleken, daarbij gebruik makend van drie opstellingen: een locomotiecompensator, een dubbele zogenaamde 'pitfall' olfactometer en een twee- keuze olfactometer **(hoofdstuk 2).** De kever reageerde in de verschillende opstellingen zowel op de geur van gefermenteerd 'pseudostam'weefsel van de bananenplant als op het aggregatieferomoon. Het meest aantrekkelijk was echter een combinatie van die geuren. De locomotiecompensator is aan te bevelen om de respons van de kever op signaalstoffen te evalueren in een geen-keuze situatie, terwijl de andere twee olfactometers in een twee-keuze situatie kunnen worden gebruikt. Andere laboratorium- en veldproeven **(hoofdstuk 3)** bevestigden dat het feromoon het meest attractief is voor de kevers als het wordt aangeboden in combinatie met plantengeuren.

Daarnaast werd, wederom in laboratorium- en veldproeven, de efficiëntie van het feromoon, het effect van de biologie van de plaag, verschillende vangstparameters, teeltsystemen en omgevingsfactoren op de aantrekkelijkheid van het aggregatieferomoon voor de bananenkever onderzocht **(hoofdstuk 4-6)**. Het doel van deze studies was het verstrekken van informatie die van belang is voor het ontwikkelen van een optimale feromoonval voor het bestrijden van de bananenkever. De leeftijd van de kever had geen effect op de respons op feromonen in het laboratorium. Hier reageerden evenveel mannetjes als vrouwtjes op de feromonen maar in het veld werden significant meer vrouwtjes dan mannetjes gevangen in feromoonvallen. De respons van de kever werd niet significant beïnvloedt door de dichtheid aan kevers in het veld. De vangstefficiëntie nam af naarmate de afstand tot de feromoonval toenam. Er bleek geen relatie te bestaan tussen temperatuur of windsnelheid en de grootte van de vangst in feromoonvallen. Er bestond een positieve correlatie tussen de relatieve vochtigheid (niet regen) en de vangst in feromoonvallen. Daarnaast werden er meer kevers gevangen in feromoonvallen die bedekt waren met

bananenbladeren dan in vallen zonder bladeren. De vangst in feromoonvallen was over het algemeen hetzelfde in percelen waarin de bodem rondom de bananenplanten werd bedekt met droog gras als in percelen waarin dat niet werd gedaan. Deze bedekking van de bodem met droog gras (om vocht zodoende beter vast te houden) gaat dus prima samen met het gebruik van feromoonvallen.

Het effect van verschillende dichtheden aan feromoonvallen op de bananenkever populatie en de schade aan de banenplanten werd onderzocht in een experiment bij de bananentelers zelf **(hoofdstuk 7)**. Veranderingen in de keverpopulaties en de schade aan de wortelstronken dankzij het gebruik van feromoonvallen waren verwaarloosbaar. Een verdubbeling van het aantal vallen veroorzaakte een verwaarloosbare toename in het aantal gevangen kevers per ha per maand, van 0.4% tot 0.6%. De schade aan de planten nam zelfs helemaal niet af bij deze verdubbeling. Het gebruik van feromoonvallen in een plantage van de teler zelf was niet effectief bij de dichtheid aan vallen die werd aanbevolen door de leverancier, nl. 4 vallen per ha.

In het laboratorium werd de respons van twee natuurlijke vijanden van de bananenkever, *Dactylosternum abdominale* (Coleoptera: Hydrophilidae) en *Pheidole megacephala* (Hymenoptera: Formicidae), op geur van de pseudostam van de bananenplant (kairomonen) en het synthetische feromoon van de bananenkever onderzocht in een twee-keuze olfactometer **(hoofdstuk 8).** *Dactylosternum abdominale* reageerde zowel op geur van gefermenteerde pseudostamweefsel als op Cosmolure+, terwijl *P. megacephala* alleen reageerde op de geur van gefermenteerde pseudostamweefsel. Voor de natuurlijke vijanden van de bananenkever lijkt de geur van gefermenteerde pseudostamweefsel belangrijker te zijn dan de vluchtige stoffen die door de bananenkever zelf worden geproduceerd om de kever en zijn habitat te vinden. Er werd in het veld ook geen bewijs gevonden dat erop duidde dat de verdeling van natuurlijke vijanden rondom de vallen werd beïnvloed door het feromoon.

Vervolgens werd het gebruik van feromoonvallen ter verspreiding van de entomopathogene schimmel, *Beauveria bassiana,* onderzocht **(hoofdstuk 9).** De kevers aggregeerden zowel op bananen planten waarop feromoonvallen waren geplaatst als op aangrenzende planten. Geïnfecteerde kevers verspreidden de schimmel vervolgens naar oorspronkelijk ongeïnfecteerde individuen in het veld. Er was significant meer sterfte onder de kevers in de percelen waar de schimmel was gebruikt in combinatie met het aggregatieferomoon dan in de percelen waar de schimmel werd ingezet zonder dat daarbij gebruik werd gemaakt van het feromoon. De data laten zien dat het aggregatieferomoon van de bananenkever kan worden gebruikt om de verspreiding van *B. bassiana* te vergemakkelijken. Dit zou een veelbelovende methode kunnen zijn voor de bestrijding van de bananenkever.

Tot slot wordt er een overzicht gegeven van het gebruik van het aggregatieferomoon en de geuren van de bananenplant zelf in een geïntegreerde bestrijdingsvorm van de bananenkever **(hoofdstuk 10)**. Dit project heeft meer dan voldoende experimenteel bewijs opgeleverd om de toepassing van het synthetische aggregatieferomoon door telers, die in Uganda op kleine schaal bananen telen, verder te ontwikkelen. Het is daarbij vooral belangrijk om een combinatie van feromoonvallen met entomopathogene schimmels en nematoden verder te exploiteren.

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*William Tinzaara*

*Wageningen, 25 February 2004*

# Curriculum vitae

William Tinzaara was born in Katenga, Kabale District in South Western Uganda on November 30, 1965. He attended Katenga and Bwama Primary schools, and later joined St. Mary College Rushoroza (1981-1984) and Kigezi High School (1985-1987) for his ordinary and advanced level secondary education respectively. In 1990, he graduated at Makerere University with B.Sc majoring in zoology (entomology and wildlife biology) and in the same year he registered for a Postgraduate Diploma in Education. He registered for M.Sc in the Institute of Environment and Natural Resources at Makerere University (MUIENR) in 1991 and at the same time he was teaching statistical methods and entomology at Uganda Polytechnic Kyambogo (now Kyambogo University). He worked as a Research Assistant at MUIENR on a Rockefeller Funded Project: Use of pesticides in a banana cropping system in Uganda. This project formed a basis for his M.Sc thesis entitled: Effect of pesticides on soil macro-fauna in a banana agro-ecosystem in Uganda. He completed his M.Sc degree in 1994 and in the same year, he was appointed as a Research Assistant by the National Banana Research Programme, NARO, to conduct research on the management of the banana weevil. He was appointed a Research Fellow with International Institute of Tropical Agriculture (IITA) in 2000 to undertake a Ph.D programme supported by a grant from Rockefeller Foundation through IITA and the Wageningen University Sandwich Fellowship. The study was a sandwich arrangement with the first year (2000-2001) spent at Wageningen University where he did courses in Statistical Methods and Experimental Design, Insect Biology, Integrated Pest and Vector Management in the Tropics, Pest and Disease development, and Insect Plant Interactions. During his Ph.D period, he attended several conferences and visited research and academic institutions in The Netherlands, Germany, Denmark, Sweden and Belgium where he had an opportunity to interact with several chemical ecologists. This doctoral thesis is a result of the research that was conducted at Kawanda Agricultural Research Institute and IITA Sendusu in Uganda (2002-2004) and keenly supervised by Dr. Clifford S. Gold, Prof. Dr. Marcel Dicke and Prof. Dr. Ir. Arnold van Huis.

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# List of publications

## A] Published in Journals & Proceedings


**Tinzaara, W.,** Gold, C.S., Nankinga, C., Dicke, M., van Huis, A., Ragama, P.E. &. Kagezi, G.H. 2004. Integration of pheromones and the entomopathogenic fungus for the management of the banana weevils. *Uganda Journal of Agricultural Sciences*, 9:621-629.

Gold, C.S., Night, G., Ragama, P.E., Kagezi, G.H. & **Tinzaara, W**. 2004. Field distribution for banana weevil adults in cooking banana stands in Uganda. *Insect Science and its Application*, 24:242-248.

**Tinzaara, W.,** Dicke, M., van Huis, A., van Loon, J.J.A. & Gold, C.S. 2003. Different bioassays for investigating orientation responses of the banana weevil, *Cosmopolites sordidus* (Germar) show additive effects of host plant volatiles and the synthetic pheromone. *Entomologia Experimentalis et Applicata*, 106:169-175.

**Tinzaara, W.,** Dicke, M., van Huis, A. & Gold, C.S. 2002. Use of infochemicals in pest management with special reference to the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae): a review. *Insect Science and its Application*, 22: 241-261.

**Tinzaara, W.,** Gold, C.S, Dicke, M., van Huis, A. & Nankinga, C. 2002. Use of infochemicals for the management of the banana weevil in Uganda. In: Tenywa, J.S., Nampala, M.P., Kyamanywa, S. & Osiru, M (eds). Integrated Pest Management Conference Proceedings, 8-12 September 2002, Kampala, Uganda, pp: 244-255.

Kagezi, G.H., **Tinzaara, W.,** Gold, C.S., Nankinga, C.M., Tushemereirwe, W., & Ragama, P. 2002. Field evaluation of pheromone-enhanced traps for management of the banana weevil, *Cosmopolites sordidus*, in Uganda. In: Tenywa, J.S., Nampala, M.P., Kyamanywa, S. & Osiru, M (eds). Integrated Pest Management Conference Proceedings, 8-12 September 2002, Kampala, Uganda, pp: 244-255.

**Tinzaara, W.,** Nankinga, C., Kashaija, I. & Tushemereirwe, W. 2002. Studies on the efficacy of some biorational insecticides against the banana weevil *Cosmopolites sordidus* (Germar). *Uganda Journal of Agricultural Science*s, 7:31-35

**Tinzaara, W.,** Kashaija, I., Tushemereirwe, W. & Nankinga, C. 2002. Comparative studies on chemical, hot water and cold water treatments of banana suckers to control the banana weevil, C*osmopolites sordidus (G*ermar) and the effect of paring suckers on banana nematodes in Uganda. *Uganda Journal of Agricultural Science*s, 7: 43-47

**Tinzaara, W**. & Tukahirwa, E. M. 2002. Farmers' perceptions of pests and pesticide usage in Masaka District, Uganda. *MUARIK BULLETIN*, 5:44-52

**Tinzaara, W**., Tushemereirwe, W. & Kashaija, I. 2000. Efficiency of pheromones and trap types in the capture of the banana weevil C*osmopolites sordidus* Germar in Uganda. *Uganda Journal of Agricultural Science*s, 5: 92-97

Gold, C.S., Ssennyonga, J., Okech, S.H., Bagamba, F., Kiggundu, A., **Tinzaara, W**., Masanza, M. & Nampala, P. 1999. Cultural control of banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in Uganda. *African crop Science Conference Proceedings*, 4:555-562

**Tinzaara, W**., Tushemereirwe, W. & Kashaija, I. 1999. The potential of using pheromone traps for the control of the banana weevil *Cosmopolites sordidus* Gemar in Uganda. In: Frison, E.A., Gold, C.S., Karamura, E.B. and Sikora, R.A. (eds). Mobolizing IPM for sustainable banana production in Africa. Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28 November 1998, pp: 327-332.

**Tinzaara, W.,** Karamura, E.B. & Tushemereirwe, W. 1999. Preliminary observations on natural enemies associated with the banana weevil *Cosmopolites sordidus* Germar in Uganda. *INFOMUSA*, 8: 28-29

Wiktelius, S.,Chiverton, P.A., Meguenni, H., Bennaceur, M., Ghezal, F., Umeh, E. D.N., Egwuatu, R.I, Minja, E., Makusi, R., Tukahirwa, E., **Tinzaara, W**. & Deedat, Y. 1999. Effects of insecticides on non-target organisms in Africa agroecosystems: a case for establishing regional testing programmes. *Agriculture, Ecosystems, and Environment*, 75: 121-131.

**Tinzaara, W.** & Tukahirwa, E.M. 1996. The effects of carbofuran insecticide on non-target soil invertebrates in a banana agro-ecosytem. *MusAfrica*, No. 10 September 1996, pp: 111

Tukahirwa, E.M.; **Tinzaara, W** & Kiremire, B.T. 1997. Adverse effects of Lindane in a maize agro-ecosystem in Uganda. In: Organochlorine insecticides in African Agroecosystems. Proceeddings of a final Research Coordination Meeting Organised by joint FAO/IAEA Division of nuclear Techniques in food and Agriculture, held in Entebbe, Uganda, 27 Nov - 1 Dec 1995, pp: 189-197.

**Tinzaara, W**. 1995. *The effect of soil macrofauna on soil properties in a banana cropping system.* In: Mwenja, G. and Woomer, P. (eds), The Biological Management of Soil Fertility: Proceedings of the Fifth Tropical Soil Biology and Fertility Programme Network Meeting, Entebbe, Uganda, 15 – 19 May 1995, pp: 23-24

**B] Submitted**

Tinzaara, W**.,** Gold, C.S, Dicke, M., van Huis, A. Olfactory response of banana weevil predators to volatiles from banana pseudostem tissue and synthetic pheromone, (to *Journal of Chemical Ecology)***.**

Tinzaara, W., Gold, C.S., Dicke, M., van Huis, A & Ragama, P.E. Factors influencing pheromone trap effectiveness in attracting the banana weevil, *Cosmopolites sordidus, (*to *International Journal of Pest Management)*.

Tinzaara, W., Gold, C.S., Kagezi, G.H., Dicke, M., van Huis, A., Nankinga, C.M., Tushemereirwe, W. & Ragama, P.E. Effect of pheromone trap densities against banana weevil, *Cosmopolites sordidus*, populations and their impact on plant damage in Uganda, (to *Journal of Applied Entomology).*

Tinzaara, W**.,** Barekye, A., Gold, C.S., Nankinga, C., Kagezi, G.H., Ragama, P., Tushemereirwe, W. & Blomme, G. 2004. Use of cultural practices for the management of the banana weevil, C*osmopolites sordidus* (Germar) and nematodes in Masaka District, Uganda. *African Crop Science Conference Proceedings* (accepted).

**C] To be submitted**

Tinzaara, W., Gold, C.S, Dicke, M., van Huis, A & Ragama, P.E. Host plant volatiles enhance the responses of the adult banana weevil, *Cosmopolites sordidus*, to the synthetic aggregation pheromone.

Tinzaara, W., Gold, C.S, Dicke, M., van Huis, A & Ragama, P.E. The influence of age, female mating status and density of the banana weevil, *Cosmopolites sordidus*, on its response to aggregation pheromone.

Tinzaara, W., Gold, C.S, Dicke, M., van Huis, A & Ragama, P.E. The effect of mulching on banana weevil, *Cosmopolites sordidus*, movement relative to pheromone-baited traps.

Tinzaara, W., Gold, C.S, Dicke, M., van Huis, A & Nankinga, C.M. Use of pheromone-baited traps enhances dissemination of entomopathogenic fungi to control the banana weevil, *Cosmopolites sordidus*

Tinzaara, W., Gold, C.S., Dicke, M., & van Huis, A. An overview of infochemicals and management of the banana weevil, *Cosmopolites sordidus*.

**D] Book(s)**

Tushemereirwe, W., Kashaija, I., **Tinzaara, W**., Nankinga, C. & New, S. 2001. Banana Production Manual: A guide to successful banana production in Uganda. NARO and ACD-IDEA, First Edition, 71pp.

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