BEHAVIOURAL RESPONSES OF COCOA MIRIDS, *Sahlbergella singularis* Hagl AND *Distantiella theobroma* Dist. (HETEROPTERA: MIRIDAE), TO SEX PHEROMONES.

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DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not currently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

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ABSTRACT

The mirids, *Sahlbergella singularis* Hagl and *Distantiella theobroma* (Dist) (Heteroptera: Miridae), are major insect pests of cocoa, a valuable crop in West Africa. Their control by the application of insecticides is problematic in terms of safety and cost. Therefore, this study was undertaken to determine the potential for use of mirid sex pheromone trapping as an alternative, environmentally-acceptable method of managing the mirids. Based on the behavioural responses of the mirids to pheromones in traps, parameters were standardised for efficient performance of the traps. A range of five blends of the synthetic pheromone, the diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and the monoester, hexyl (*R*)-3-hydroxybutyrate, impregnated in polyethylene vials were assayed with a blank control. Blends of 1000:500 µg and 1000:1000 µg respectively attracted significantly higher numbers of male *S. singularis* than other blends and the lure attracted male mirids optimally for four weeks with minimal reduction in eight weeks. Field bioassays were conducted to determine the appropriate trap design for pheromone trapping from four models; 2.5 litre and 4.5 litre plastic water bottles, sticky plastic plates, cylinder and standard rectangular traps. All models were equally effective. A field experiment was conducted with sticky glue on the outside of the traps. Combined inside and outside surfaces caught more mirids than the inside surface alone which caught only about 23% of the male mirids. Three field experiments using two different experimental designs were conducted to determine optimal height for trap placement. Traps placed inside the canopy attracted significantly more mirids than below 2.7 m height from the ground. The potential for mass trapping of mirids as a method of control was studied through three mass trapping experiments on research plantations and smallholder farmers' farms. Catches of male *S. singularis* in pheromone traps were significantly reduced in mass-trapped fields but pheromone trapping did not control mirid numbers or affect damage on cocoa. Densities of 150 and 230 traps/ha were found to be optimal for trapping *S. sahlbergella* and *D. theobroma* respectively. Catches of male *S. singularis* in pheromone traps, however, predicted the magnitude of total mirid populations, and also shoot and pod damage in cocoa farms, albeit inconsistently.

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Chapter 1
GENERAL INTRODUCTION

1.1 THE COCOA PLANT

The cocoa plant species *Theobroma cacao* L, belongs to the flowering plants division of Magnoliophyta, order Malvales and the family Sterculiaceae (Cronquist, 1981; Wood and Lass, 2001). Its origins are traced to the upper regions of the Amazon basin and other tropical areas of South America. It is believed to have been introduced to Ghana from Fernando Po (now part of Equatorial Guinea) by Tetteh Quarshie, a trader from the Gold Coast, as Ghana was named pre-independence. It grows best in the geographic band roughly between 10° and 15° north and south of the equator and it is now cultivated predominantly in West Africa, Latin America and South East Asia (World Bank Report, 2011). The plant is cauliflorous, and favourable conditions for its growth are humid rainforests, constant but moderate temperatures (25ºC), ample rainfall (102-203 cm/year) and rich well drained soils (http://thechocolatereview.com/where-does-chocolate-come-from-/where-does-chocolate-come-from.html, cited on 6th January, 2013). Referred to as the "food for the gods", *T. cacao* is the source of cocoa used to produce chocolate.

Cocoa is an important export crop of Ghana and other countries of the West (Côte d'Ivoire, Nigeria, Cameroun, Togo and Sierra Leone) and Central (Cameroun) African cocoa belt. Countries across this belt account for more than two-thirds of global production with Ghana and Côte d'Ivoire alone supplying about 40% of the total global production (World Cocoa Foundation, 2010). It is the second largest export out of Ghana (SGER, 2006) and accounts for more than 9% of agricultural Gross Domestic Product (GDP) according to the Bank of Ghana. Current estimates put 1,212,000 ha of land in the cocoa-producing area under cocoa cultivation (Figure 1.1), with about 800,000 mainly smallholder families, i.e. 60% of

the total agricultural labour, involved in its production (Appiah, 2004; Anim-Kwapong and Frimpong, 2004).

For 66 years (1970-1977), Ghana was the leading producer, with 30-40% of the world's total (Bateman, 1988), before losing that position due to decline in production. Paramount among the reasons for the decline were the ravages caused by cocoa insect pests, mainly mirids, and diseases, mainly swollen shoot caused by cocoa swollen shoot virus and black pod caused by the fungi *Phytophthora megakarya* and *P. palmivora.*

Figure 1.1 Cocoa growing area of Ghana (sourced from Cadbury skills space at www.skillsspace.co.uk/geography/cocoa/ghana_cocoa_region.asp).

1.2 MIRID PESTS OF COCOA

Mirids (formerly known as capsids), are sap sucking plant feeders. They belong to the Order Hemiptera, sub-order Heteroptera and to the family Miridae. Members of this family have a well-developed cuneus in the forewing that separates them from other families. Members of five mirid genera viz, *Sahlbergella, Distantiella, Bryocoropsis, Daniela* and *Helopeltis* attack cocoa in West and Central Africa. *Sahlbergella* is the most widespread, attacking cocoa from Sierra Leone in West Africa to Zaire and Central Africa Republic in the East (Padi and Owusu, 2001). In West Africa, two species of mirids*, Sahlbergella singularis* Hagl and *Distantiella theobroma* (Dist.) are the major pests with the former being dominant in Ghana (Dungeon, 1910; Owusu-Manu, 1985; Owusu-Manu, 1996; N'Guessan and Coulibaly, 2000; Sounigo *et al.,* 2003; Babin *et al*., 2008). Two other species *Bryocoropsis laticollis* Schum and *Helopeltis* spp. also occur in Ghana. They feed mainly on pods but their feeding punches are superficial and for that reason they are considered as minor pests (Raw, 1959). In Ghana temporal distribution of mirids is in the main characterized by low populations from February to June followed by gradual build up in July to high numbers from August to January, with peak between November and December (Gibbs *et al.,* 1968; Owusu-Manu and Somuah, 1989). Similar temporal distribution essentially exists in Côte d'Ivoire and Nigeria. However, in Cameroun populations may peak earlier in October (Lavabre *et al*., 1963; Collingwood 1971b; Anikwe *et al*., 2010). Spatially, mirids are strongly aggregated in distribution (Williams, 1953b; Youdeowei, 1965; Lotodé, 1969; Babin *et al.,* 2010), particularly in areas where a break occurs in the canopy of a plantation (Entwistle, 1972; Babin *et al.,* 2010).

1.2.1 Life cycle

The six-week life cycles of *S. singularis* (Figure 1.2) and *D. theobroma* (Figure 1.3) are very similar with only minor differences. Mating is mediated by sex pheromones (King, 1973; Padi *et al.,* 2002). Female mirids first adopt a 'calling' posture to attract males from about three to five days into adulthood (Collingwood, 1971a). In resting, they align themselves horizontally to the surface of their

support but this position changes to an inclined one, with the tip of the abdomen raised about 45º when calling, and only terminates with mating (Collingwood, 1971b). After mating, oviposition in *S. singularis* begins after four to eleven days and continues for about 30 to 40 days to lay up to about 200 eggs, while in *D. theobroma* it begins after four to ten days but it takes the same period to lay about 100 eggs (Williams, 1953a). The eggs, which are laid with a saw-like ovipositor under the bark of young shoot or in the cortex of a pod, incubate for between 13 and 18 days for *D. theobroma* and a day or two more for *S. singularis*. There are five nymphal stages (Figure 1.4) with a total period of between 14 and 23 days for *D. theobroma* and 16 and 23 for *S. singularis* (Anon., 1946; Williams, 1954). Though females die soon after laying all their eggs, they would have lived about two times longer than the males (Anon. 1946) which could be over 60 days in *S. singularis* (Babin *et al.,* 2011). In both species there are slightly more females than males. An international capsid research team in Ghana reported overall sex ratio of 49% males to 51% females in *D. theobroma* which may drop to 45% to 55% in the dry season respectively, and about 40% males to 60% females in *S. singularis* (Collingwood, 1971b). However, Babin *et al.* (2008) reported the male to female ratio in *S. singularis* to be 1: 0.71 respectively.

Figure 1.2 Dorsal view of adult female *Sahlbergella singularis*.

Figure 1.3 Lateral view of adult female *Distantiella theobroma*
(Picture: Courtesy Nick Jessop)

Figure 1.4 Dorsal view of nymph *Sahlbergella singularis*
(Picture: Courtesy Nick Jessop)

1.2.2 Damage caused by mirids

Mirids do significant damage to cocoa although they occur in low numbers, usually of about 1000-2000 per hectare from routine hand-height collections (Collingwood, 1971b). According to Owusu-Manu (1985), a mean of 6 mirids per 10 trees represents a high and damaging population level. Both adults and nymphs feed on pods and soft branches by piercing the plant tissue with their pin-like mouthparts to suck sap which causes injury to the tissues (Williams, 1953a; Entwistle, 1972; Collingwood, 1977). The injury results in dark markings, “lesions’’ (Figures 1.5 and 1.6), which are usually infected by parasitic fungi, notably *Albonectria* (*Calonectria) rigidiuscula* (Berk. and Broome), to cause secondary damage, which eventually leads to canker and die-back in shoots (Cotterell, 1926; Crowdy, 1947). Extensive feeding on fan branches results in degradation of the canopies of several trees to form a “pocket” (Figures 1.7 and 1.8) eventually. It begins as “blast” (Figure 1.7) where dead leaves remain on trees, then progresses to “staghead” (Figure 1.8) where the crown becomes thin with many leafless branches (Wright, 1938). Seedlings also suffer consequences of mirid attack. Those damaged by mirids may fail to establish or delay in bearing several years (Williams, 1953a). Pods less than three months old may wilt and die but older ones are seldom affected (Gerard, 1968).

Inadequacy of records, and the difficulty in isolating losses by fungal and viral diseases complicate the determination of estimates of losses due to mirids only, and may be combined (Lass, 2004). Damage by mirids only are estimated at 25-30% per annum by Wills(1962), about 25% by Stapley and Hammond (1959) and as high as 75% if cocoa is left unattended to for over three years (Anon., 1951). Indeed, current figures from a World Bank (2011) report on the crop losses due to mirids show increase in annual losses in both acreage and revenue from 2008 to 2010. A total crop loss of 64,283 metric tonnes resulted in US$ 135,251.086 loss in revenue in 2008. In 2009 a crop loss of 64,442 metric tonnes cost the nation US$ 154,613,581 while an 83,400 metric tonne crop loss in 2010 resulted in a loss of US$ 225,346,638 in revenue.

Figure 1.5 Mature and immature pods fed on by mirids

Figure 1.6 Mirid feeding lesions on shoot

Figure 1.7 Mirid infested cocoa plants showing ‘blasts’ in ‘mirid pocket’

Figure 1.8 Mirid pocket showing ‘staghead’
(Picture: Courtesy CRIG)

1.2.3 Chemical control of mirids

Since the 1950s, the main method of control recommended by the Cocoa Research Institute of Ghana (CRIG) has been the foliar application of chemical insecticides (Graham 1908; Dungeon 1910; Collingwood and Marchart 1971; Owusu-Manu-2001; Padi and Owusu 2001) (Figure 1.9).

Figure 1.9 Application of insecticide on cocoa.

Farmers are advised to apply CRIG recommended insecticides as aqueous emulsions or soluble concentrates on mature cocoa trees with recommended mist-blowers and at 55-56 litres per ha (Stapley and Hammond 1957). The recommended frequency is four times in a year, at monthly intervals from August to December omitting November. Over the years several insecticides have been applied to control mirids; these include, for example, nicotine, dichloro-diphenyl-trichloroethane (DDT) (Nicol, 1953), malathion (Johnson *et al.,* 1970), dimethrin (Prins, 1965), dioxocarb, promecarb (Marchart, 1971), cypermethrin, propoxur,

(Owusu-Manu, 1985), imidacloprid, bifenthrin (Adu-Acheampong and Ackonor, 2005), and natural pyrethrum in combination with deltamethrin (Cudjoe *et al.,* in press). In applying these insecticides, investigations have always been conducted to certify that in addition to high efficacy in causing acceptable levels of mortality by CRIG standards (i.e. < 95% mortality) they should also have other desirable qualities. These desirable qualities include short persistence, volatility to ensure good coverage on application, specificity against mirids and safety to the user. Others are that the application of the insecticides should not taint or leave residues in beans, nor encourage the emergence of other pests. The cost must also be affordable to the farmer.

In as much as insecticide application can be credited with controlling mirid infestation over the years, ensuring their desirability has not always been possible and might threaten the sustainability of cocoa production. The normal application of carbaryl or fenitrothion destroyed colonies of the predatory ants *Oecophylla longinoda* (Latreille) and *Micromiscoides* according to Gerard (1964). Marchart (1969) also reported that doubling the normal application rates of propoxur depressed *O. longinoda* populations by 75%. Apart from the environmental unfriendliness and human health consequences of insecticide treatments (Murray and Lopez, 1996; Bouwman *et al*., 2006), the high cost of insecticides has led to less adoption of the CRIG recommendations (Davis, 2001), resulting in indiscriminate use of cheap and highly toxic insecticides (Ackonor *et al*., 2006). The indiscriminate use of large quantities of insecticides led to resistance in mirids (Dunn, 1963). It has led to the destruction of natural enemies to shift the status of hitherto minor pests into major ones, as happened in the case of the stink bug, *Bathycoelia thalassina* (Herrich-Schaeffer) (Hemiptera: Pentatomidae) that became a major pest of pods after long use of DDT (Owusu-Manu, 1974). Increased pod and shoot damage by *Marmara* spp (Lepidoptera: Gracillaridae) and *Eulophonotus myrmeleon* Fldr. (Lepidoptera: Cossidae) respectively also occurred after the application of dieldrin at high rates (Entwistle *et al.,* 1959) as did the outbreak of *Pseudopteraptus devastans* Dist. (Heteroptera: Coreidae), a pest of cocoa pod after the long use of carbaryl (Sevin) (Lodos, 1967).

The indiscriminate use of insecticides may also have dire consequences for cocoa exports. A 2,000 tonne shipment of cocoa to Japan from Ghana was rejected in 2006, due to the detection of illegal insecticide residues. The risks of current practices, coupled with the sophistication of the consumer, demanded a search for alternative bio-rational methods, such as the use of semiochemicals that would reduce or, if possible eliminate, the use of chemical insecticides.

1.3 SEMIOCHEMICALS

Semiochemicals are compounds produced by animals or plants that bear information which cause modification of behaviour in other individuals. They are used in communication in both invertebrates and vertebrates. Based on their use within or between species, semiochemicals are categorised either as pheromones (Karlson and Lüscher, 1959) or allelochemicals (Whitaker, 1970), respectively. Allelochemicals are further categorised as allomones, kairomones, synomones or apneumones depending on whether the producer or receiver or both, become beneficiary of the interaction (Nordlund and Lewis, 1976). Thus, kairomones benefit the receiver only, allomones the emitter only and synomones, both emitter and receiver. Apneumones, though benefitting the receiver only, are produced from non-living sources.

1.3.1 Pheromones

Communication between animals of the same species, mediated by chemicals was recognised in ancient times (Schneider, 1999), but it was not until 1932 that Bethe proposed the term ‘’ectohormones’’ to represent the chemicals. However, contrasting processes in the production of ‘ectohormones’ and hormones led Karlson and Butenandt (1959) to introduce the name ‘pheromones’ (Greek*; pherein,* to excite and *horman,* to carry) to represent chemical substances that are secreted by an animal to the outside which when perceived by individuals or group of the species elicit a specific behavioural response. Studies since then

have shown that conspecific response to pheromone need not be obligatory since response by closely related species is possible as exemplified by the cocoa mirids, *D. theobroma* and *S. singularis* and other plant bugs such as *Phytocoris difficilis* Knight and *Phytocoris breviusculus* Reuter (Downham, *et al.*, 2002; Zhang and Aldrich, 2003b).

Pheromones have been studied widely in insects but reviewers differ on their scheme of classification which is essentially based on the behaviour of the receiving insect. For example, Wilson (1968) identified seven classes, and Butler (1970) categorised them into six, just as Shorey (1973). The classes had more differences than similarities. Usually, however, classes of insect pheromones include sex, aggregation, epideictic or dispersal, alarm, trail or recruitment and maturation pheromones as well as those associated with social insects (e.g. Birch and Haynes, 1982; Jutsum and Gordon, 1989).

1.3.2 Sex pheromones

Sex pheromone is a chemical produced by an individual to elicit a sequence of behavioural responses in the opposite sex of species that will eventually end in mating or copulation between the two (Karlson and Butenandt, 1959; Karlson and Luscher, 1959). They are classified into components according to their effective distance of attraction; primary sex components attracting the opposite sex from far distances in upward oriented movement while the secondary component(s) attract from close proximity for close range courtship rituals (Roelofs and Arn, 1968).

1.3.3 Perception of sex pheromones

Sex pheromone perception in insects is very fundamental to the success of pest management programmes because it determines the overall behavioural pattern of response to the pheromone. Sex pheromones odorant molecules are perceived mainly by receptive sensilla on the antenna of insects which have been adapted structurally for this function. Basically, the mechanism of perception starts with

antennal sensillum filtration of air for the entry of odorant molecules through peripheral pores on its walls; and the binding of lipophilic odorants with odorant-binding proteins for transportation across lymph to the dendrites (Vogt, *et al*., 1985; Vogt, 1995; Pelosi, 1996; Steinbrecht, 1996). It continues with intercellular events on the dendrite which results in the generation of corresponding electrical messages (reviewed by Jacquin-Joly and Merlin, 2004). Electrical messages are received at specific sites of the glomeruli for translation in the brain. Studies on the olfactory reception of the insect brain (Galizia, *et al*., 1999) and the functional specialization of the olfactory glomeruli (Hansson, *et al*., 1992; Gao, *et al*., 2000; Voshall, *et al*., 2000) elucidated clearly the specificity of odour coding on glomeruli. These studies confirmed earlier reports that sex pheromone detection occurred in specific glomeruli in the macroglomerus complex, (MGC) (Koontz and Schneider, 1987) and may be the basis of the discriminatory behaviour of insects to pheromones of other species as well as synthetic blends of its pheromones.

The mechanisms of pheromone perception have been studied in detail in groups such as Lepidoptera, Diptera and Coleoptera (Vogt, 1995; Carlson, 1996; Pelosi, 1996; Steinbrecht, 1996; Breer, 1997). It is apparent from these studies that antennal form, which reflects the total surface area and number of sensilla, correlates with the sex pheromone sensitivity of the individual (Kaissling, 1971).

1.4 SEX PHEROMONES IN THE HETEROPTERA

Compared with the Lepidoptera, studies on pheromone chemistry in this order are a relatively recent phenomenon. Sex pheromones, however, have been identified in species from several groups including the broad-headed bugs (Alydidae), shield bugs (Scutelleridae), seed bugs (Lygaeidae), assassin bugs (Reduviidae), stink bugs (Pentatomidae) and plant bugs (Miridae) (reviewed by McBrien and Millar, 1999).

1.4.1 Identification of sex pheromones of mirids

Sexual communication mediated by pheromones was suspected in several mirid species (reviewed by McBrien and Millar, 1999) and attempts were made to identify the chemicals involved with mixed results. Identification of pheromones for *Lygus* species in particular has been very frustrating (McLaughlin, 1998; Ho and Millar, 2002). There is overwhelming evidence that sexually mature female *Lygus hesperus* Knight, *Lygus lineolaris* (Palisot de Beauvois), *Lygus desertinus* Knight and *Lygus elisus* Van Dezee, use sex pheromones to attract males (Graham, 1987, 1988). However, repeated attempts at confirming the identities of the compounds involved have failed (McLaughlin, 1998; Ho and Millar, 2002). Other species with demonstrable female sex pheromone attraction but no components yet identified include the cocoa mirids *Helopeltis clavifer* (Walker) (Smith, 1977) and *H. theobromae* (Virdiana, 2011), the green apple bug *Lygocoris communis* (Knight) (Bolvin and Stewart, 1982) and the apple brown bug *Attractotomus mali* (Meyer) (Smith and Gaul, 1994). Few mirids, however, have their sex pheromones completely identified.

The first report of complete identification and bioassay of mirid sex pheromones was by Smith *et al*. (1991) for the mullein bug*, Campylomma verbasci* Meyer. This followed an earlier report by Thistlewood *et al.* (1989a) which suggested the release of sex pheromones by the female. Since then sex pheromones have been identified for the plant bugs, *Phytocoris relativus* Knight (Millar *et al.,*1997), *Phytocoris californicus* Knight (Millar and Rice, 1998) and *P. difficilis* and *P. breviusculus* (Zhang and Aldrich, 2003b). Also identified are the sex pheromones of the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Kakizaki and Sugie, 2001), the cocoa mirids, *D. theobroma* and *S. singularis* (Downham *et al.,* 2002), the European tarnished plant bug, *Lygus rugulipennis* Popp. (Innocenzi *et al.,* 2004), the sorghum plant bug, *Stenotus rubrovittatus* (Matsumura) (Yasuda *et al*., 2008) and the green mirid, *Creontialdes dilutus* (Stål) (Hemiptera: Miridae) (Lowor *et al*., 2009).

Metathoracic scent glands are characteristic feature in the Heteroptera producing a plethora of volatiles, for example, for defence in the stink bugs (Pentatomidae)

and the leaf footed bugs (Coreidae) (Staddon, 1986; Aldrich, 1988) and sexual attraction in female mirids (Aldrich, 1988; Millar and Rice, 1998; Zhang and Aldrich, 2003b), as well as alarm and anti-sexual olfaction in male mirids (Zhang and Aldrich, 2003a). However, the sources of mirid sex pheromones identified varied among the species. Strong *et al.*(1970) postulated that the spermatheca might be the source in *Lygus* species, especially *L. hesperus,* based on the reported absence of a metathoracic scent gland (MSG) in the species, as a result of feeding on plants containing methylpurines such as caffeine (reviewed by Aldrich, 1988). The source remains unknown in others such as *L. rugulipennis*, where volatiles were collected from aeration as well as extraction from abdomen and thorax (Innocenzi *et al*., 2004), in *S. rubrovittatus*, where whole body volatiles were collected in solvents (Yasuda *et al.,* 2008), and in *C. dilutes* where air collections and extracts were obtained from whole bodies (Lowor *et al*., 2009). In contrast, other studies determined the thorax as the exclusive source of sex pheromones in *Phytocoris* species *P. relativus, P. californicus* and *P.* difficilis without identifying the glands (Millar *et al.,* 1997; Millar and Rice, 1998; Zhang and Aldrich, 2003b), while the head and thorax were also implicated in *C. verbasci* (McBrien and Millar, 1999). Kakizaki and Sugie (2001) extracted sex pheromones from the whole body of *T. caelestialium*. The source in cocoa mirids *D. theobroma* and *S. singularis* also remains unknown but might exclude the MSG since caffeine is a major component of their diet.

1.4.2 Chemical composition of mirid sex pheromones identified

Identified mirid pheromones are relatively simple compounds unlike those of other Heteroptera (McBrien and Millar, 1999), and consist mainly of esters with some similarities in the volatile profiles. In the four species of the genus *Phytocoris* reported, hexyl acetate was the major component combined in the ratio 2:1 with the minor component (*E*)-2-octenyl acetate in three species *P. californicus, P. difficilis* and *P. breviusculus* (Millar and Rice, 1998; Zhang and Aldrich, 2003b). The additional minor components, (*E*)-2-octenyl butyrate and (*E*)-2-hexenyl acetate, however, were unique to *P. relativus* (2:1) and *P. difficilis* (2:1:1.5)

respectively (Smith *et al.,* 1991; Zhang and Aldrich, 2003b). Similarly in the cocoa mirids *D. theobroma* and *S. singularis*, sex pheromone components, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and hexyl (*R*)-3-hydroxybutyrate collected by entrainment from *D. theobroma* in the ratio 2:1, was shared by the two species (Downham *et al.,* 2002). All major components of mirid pheromones were produced by the female and all minor by both sexes except in *C. verbasci* in which the female produced both the major, butyl butyrate, and minor, (*E*)-crotyl butyrate, components combined in 2:1 ratio (Smith *et al*., 1991), and *S. rubrovittatus* in which the female also produced the major components hexyl butyrate and (*E*)-2-hexenyl butyrate, as well as the minor (*E*)-4-oxohex-2-enal (Yasuda *et al*., 2008). Another exception is *T. caelestialium* where both sexes produced hexyl hexanoate, (*E*)-2-hexenyl hexanoate and octyl butyrate (1000:400-500:10-100) albeit in low quantities by the male.

In mirid species the female is the attractive sex attracting mature males (McBrien and Millar, 1999; Kakizaki and Sugie, 2001). Attraction to virgin females, however, did not guarantee male attraction to the synthetic isomers of female sex pheromones. All the synthesised lures of the mirid species above attracted only males in significant numbers in the field showing their potential use in pest management. However, those of *L. rugulipennis,* hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (1.5:1:1.08) attracted about equal numbers of both males and females in traps (Innocenzi *et al.,* 2004). Nevertheless, this does not invalidate its importance in pest management as the observation suggested some aggregation function of *L. rugulipennis* pheromones. The observation in *L. rugulipennis* follows a precedent in *L. hesperus* (Ho and Millar, 2002). The explanation by Innocenzi *et al.* (2004) that the compounds might be insufficiently attractive would appear plausible considering the fact that the suspected source of production (Strong *et al.,* 1970) was not targeted.

1.4.3 Collection of mirid sex pheromones

The identification of sex pheromone compounds goes through processes which start with the collection and separation of the compounds and terminates with

insect responses to the compounds. Pheromone extracts are prepared in several ways (Evans *et al.,* 1990), the most useful method for Heteropteran bugs being aeration or entrainment according to McBrien and Millar (1999). In this method, live virgin female(s) and food are enclosed in a chamber with a suitable adsorbent (e.g. an activated charcoal filter), humidifier and a volatiles collector. Air is pulled through the chamber after which the collector is eluted with a solvent and the extract concentrated for analysis. The results are most representative of what the insect actually produces and very useful but other compounds not targeted may also be released if too many insects are entrained at a time. This method has been extensively used to obtain the sex pheromones of mirids such as *C. verbasci* (Smith *et al.,*1991*), P. relativus* (Millar *et al.,* 1997), *L. rugulipennis* (Innocenzi *et al.,* 2004) as well as *D. theobroma* and *S. singularis* (Downham *et al.,* 2002). The other methods involve the direct extraction of compounds from whole insects or part(s) of it, e.g. pheromone gland or thorax, either ground or intact with suitable solvents. Though a large fraction of the mixture may not be necessary from an olfactory point of view, the methods have been employed variously either on their own or in combination with the aeration method, in obtaining sex pheromones from several mirids including *T. caelestialium* (Kakizaki and Sugie, 2001) and *P. difficilis* (Zhang and Aldrich, 2003b).

1.4.4 Electrophysiological analysis of pheromones

After the extracts have been obtained, the behaviourally active compounds are determined through electroantennogram (EAG) assay (Roelofs, 1984). The method is shortened by a linkage between gas chromatography (GC), coupled GC-mass spectrometry (GC/MS), and GC-electroantennographic detection (GC-EAD). GC-EAD is a biosensor in which an insect antenna is the sensing element. Developed by Schneider (1957) and widely used since then, it essentially works first by placing a recording electrode over the cut end of the antenna and a reference one at the base of it followed by GC analysis. Analysis in the GC starts with injection in splitless mode onto a capillary column which has a temperature programme and a carrier gas. GC column effluent constituents of the extract are

separated equally in a Y-connector with one part directed to the GC detector and the other directed into a humidified air stream passing over the antennal preparation. Amplified antennal responses and the GC detector signals are simultaneously recorded. The EAD peaks matched with those detected by the GC through the flame ionisation detector, leads to the characterisation of the electrophysiologically active compounds. By this method a compound not eliciting an electrophysiologically active response can be excluded as an olfactory cue mediated by antennal reception.

Once the electrophysiologically active peaks of the GC have been detected, the next step is the characterisation of their chemical structures which is done through coupled GC-MS (Heath and Tumlinson, 1984), a very powerful tool for separating and quantifying components of an extract according to Rose (1990). Spectra are obtained in electron impact mode and compounds are identified by matching their mass spectra and GC retention times with those of authentic standards.

Pheromones in groups such as Lepidoptera, Coleoptera and Diptera have been widely analysed by GC-EAD. Among the Heteroptera it has been employed in the analysis of pheromones of several species including the broad headed bugs (Alydidae) (Numata *et al*.,1990; Leal *et al*.,1995), the seed bugs (Lygaeidae) (Aldrich *et al*., 1997) and the stink bugs (Pentatomidae) (Leal *et al*., 1998). In mirids, pheromones of all species identified above were assayed through GC-MS and/or GC-EAD. In addition, electroantennographic activity has been determined for *L. hesperus* and *L. lineolaris*

The chemical identity and electrophysiological activity of a compound is of little value in pest management if there is no behavioural response to it. Hence results of GC-MS and GC-EAD are confirmed in the field in behavioural bioassays. Some early workers skipped EAD assays for direct confirmation in the field. Thus, Smith *et al.* (1991) and Millar *et al.* (1997) proceeded to test synthetic lures of *C. verbasci* and *P. relativus* respectively, in the field without prior EAD assay and succeeded in attracting males. Recent workers routinely conducted EAD assays prior to field trials. Kakizaki and Sugie (2001) conducted field trials with synthetic lures based on GC-EAD results of the components of the female sex pheromones

of *T. caelestialiium* and attracted males. Similarly, Zhang and Aldrich (2003b) synthesised the lures of *P. difficilis* which not only attracted its males but those of *P. breviuculus* as well. By the same process the synthetic lures of the cocoa mirid *D. theobroma* attracted males of a related species *S. singularis* (Downham *et al.,* 2002).

However, electroantennographic activity does not necessarily mean successful mate attraction in the field. The literature is replete with instances among mirids especially *Lygus* spp where synthetic lures of EAD active compounds failed to attract sufficient males or none at all in the field. *L. hesperus* (Ho and Millar, 2002), *L. rugulipennis* Poppius (Innocenzi *et al.,* 2004) and *L. lineolaris* (Palisot de Beauvois) (Zhang *et al.,* 2007) all failed in field trials even though they had shown EAD activity.

1.5 USES OF SEX- PHEROMONES IN PEST MANAGEMENT

The use of sex pheromones to attract mates (Karlson and Butenandt, 1959; Karlson and Luscher, 1959) in reproduction has been exploited to control insect pests either directly or indirectly. Indirectly, sex pheromones are most commonly used to monitor insect populations and directly through mass trapping of large numbers of pests from population or disruption of mating or luring and killing (Jutsum and Gordon, 1995; Witzgall *et al.,* 2010)

1.5.1 Monitoring

The ability of sex-pheromone baited traps to catch insects even at low populations underpins the use of traps in rapid, sensitive and selective monitoring programmes. Mirid populations are monitored mainly for three reasons; to track incidence of species that chronically infest crops, to detect immigration of species into crops and to determine economic threshold for the application of control measures against the mirid pest (McBrien and Millar, 1999). Achievement of any of these objectives depends on effective trapping of the mirid pest. However,

several factors affect the trapping of mirids and they must be considered in the development of trapping programmes. Among these are the synthetic pheromone blend, pheromone dispensers and formulations, dosage and active range of the lures. Others are the design of the trap and the seasonality of the insect. Ho and Millar (2002) failed to attract either sex of *L. hesperus* with the insect's volatiles as was reported in an earlier work on *L. lineolaris* (Hedin *et al.,* 1985). Their conclusion is that mirids can be monitored only by accurate synthetic isomers of sex-pheromones with clear pattern of ratios whose biological functions have been proved in bioassays regardless of positive EAD responses. With the exception of *L. rugulipennis* (Innocenzi *et al.,* 2004), components of all the identified mirid sex pheromones are reproducible in clear ratios and by extension suitable for monitoring, but only butyl butyrate and (*E*)-2-butenyl butyrate in 2:1 ratio in *C. verbasci* (Smith *et al.,* 1991) has been reported in a monitoring programme.

Successful monitoring is achieved when the attractant is released at a constant rate for a long period (Wall, 1989) which may depend on the dispenser used in the formulation. Several dispensers used to trap bugs have been reviewed by McBrien and Millar (1999). However, in mirids three main dispensers were used in field trapping. Millar *et al*. (1997) used impregnated septa to trap *P. relativus* and Zhang and Aldrich (2003b) also used same in the trapping of *P. difficilis* and *P. breviuculus*. Smith *et al.* (1991) and Kakizaki and Sugie (2001) used capillary tubes to trap *C. verbasci* and *T. caelestialium* respectively. In the field trapping of *D. theobroma* and *S. singularis,* impregnated polyethylene vials were used (Padi *et al.,* 2002). Inappropriate dispensers cause the chemical components to be released either too quickly or too slowly and targets are missed.

While rubber septa formulation of sex pheromones of *L. rugulipennis* failed to attract males, loadings of the same lures in capillary tubes appears to work according to Innocenzi *et al.* (2005). Also a single dispenser formulated with compounds of very diverse volatilities is not effective due to unequal releases resulting in changes in blend composition (McBrien and Millar, 1999). The review by McBrien and Millar (1999) showed that mirids respond to large doses but subsequent studies have shown that a mirid's response to doses and release rates vary between the species. Thus while *C. verbasci, P. relativus*, *P.*

californicus and *T. caelestialiium* respond to large doses and release rates (McBrien *et al*., 1994; Millar *et al*., 1997; Millar and Rice., 1998; Kakizaki and Sugie, 2001), *P. difficilis*, *P. breviuculus*, *D. theobroma* and *S. singularis* respond to low release rates (Zhang and Aldrich, 2003b; Downham *et al.,* 2002; Padi *et al*., 2002). It appears, therefore, that a wide range of testing is required in determining dosage and release rates of mirid pheromones.

Catches in traps are affected by the effective range of the lures. Long range lures such as those releasing pheromones produced by *P. relativus* and *C. verbasci*, give consistently large catches in traps, while short range ones give highly variable catches especially in aggregated populations such as *D. theobroma* and *S. singularis* (Padi *et al.,* 2002). In short range lures catches may be improved with shortened distances between adjacent traps.

Mirids may fly straight into traps according to McBrien and Millar (1999), therefore the size of the trap opening may affect the trap catches and hence monitoring. McBrien *et al.* (1994) reported the superiority of wider opening wing traps over narrow ones in the capture of *C. verbasci*. Sticky surfaces have usually been used to capture mirids and they cannot pull away because of their small sizes but other retention substances such as water have also been reported (Kakizaki and Sugie, 1997). Studies on the influence of colour on trap catches in monitoring are inconclusive but height of trap placement are reported to affect trapping. McBrien *et al.* (1996) reported the effectiveness of traps at 1.5 m above ground as against placement at 2.5 m. Also Sarfo *et al.* (2007) found optimum catches by sticky traps at 1.8 m in the cocoa canopy. The period of monitoring is determined by the purpose. Monitoring for the determination of threshold levels done at periods of low population may not be useful since catches are often low (Sarfo *et al.,* 2007) and threshold levels so determined inaccurate.

Potentially all identified mirid pheromones with proven biological activity in the field can be used in monitoring the insect, and indeed, that was the prime reason in most cases. For example, Millar *et al.,* (1997) identified the sex pheromones of *P. relativus* specifically for the development of a rapid and sensitive method of detecting the pest infestation in pistachio orchards in California. The identification

of the sex pheromones of *C. verbasci* by Smith *et al.* (1991), was motivated by the desire to find a reliable method of monitoring the incidence of the insect after promising results had been reported by Smith and Borden (1990) using a virgin female. Also, McBrien *et al.* (1994) developed traps to monitor the same insect in Canada. For improvement in management, Zhang and Aldrich (2003b) suggested the use of synthetic sex pheromones of *P. difficilis* to sample and monitor the populations of the insect instead of the tedious and time consuming methods of beating tray and sweep-net sampling (Ho and Millar, 2002). Kakizaki and Sugie (2001), after identifying the sex pheromones of *T. caelestialium*, recommended their use in monitoring. However, serious attempts at developing threshold levels after monitoring mirid populations have only been reported in *C. verbasci* (McBrien *et al.,* 1994; McBrien *et al.,* 1996). One of the main aims of synthesizing the sex pheromones of *D. theobroma* and *S. singularis* was for the development of threshold levels to time control measures (Padi *et al.,* 2002).

Monitoring with sex pheromone baited traps is of tremendous value, nonetheless it may not give accurate measure of populations. In mirids only males are caught leaving females and immatures, such that additional methods may be required to assess the total populations.

1.5.2 Mass trapping

Mass trapping insects in pheromone baited traps to suppress the population to economically acceptable levels is a direct method that has been applied in several insects including the Lepidoptera (e.g. Zhang *et al*., 2002), Diptera (e.g. Suckling *et al*., 2007) and Coleoptera (e.g. Allou *et al*., 2006). Documented use of bio-rational methods in CABI Abstracts reviewed by El-Sayed *et al.* (2006), shows mass trapping as being only second to mating disruption in the use of bio-rational techniques of control. However, there was no mass trapping of any mirid or Heteroptera for that matter reported. This review is, therefore, on other insects but with the assumption that the principles involved would be relevant to the mirids also.

Optimization of the blend, dosage and release rate contributes to the success of mass trapping by increasing the competitiveness of the attractant against the natural sources of attraction such as calling females in the field (Mottus *et al.,* 1996). Thus, synthetic lures that contain all the components of sex pheromones elicit strong response from the males, compete better and consequently are more successful in mass trapping than those that may have some components missing (El-Sayed and Trimble, 2002; El-Sayed, 2006, http//www.pherobase. com, cited 20th July, 2007).

Like monitoring, mass trapping is also affected by the trap design with traps having the capacity to retain high numbers of insects being successful. Also success in mass trapping has been achieved when there is high ratio of traps to wild females (El Sayed, 2006) but the density to be deployed has always been in dispute. Both higher (Sternlicht *et al.,*1990) and lower densities (Pasqualini *et al.,* 1997) have been advocated. However, higher densities not only disorient the males but might impose an economic burden as well (e.g. Roelofs *et al.,* 1970).

The eco-biology of the pest as well as the lay out of the crop may determine the success or otherwise of mass trapping. Success has been reported of the technique in several species especially Lepidoptera and Homoptera when the pest density is low and the crops isolated, without the possibility of re-infestation from immigration (Madsen *et al.,* 1976; Huber *et al*., 1979; Madsen and Carty, 1979; Sternlicht *et al.,* 1990). Also, those pest species with only one generation in a year have an advantage since trapping would be needed only once in a year (Sternlicht *et al.,* 1990; Mottus *et al.,* 1996; Zhang *et al.,* 2002). In contrast, by requiring continuous trapping for a greater part of the year, multivoltine pests are a disadvantage (Teich *et al.,* 1979; Sternlicht *et al.,* 1990). Less mobile as well as monophagous pests also present an advantage over the highly mobile polyphagous pests because monophagy reduces the risk of re-invasion of treated crops by unmated females from surrounding crops (Sternlicht *et al.,* 1990). Mating habits of the pest are of significance in determining the success or otherwise of mass trapping. While one-time mating keeps the reproduction capacity of the population low, males mating with several partners achieve the opposite (Hagley, 1978).

Several methods are used to evaluate the success or efficacy of mass trapping. They include monitoring decline in populations from trap catches (Faccioli *et al*., 1993; Moraal *et al.,* 1993; Suckling *et al.,* 2007) or damage (Zhang *et al*., 2002; Allou *et al*., 2006; Suckling *et al*., 2007) or sex ratio of the population (Howell, 1980). Zhang *et al.* (2002) evaluated the suppression of male *Cydia trasias* (Meyrick) (Lepidoptera: Olethreuidae) on *Sophora japonica* L trees in Beijing from the mating rate of the female and the decline in damage.

In attempts to enhance the success of mass trapping, the technique has been combined with the application of insecticides but the results have been mixed, ranging from success, partial success and complete failure (Teich *et al.,* 1979; Hagley, 1978; Yamanaka *et al.,* 2001).

1.5.3 Mating disruption

The process of coming together of opposite sexes of insects mediated by sex-pheromones can be derailed by interfering with the normal communication mechanisms of the insects, thus disrupting mating and potentially reducing reproduction. Some programmes may combine mating and conventional insecticide application to suppress populations (Walton *et al*., 2006).

The mechanisms by which mating can be disrupted, however, don't lend themselves to easy understanding and many authors have outlined different views (Bartell, 1982; Cardé and Minks, 1995; Sanders, 1997), but three likely modes of action are:

- False trail following when the insect is presented with more small point sources of analogous synthetic lures than the wild females in the field so that more males are attracted to the lures than to the females. Male *Pectinophora gossypiella* are reported to attempt to mate with pheromone dispensing hollow fibres in the field (Brooks *et al.,* 1979).
- Continuous non-oriented flight by males resulting from inability to discriminate female odour trails from the high levels of synthetic lure. The males may drop dead from loss of energy.

- Inability to perceive female odours because of adaptation by antennal receptors and habituation of the central nervous system to a lure overdose and therefore, makes no flight to mate (Jutsum and Gordon, 1989).

Various dispenser formulations have been developed for pheromone release in mating disruption (reviewed by Howse *et al.,* 1998) but the lure may also be deployed as spray application. The quantity of dispensers or loading varies for individual species (e.g. McBrien *et al.,* 1996; 1997; Kakizaki, 2004). Irrespective of the quantity, however, the dispensers should be slow, stable and long term in releasing pheromones to ensure saturation of fields for longer periods. Conditions in the field, nonetheless, may militate against this because of the acceleration of release rate of pheromones by wind, solar radiation and rain (Bierl *et al.,* 1976; Bierl-Leonhardt *et al.,* 1979), which may adversely affect mating disruption programmes. Also of adverse effect is increased pest density (Cardé and Minks, 1995), hence the suggestion by Walton *et al.* (2006) to include insecticide sprays or any other method(s) to lower initial mealy bug density before the deployment of pheromones, for optimum results. Most mating disruption programmes are carried out on large isolated plots to prevent immigration of mated females and emigration of unmated males to ensure success.

The success of mating disruption is usually assessed by the reductions in both population density and damage, as well as the suppression of subsequent generation. Reduction in trap catches may, however, not be reflective of either population suppression or reduction in damage because of the specificity of pheromone trapping which usually leaves other stages and/or sex in the population (Kovanci *et al.,* 2004; Lykouressis *et al.,* 2005). A combination of methods may therefore be necessary to assess the efficacy of mating disruption.

Following the identification of the sex pheromone of some mirid species, studies aimed at utilizing them for direct control through mating disruption have been reported. Investigations began with experiments by Judd *et al.* (1995) to test the responses of *C. verbasci* to various ratios of its synthetic lure components in orchards. The authors found that the responses were biased towards ratios rich in one component or the other than the natural ratio which was 94:6 butyl butyrate

and (*E*)-crotyl butyrate. Their conclusion, though not definitive, said that individual components appeared to disrupt pheromone communication by sensory imbalance and modified interpretation of blend ratios while disruption by complete blend may involve false trail and camouflage among others. However, McBrien *et al*. (1996) found no disruption by the individual components but demonstrated for the first time mating disruption by the complete blend of *C. verbasci* in orchards. In further studies, McBrien *et al*. (1997) again reported for the first time a reduction of population density of *C. verbasci* in apple orchards in the Okanagan valley of British Columbia. In experiments conducted for two years with between 500 and 1000 polyurethane dispensers per hectare each loaded with 118 mg of synthetic sex pheromone; 16:1 butyl butyrate and (*E*)-crotyl butyrate, there was 71-85% reduction in first generation nymphs of over wintering populations treated in the autumn with 1000 dispensers. The percentage reduction from 500 dispensers was lower. However, the authors did not find the results consistent enough to recommend the commercialisation of the lure in pest management programmes.

The potential of mating disruption has also been demonstrated in *T. caelestialium* in cages and field by Kakizaki (2004). From cage experiments, he showed that a single dispenser loaded with 50 mg of the synthetic pheromone, hexyl hexanoate, (*E*)-2-hexenyl hexanoate and octyl butyrate in 100:40:3 ratio, reduced the mating rate as well as the next population of the insect. In both small and large scale field trials with 9 dispensers loaded with 50 mg applied in 10 m x 10 m and 200 dispensers loaded with 300 mg in 100 m x 100 m respectively, he showed that the complete pheromone suppressed the population densities of the pest more than any of the first two components or a 40:30 mixture of the two (Kakizaki, 2004).

Insects other than Heteroptera, such as Lepidoptera and Coleoptera, have been the target of mating disruption programmes (Cardé and Minks, 1995; Suckling 2000; Sciarappa *et al.,* 2005; Witzgall *et al*., 2010) but more trials are expected in mirids as the sex pheromones of economically important species are progressively identified.

1.6 BACKGROUND TO MIRID PHEROMONE STUDIES IN GHANA

The identification of semiochemicals of insects has provided the impetus for their utilization in monitoring and control of major pests of economic importance, which otherwise may be too difficult or impossible to control. They have also been used as replacement for control by toxic synthetic insecticides to reduce hazards to the environment, and also to offer an affordable alternative. Identification of mirid pheromones, though recent relative to Lepidoptera, has led to their utilization in monitoring pest incidence and determination of threshold (Smith *et al.,* 1991) and in demonstrating mass trapping and mating disruption (McBrien *et al.,* 1997; Kakizaki, 2004; Sarfo *et al.,* 2007). Utilizing the sex pheromones was therefore of priority consideration when it became imperative to develop safe alternatives to chemical control methods in the management of the cocoa mirid pests in Ghana.

Consequently, since 1998, CRIG has in collaboration with the Natural Resources Institute (NRI), UK, CABI Bioscience, UK and its African Research Centre in Nairobi, Kenya, embarked on a study to use the female sex pheromones of cocoa mirids not only to develop a more environmentally acceptable management of the pests but also an affordable one.

1.6.1 Identification of sex pheromones of cocoa mirids

The studies started with the confirmation of earlier observation by King (1973) that *D. theobroma* males were attracted to traps baited with conspecific virgin females and a further similar demonstration with *S. singularis* in 1999 (Padi *et al.,* 2002). In field experiments at CRIG, virgin females of *S. singularis* over a week old were enclosed with cut shoots of cocoa (chupons) in glass tubes closed at both ends with nets, and suspended in sticky delta traps (Figure 1.10).

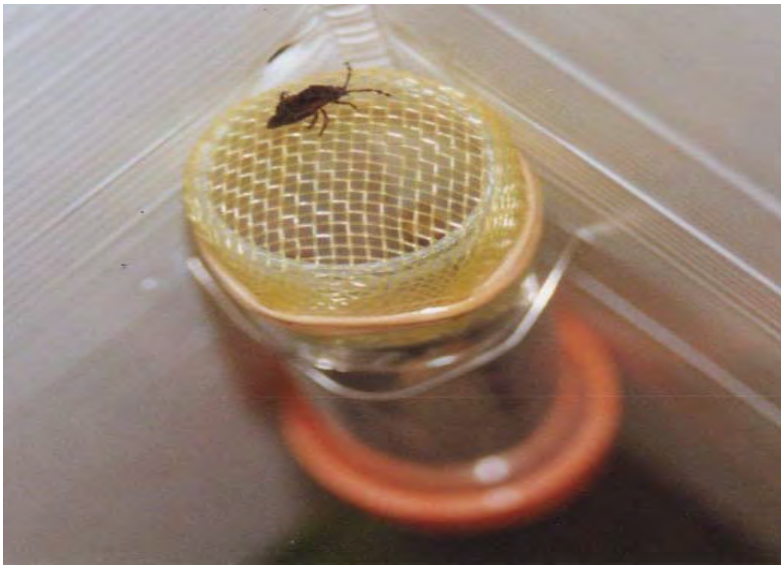

Figure 1.10 Male *Sahlbergella singularis* attracted to virgin female (inside the tube) in a delta trap. (Picture: Courtesy David Hall)

Tested against blank sticky delta traps, the female-baited traps caught only conspecific males with no catches in the control. This demonstrated the involvement of sex pheromones in mediating communication processes between the two sexes, and served as motivation for the work since identification through this route has precedence in mirids (e.g. *C. verbasci*, Thistlewood *et al.,* 1989a; Smith *et al.,* 1991).

In the collection of sex pheromones, virgin female and male *D. theobroma* and *S. singularis* reared from nymphs in the laboratory at CRIG provided material for entrainment and GC-EAD analyses at NRI. Females and males were entrained singly and in groups and volatiles collected. Initial collections showed no obvious consistent differences between the volatiles from the sexes because sexually immature adults had to be used due to early mortality. Therefore, no responses were elicited by these collections and not until mature adults over a week old were used. By consideration of mass spectral and chromatograghic data and after synthesis of about 40 standards, two EAG-active components, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and hexyl (*R*)-3-hydroxybutyrate in the ratio 2:1, were successfully identified and synthesised from *D. theobroma*, although both components were subsequently detected in volatiles from female *S. singularis*

(Downham *et al.,* 2002). The method used in collecting volatiles from whole insects is very useful and usually employed in mirids (see section 1.4.3). But considering the difficulty posed in this case from the similarity of volatiles, the small amounts of pheromones produced and the labour in invoking responses in the electrophysiological bioassays, perhaps focusing on suspected organs of pheromone production (see section 1.4.1) could improve the process.

1.6.2 Responses of mirids to synthetic lures

Results of field bioassays for the biological activity of the synthetic lure conducted in CRIG plots showed attraction of males to pheromone-baited traps to the exclusion of unbaited ones. The attraction of males of both species to the same lure suggested the sharing of common pheromone by the two. However, this argument is undermined by the attraction of only conspecific males by females of both species. Being related species (Entwistle, 1972), their sex pheromones may share common major component but different minor ones as exists in *Phytocoris* spp. already mentioned in the section on Mirid pheromones above. They would therefore, respond albeit weakly, to closely related lure of their pheromone or generic blends (Gronning *et al.,* 2000; Payne, 1971) usually resulting in low trap catches.

1.6.3 Dispensers for synthetic pheromone

The effects of dispensers on the release rates of lures and the influence of release rates on insect trapping have been discussed under the section on Monitoring above; the synthetic lure of the cocoa mirids appear to follow the general trend. Comparison of release rates of the synthetic lure formulated as a slow releasing polyethylene vial and rubber septum, demonstrated the unsuitability of the rubber dispensers for monitoring purposes as they released faster than the vials. Furthermore, both dispensers not only showed the inverse

relationship between aging of the lure and the release rate, but also variation of the two components over time due to differential release (Downham *et al.,* 2002).

1.6.4 Field evaluation of synthetic lures

Field evaluation of the synthetic lure components started in 2001 with the impregnated polythene vial dispensers. A series of validation trials conducted with blends of the *R* enantiomers of diester and monoester in various amounts ranging from 1000:0 through 1000:1000 to 0:1000 µg gave inconclusive results. Thus, consistently only the monoester alone was found unattractive to males. Each of all other blends tested attracted males as much as the other (Figure 1.11). Though the 1000:1000 blend appeared to catch more, this could not be proved statistically. Catches were skewed in few replicates with few traps contributing most of the catches. But the experiment could be improved for more reliable results by increasing the replication and consistently moving and changing traps and lures respectively which were lacking previously. However, the exclusive attraction of males to the baited traps indicated the potential use of the lure in pest management which needs investigating.

Figure 1.11 Males of both *Sahlbergella singularis* and *Distantiella theobroma* caught in a delta trap

1.6.5 Effect of lure dosage on mirid attraction

Increasing the dose by multiplying the lure did not increase catches. This was demonstrated with the two dispensers, polyethylene vial and rubber septum, in field trapping experiments (Padi *et al.,* 2002). Three traps were baited with vials containing the 1000:500, 1000:50 or 1000:0 blends. Another set of three traps were baited with rubber septa dispensers containing ten times the loading; 10000:5000, 10000:500 or 10000:0 to give high release rate of pheromone. The experiment was replicated 4-fold. Unlike the former, the latter set attracted insignificant number of male mirids. The results were not surprising because similar observations have been reported by Kakizaki and Sugie (2001) on the synthetic lure of *T. caelestialium.* However, they may be less reliable because the duration for the experiment was too short and the populations of mirids also were low at the time of the trial. However, the results are very important, because by attracting no males while the 1 mg loading did attract, showed that male mirids were present in the area, and that the inability to attract suggested an interference of odour communication processes among males by the unnatural multiple doses. This inadvertently provides evidence for possible manipulation of the lure to cause disruption in the mating process of the insects for the purpose of control.

1.6.6 Effect of lure loading on mirid attraction

Reducing the lure loading to quantities less than 1 mg, also reduced the attractiveness of the lures. In field experiments in Ghana carried over six months with sticky traps baited with the 1000:500 blend, lure loadings were reduced from 1mg to 0.1mg, 0.01mg and a release from a capillary tube. Out of a total of 111 male mirids captured, data analysis showed that mean trap catches dropped from 16.6 to 4.0, 0.2 and 1.4, respectively (Padi *et al.,* 2002). The capillary loading was unknown but the results may support the observation by Millar *et al.* (1997) that fast releasing rubber septum is unsuitable as a dispenser for mirid trapping. However, what this experiment and the one on increased lure loadings failed to show was the response of the male mirids to graduated increase of loading from 1mg.

1.6.7 Effect of aging on lure attractiveness to mirids

In a related experiment (Padi *et al.,* 2002), the effect of aging of lure on male attraction was investigated at CRIG in which male attraction to a set of lures left in traps continuously for three months was compared to attraction to a set of lures changed at biweekly intervals. The results which were similar to the observation by Millar *et al.* (1997) on the synthetic lure of *P. relativus*, showed decreased lure attractiveness with age. The results demonstrated the effects of load reduction and hence consistent with those above. But because of low population at the start of the experiment, the critical age at which the lures became significantly less attractive was not determined but arbitrarily set at 4 weeks. Yet for optimum field trapping, attractiveness of lure is paramount and should be consistent, thus the critical age of loss ought to be known.

1.6.8 Traps for cocoa mirids

In experiments to determine the suitability of traps for mirid pheromone trapping in cocoa, three traps were tested at CRIG. They consisted of two types of sticky traps; the delta trap and the 'New Rectangular Trap' (NRT) especially designed by NRI and CRIG, and a funnel trap. Sticky traps effectively collected mirids as against precipitation by the funnel traps (Padi and Sarfo, 2002). Further testing of various designs of the delta and NRT in white and green colours consistently showed the superiority of white NRT over all others tested. However, the range of types and designs was not wide enough thus excluding other potentially suitable traps and designs. The cost of the traps also might be reduced by finding local substitutes for the traps.

Trap placement is very important in pheromone trapping since it can influence trap efficiency (Boucher *et al.,* 2001; Edde *et al.,* 2005). The optimum trap height for capture of male mirids determined in the field at CRIG with NRT, using virgin females as lure, was 1.8 m in the canopy (Sarfo *et al.,* 2007). But it would be necessary to confirm this with the synthetic lure since there could be differences between them as reported by Yonce *et al.* (1976) in the lesser peachtree borer.

1.6.9 Potential for mass trapping of mirids

The underlying mechanisms may not be easily understood but density of traps affects their efficiency (McNally and Barnes, 1981). Field experiments at CRIG using 1 mg loading of 1000:50 blend in NRT deployed in densities equivalent to 50, 100 and 150 per hectare showed the lowest mean trap catches at the highest density. The results were interpreted to mean a competition between the pheromone traps for the available male mirids which became more intense as the density increased and the distances between the traps shortened (Sarfo *et al.,* 2007). There are other possibilities though. For example, the results could be interpreted to indicate interference from pheromone plumes of neighbouring traps in close proximity to each other or a significant trapping out effect at the highest density. These possibilities can only be tested on larger experimental plots.

1.7 AIMS, HYPOTHESIS AND OBJECTIVES

Results of the evaluation of the synthetic lure and pheromone traps in field studies in Ghana in the earlier CRIG/NRI/CABI/DFID mirid project were not conclusive enough for use in the development of reliable standard methods of control, due mostly to the high variability in trap catches. However, the results suggest the potential use of mirid pheromone in improving the management of the pest. The present study was, therefore, aimed at validating the results and utilizing them for the control of the cocoa mirid pests, with the hypothesis that pheromone trapping can be used to manage mirids. Thus, the objectives of the study are:

- To optimize the trapping parameters i.e. pheromone blends, lure longevity, trap design and placement for the two species of cocoa mirids in Ghana.
- To test whether control of mirids is possible by the technique of mass trapping with pheromone traps.
- To determine the potential of pheromone trapping for monitoring mirid numbers and damage.

Chapter 2

IMPROVEMENT OF SYNTHETIC PHEROMONE BLEND

2.1 INTRODUCTION

The sex pheromone components of cocoa mirids were identified and synthesized at NRI from volatiles entrained from the female *D. theobroma* during the CRIG/NRI/CABI project on mirid pheromones. The pheromone consists of two components: a diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate (I) and the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate (II), in the ratio 2:1 (Downham *et al.,* 2002). The pheromone is attractive to males of both *D. theobroma* and *S. singularis* in the field.

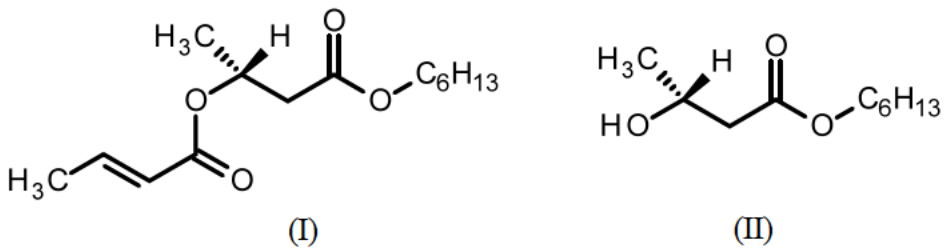

Figure 2.1 Structures of components of the female sex pheromones of *D. theobroma* and *S. singularis*, diester hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate (I) and the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate (II).

Following the identification and synthesis, a series of validation trials conducted with the pheromone blends of the *R* enantiomers of the diester and monoester compounds could not confirm any of the blends as the best statistically, although the 1000:50 diester : monoester blend caught the most male mirids (Padi *et al.,* 2002). The experiments involved only *S. singularis* due to the unavailability of *D. theobroma* and also the populations of mirids available were very low. A further field trial by Sarfo and Ackonor (2007a) also failed to determine a single most attractive lure as the 1000:1000 and 1000:500 blends gave non-significant highest catches out of the lures tested.

In initial field trials testing the response of mirids to the synthetic pheromones in the CRIG/NRI/CABI mirid pheromone project, lures were left in the field for indeterminate periods. Later on, trials conducted on the longevity of the lures established loss of lure attractiveness with age. The critical point when the lure ceases to give optimal catches was not determined from the trial but a suggestion was made to keep lures for one month in traps before changing them.

The pheromone blend, as a matter of priority ought to be refined to ensure optimal reception by the mirids. The amount that should be dispensed and the effective-life before replacement must all be determined, to enhance the development of consistent and efficacious pheromone trapping method. The objectives of these studies therefore, were to evaluate the effects of pheromone blends and lure age on the capture of male *D. theobroma* and *S. singularis,* to determine the blend with optimal catches and the length of time such blend can be used in trapping. The optimal blend will be selected for the development of pheromone trapping method for the cocoa mirids.

In order to achieve the objectives, two experiments were conducted at Akwadum in Ghana on farmers' farms. The first experiment was used to test the attractiveness of a range of synthetic pheromone blends of the cocoa mirids and the second evaluated the attractiveness of the selected lure as it aged.

2.2 MATERIALS AND METHODS

2.2.1 Study sites and experimental plots

The studies were conducted in cocoa farms in one location in Ghana. Plots were selected from individual farmers' farms which had mirid infestation. There was no application of inorganic chemical insecticides and the plots were released for long experiments.

Optimisation of synthetic pheromone blends

The study was carried out on experimental plot selected at the Akwadum- Brong-Densuso area (06º 05' North, 0º 21' West). The area lies in the semi-equatorial forest zone in the Suhum Kraboa Coaltar district of the Eastern region of Ghana. The area experiences two rainy seasons with an annual rainfall of between 127 cm and 165 cm. Temperatures and relative humidity range between 24ºC to 29ºC and 50% to 90% respectively. The soils are generally loamy and suitable for the cultivation of cocoa. The experimental plot measured about 5 ha and was demarcated out of about 200 ha of contiguous cocoa. It cuts across farms whose owners practised organic cocoa production. Though there was no application of synthetic insecticides, the farmers applied crude neem extract, a botanical insecticide, for the control of mirids which was ineffective. The cocoa trees were over 30 years old. They consisted of irregularly planted mixed hybrids and were very tall, averaging 13 m high into the canopy. The plot was overpopulated by shade trees, far in excess of the 6 per ha recommendation by CRIG.

Determination of effective age of lure for attraction

The study site was at Akwadum and has been described above. The experiment was conducted on a farmer's farm. The farm was used to cultivate organic cocoa. The size of the farm was about 3 ha. and the age was about 10 years old. The cocoa trees were between 3.5 m and 6.5 m high and irregularly planted under shade trees. The crop consisted of mixed hybrid and the canopy was mostly closed with few open areas. With the exception of one side which was bordered by a village, the farm was bordered on all other sides by land used for the cultivation of food crops such as cassava, plantain and cocoyam. Good agronomic practices such as the clearing of weeds, removal of basal chupons and pruning were partially carried out.

2.2.2 Field experiments

Synthetic pheromone and traps

The synthetic pheromone used consisted of two components: a monoester, hexyl (*R*)-3-hydroxybutyrate and a corresponding diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate, synthesized at NRI. Dispensers were polyethylene vials (20 x 8 x 1.5 mm thick; Just Plastics, London, UK).

In the blend optimisation experiment, New Rectangular Traps (NRT) were used. These were made from white corrugated plastic sheets ('Correx'; Sign Trade Supplies, Maidstone, UK), cut and folded into open-ended boxes (38 cm long, 10 cm wide and 14 cm high) whose axis is horizontal in normal use. A smaller second sheet (38 cm long, 9.6 cm wide and 12 cm high) was folded and inserted into the trap as liner. The liner was coated with polybutene sticker (Agralan, Ashton Keynes, Wilts. UK) on all the three inner surfaces such that it formed a sticky retentive surface for capturing mirids. The lure was suspended in the middle of the trap on aluminium wire.

In the lure-ageing experiment, plastic water bottle traps were used, made from 4.5 litre plastic water bottles (26 cm high and 16 cm in diameter). Two opposite windows (7.0 cm x 20 cm) were cut out of the bottle. In use, the trap was hung bottom up with the lure suspended above water, the retention medium.

Optimisation of synthetic pheromone blend

A range of five synthetic pheromone blends made up of diester : monoester amounts 1000:0, 1000:50, 1000:500, 1000:1000 and 0:1000 µg respectively, plus a blank control, was tested in a randomised complete block design (RCBD) experiment replicated 8-fold from March 2007 to May 2008. Baited traps were suspended about 1.8 m above the ground in cocoa trees in a line 20 m apart in plots measuring 20 m x 150 m. The plots were separated by 70 m intervals. Measuring tape was used to do the measurements to the nearest cocoa tree. The positions of treatments within blocks were rotated (moved on one position within the line) every two weeks such that every treatment occupied every plot for a

fortnight in cognizance of the patchy distribution of mirids. Catches were recorded three times a week and removed and lures were replaced at monthly intervals.

Determination of effective age of lure for male attraction

Lures were aged at the insectary at CRIG as follows. Two hundred freshly prepared samples of the polyethylene vial lures were put in a New Rectangular Trap without the sticky insert and suspended in cocoa tree to age. After two weeks, a batch of 50 lures (i.e. aged two weeks) was put in a freezer, followed by another batch of 50 after two weeks (i.e. aged four weeks). This procedure was repeated for lure batches aged 8 and 12 weeks. From November 2008 to March 2009 all the four differently aged lure batches were tested together with another batch of freshly prepared lures (i.e. not aged) in a randomised complete block design (RCBD) experiment in eight replicates Traps were suspended 1.8 m above ground in trees and separated about 15 m from each other in blocks. Blocks were 70 m apart measured with a measuring tape. Each trap was inspected twice a week during which mirid catches were recorded and removed and the trap also moved forward to replace the one in front so as to reduce possible positional effects. Lures were replaced monthly.

2.2.3 Analysis of data

The data were analysed using Genstat package (9^{th} Edition). Total trap catch data per treatment per replicate were transformed to $\sqrt{(x+ 0.5)}$ to normalise the data. The raw and transformed data were subjected to analysis of variance by using the factors of replicate block and treatment. Where ANOVA indicated significant differences ($P<0.05$), differences between means were tested for significance by a Least Significant Difference (LSD) test.

2.3 RESULTS

2.3.1 Optimisation of synthetic pheromone blends

A total of 701 male mirids, mainly *S. singularis* was caught in traps in the experiment on pheromone blends from March 2007 to May 2008. Out of the number, 669 were *S. singularis* and two were *D. theobroma*. The 1000:500 blend recorded the highest number of 30 *S. singularis* in one inspection in January 2008. Catches were generally low from March 2007 but there was a highest peak from a rapid increase from December 2007 to January and February 2008 (Figure 2.2). No obvious effects on catches were observed with the monthly changes of the lures.

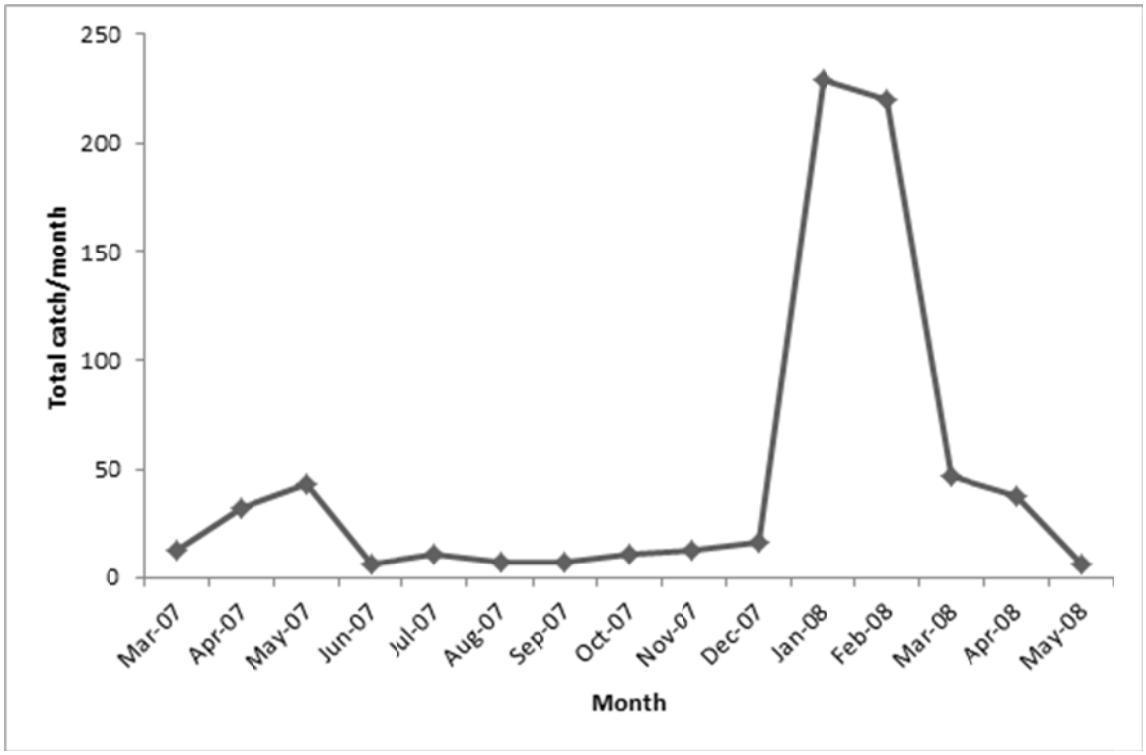

Figure 2.2 Total monthly catches of male *Sahlbergella singularis* in pheromone blend experiment (40 traps) at Akwadum; March 2007- May 2008.

Data on male *S. singularis* only were analysed because too few of *D. theobroma* were caught. Analysis was done on data for the whole period of the experiment

i.e. March 2007 to May 2008 and also from January to February 2008 when consistent and high catches (64%) were made. Figure 2.3 shows mean trap catches of the untransformed data from March 2007 to May 2008 in the different treatments of the blend.

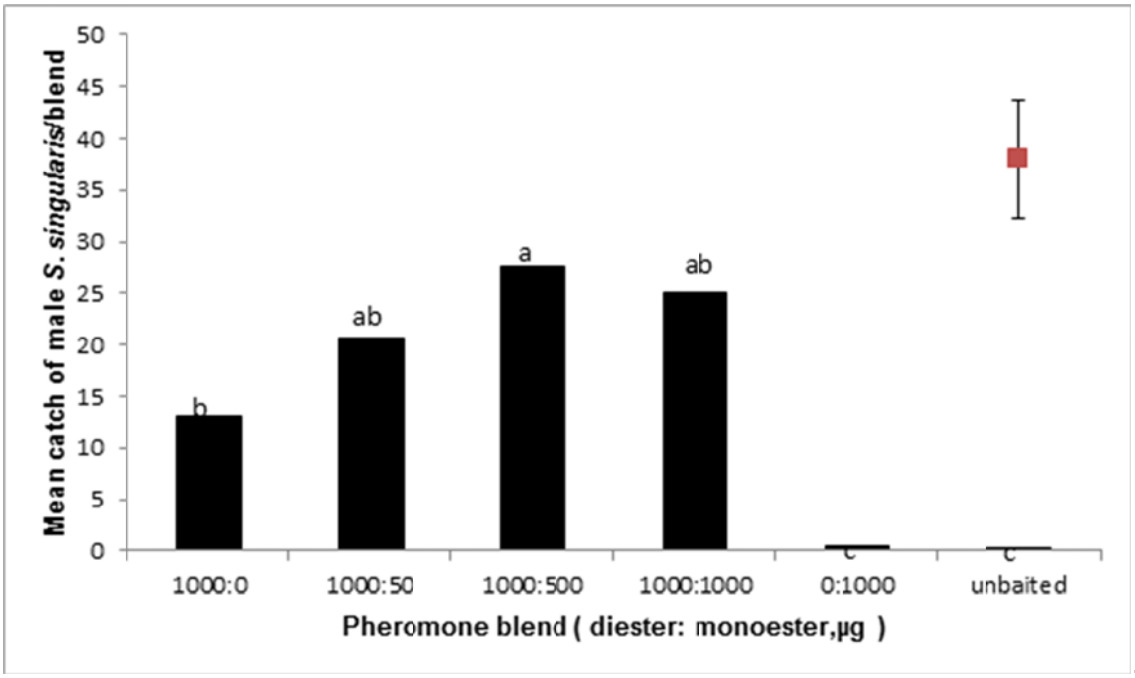

Figure 2.3 Untransformed mean catches of male *Sahlbergella singularis* per treatment in NRT traps baited with different blends of diester: monoester pheromone components at Akwadum (March 2007-May 2008; 8 replicates). Bars with different letters are significantly different (P<0.05) by LSD test after transformation of data to √(x+0.05) and analysis of variance. Error bar shows standard error of difference between means.

Catches were high in traps baited with the diester and binary blends but extremely low in the monoester and the unbaited. From ANOVA no single lure was the most attractive. Among all the treatments the binary blend 1000:500 had the numerically highest mean catch which was significantly greater than the diester alone (F=11.76, df 5, 35, P<0.001). It was however, not significantly different from the other binary blends 1000:50, and 1000:1000 (P>0.05). Catches by 1000:50 and 1000:1000 were numerically higher but not significantly different (P>0.05) from the diester alone. Catches in the binary blends and the diester alone were,

however, all significantly greater than the monoester and unbaited with the last two not significantly different (P>0.05). Thus while the monoester was not attractive to male mirids, there was attraction by the diester alone comparable to 1000:50 and 1000:1000 but less than 1000:500. From the results therefore, the major component attracted mirids but mirid attraction was maximised with the two components together in the proportion 1000:500. Analysis of total catches from January to February 2008 showed the same results.

2.3.2 Determination of effective age of lure for male mirid attraction.

A total of 259 male mirids was caught in traps with different aged lures from November 2008 to March 2009 out of which only one was *D. theobroma*. The rest were all *S. singularis*. Total monthly catches of *S. singularis* are plotted in Figure 2.4.

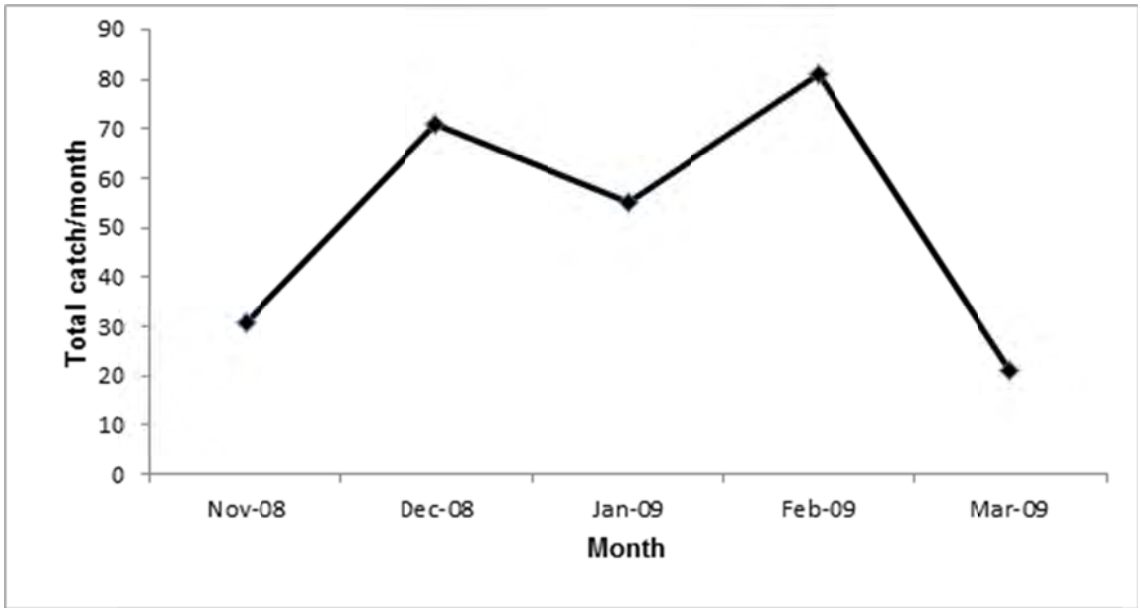

Figure 2.4 Total monthly catches of *S. singularis* males in water bottle traps baited with differently aged mirid female synthetic pheromones at Akwadum (November 2008-March 2009; 5 treatments, 8 replicates).

The highest number recorded in one day was 6 by a fresh lure and also by a 4-week old lure, all in December 2008. Consistently, high numbers were caught throughout the period especially from December 2008 to February 2009 just as in the blend experiment above in the year before. Trap catches by the different age lures are summarised in Figure 2.5.

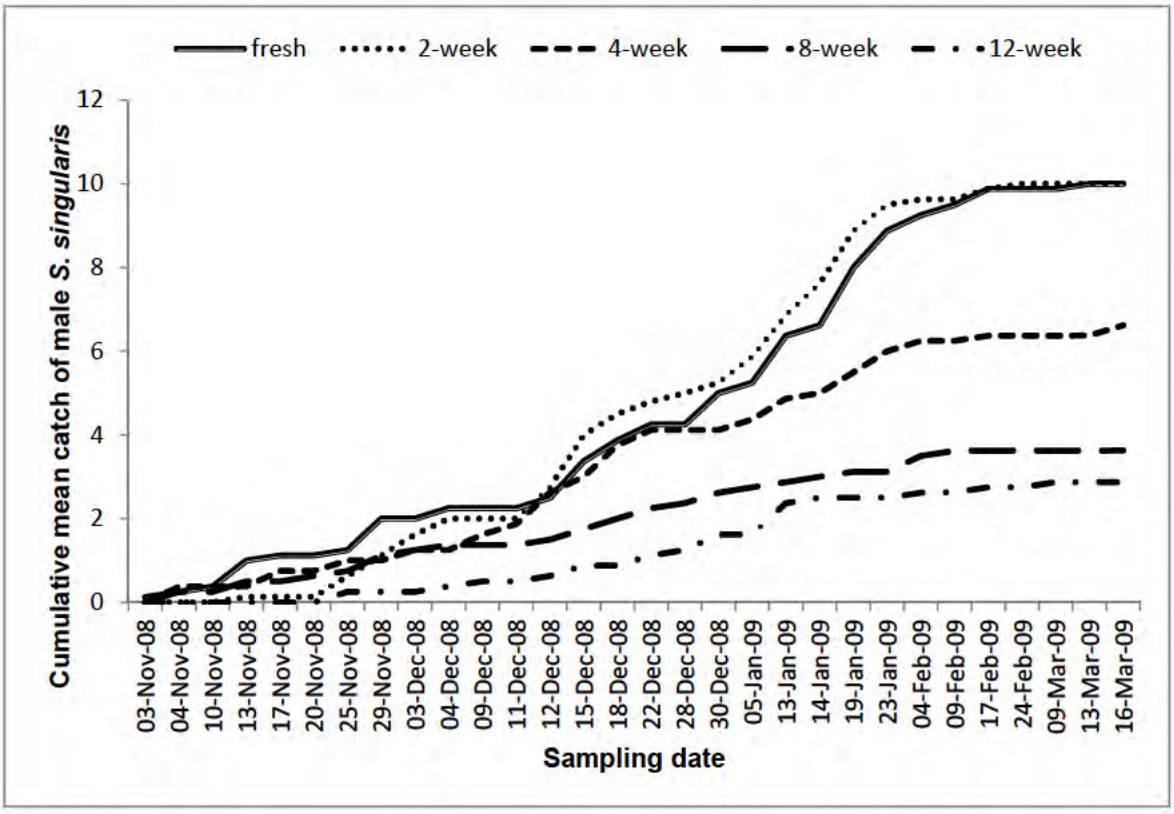

Figure 2.5 Cumulative mean catch of male *S. singularis* collected once or twice weekly from pheromone traps baited with differently aged pheromone lures replaced monthly at Akwadum (November 2008 – March 2009; 8 replicates; fresh = fresh non-aged lure, 2-week = lure aged two weeks, 4-week = lure aged four weeks, 8-week = lure aged eight weeks, 12-week = lure aged 12 weeks).

All the lures attracted male *S. singularis* from the start of the experiment and catches increased progressively and distinctly from the oldest to the less aged lures as numbers accumulated. Catches by the fresh and 2-week old lures were similarly the highest throughout the period of the experiment. Catches by 4-week

old lure were similar to fresh and 2-week lures until after 28 December, 2008 (two months) and lagged behind from thereon as catches accumulated.

Due to the insignificant number of *D. theobroma* recorded, data on male *S. singularis* only were analysed for the whole period of the experiment (November 2008 to March 2009). Figure 2.6 shows mean catches of the raw data from November 2008 to March 2009 in the treatments.

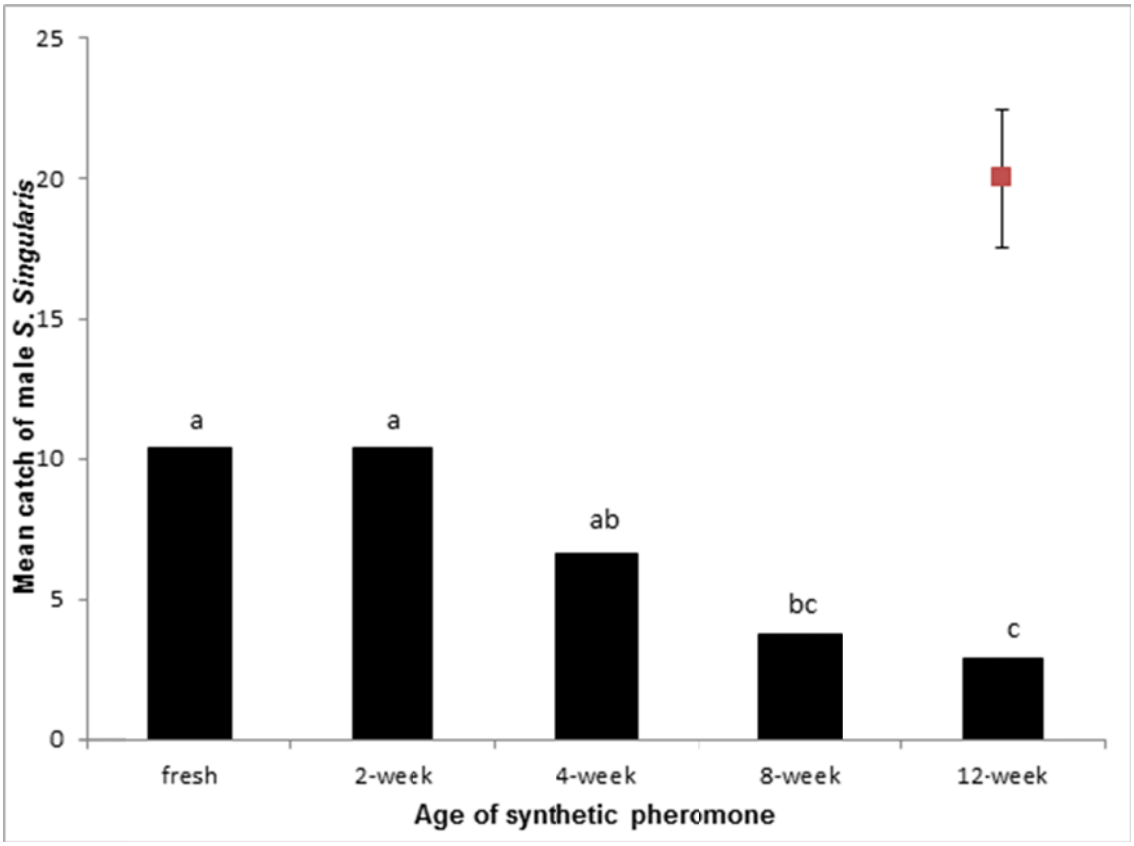

Figure 2.6 Mean catches of male *S. singularis* per treatment in water bottle traps baited with differently aged pheromone lures at Akwadum (November 2008-March 2009; 8 replicates; means with different letters are significantly different (P<0.05) by LSD test after transformation of data to $\sqrt{(x+0.05)}$ and analysis of variance; fresh = fresh non-aged lure, 2-week = lure aged two weeks, 4-week = lure aged four weeks, 8-week = lure aged eight weeks, 12-week = lure aged 12 weeks; error bar shows standard error of difference between means.).

Results of analysis of variance on untransformed data and data transformed to √(x+0.05) did not differ so results of untransformed data are presented for ease of interpretation. Generally, catches declined consistently from the highest in fresh and 2-week old lures to the lowest in 12-week old lure (Figure 2.6). ANOVA showed that mean catches in fresh lures, 2-week old lures and 4-week old lures were not significantly different (P>0.05); mean catches in 4-week and 8-week old lures also were not significantly different (P>0.05), but mean catches in the fresh, 2-week or 4-week old lures were significantly greater than mean catch in 12-week old lure (F=6.08*, df 4*,28*, P*=0.001). From the results, optimal attraction occurred when the pheromone was fresh to 4 weeks old and loss of attractiveness occurred after 8 weeks. However, the attraction of highest numbers by both fresh and 2-week old lures throughout the experiment (Figure 2.6) showed that the fresh lure can attract optimally for at least 6-weeks (each batch was tested for 4 weeks). This age falls between the 4-weeks and 8-weeks ages which could not be shown by this experiment. The results showed that loss of attraction was greater after 12 weeks. However, the similarity in catches by 12-week old and 8-week old lures suggested that the synthetic pheromone of the mirid remained attractive much longer than 12 weeks, at least 16 weeks in the field which was not investigated by this experiment. No mirids are generally caught in unbaited traps.

The relationship between treatment and months was not significant (P>0.05) and therefore attractiveness in the treatments was consistent and not affected by months. Similar results were obtained with ANOVA on the raw data.

2.4 DISCUSSION

Trap catches of mirids

The field trapping showed attraction to all blends by male mirids. This demonstrated the reported mediation of behavioural responses of adult males by volatile sex pheromones of the female mirid (Downham *et al.,* 2002). The negligible number of male *D. theobroma* caught is unusual against the backdrop that the lure was synthesized from the sex pheromones of its female. This can be

attributed partly to the reducing numbers of the species in Ghana (Owusu Manu, 1996) and particularly in the area as reported by Padi and Acheampong (2003) contrary to the reported high numbers of *D. theobroma* in the area by Ayenor (2007). The latter report could possibly have arisen from incorrect taxonomic identification due to lack of expertise by untrained farmers.

Attractiveness of blends

The blend optimization experiment was to enable the optimal blend to be selected for use as lure in pheromone trapping. The results showed that all the binary blends tested attracted mirids equally. However, by catching the highest number of mirids, the 1000:500 blend was preferred for future pheromone trapping. Preliminary results in the present experiment showed the 1000:1000 blend as the best performing one. This initially influenced the choice of that blend for field trapping trial elsewhere, but as catches accumulated the 1000:500 blend attracted more male mirids though not higher statistically.

The first test of the efficacy of the mirid synthetic pheromone was carried out at Tafo as part of the project by CRIG/CABI/NRI to develop pheromones for the cocoa mirids. In that experiment, NRT traps were baited with polyethylene vials impregnated with 1mg of diester to monoester (µg) in the ratios of 1000:500, 1000:50 and 1000:0 and tested for about two months but only a single male *S. singularis* was captured by synthetic pheromones despite catches by live virgin female-baited traps (Padi *et al.,* 2002). According to the report by Padi *et al.* (2002), a repetition of the experiment showed 1000:50 to have the highest mean attraction per trap though the catches were dominated by a single trap, and also the data were not analysed. Further testing for a period of one month also yielded low catches by synthetic pheromone traps so the data were not analysed. However, 1000:50 was numerically higher than 1000:500 and 1000:0 that were tested. This experiment was continued for another month and catches improved. The 1000:50 blend remained the highest but not significantly ($P>0.05$) different from 1000:500 which was also higher than 1000:0 but not significantly ($P>0.05$) so. By comparison, the present blend experiment was larger and spanned over a longer period to accumulate sufficient catches for analyses. The results of the

present experiment showing that 1000:500 and 1000:1000 were the highest attracting blends, therefore, contrast the predominance of 1000:50 shown in the previous experiments and in part confirm the high attraction of the 1000:500 blend above. The results also confirm the efficacy of the two binary blends, 1000:500 and 1000:1000 in attracting numerically higher mirids as observed in the experiment by Sarfo and Ackonor (2007a).

Differential release rates of the two components of the pheromone

The differential release rate of the pheromone components may help explain the attraction by the lures. In all the experiments, catches by the minor component alone have consistently been negligible whenever tested while the major component has attracted mirids on its own albeit not optimally. This could be attributed to the higher release rate of the monoester as shown in experiments by Padi *et al.* (2002). They (Padi *et al.* (2002) exposed the synthetic pheromones by removing the tops of the polyethylene vials containing them and measured the residual components in a laboratory wind tunnel at 27°C and 8 km/hr wind speed. They found that the diester had a half-life of 14.2 days as against 3.9 days for the monoester. In another experiment by Padi *et al.* (2002) in which the tops of the polyethylene vials were kept intact, the release rate of monoester was at least eight times faster than the diester. The monoester therefore, releases faster and so not much is available to attract mirids and for long. Therefore, the equal catches by the binary blends and sometimes also the diester alone, might be because for most of part of the experiment all the binary blends are left mainly with the diester only and so attract mirids equally. It was expected that the 1000:500 blend being in the same ratio of 2:1 as the diester to monoester components entrained from the female *D. theobroma*, would mimic the natural pheromone better than the rest and give significantly higher catches. This was the case with the natural blend of the pheromone components of *Prostephanus truncatus* (Horn) (Hodges *et al*., 2004). However, because of the fast release rate of the monoester, this ratio may not be sustained in the field. Therefore, the release rate of the lure should be improved further to be dispensed slower than the present one. Increasing the lure loading is not an option because from the

results of field experiment by Padi *et al.* (2002), increasing lure loading did not elicit any response from the mirids.

Highest attraction by binary blends followed by diester alone and negligible attraction by the monoester, observed in the present and previous field trials in Ghana cited above, have also been reported in field trials of the mirid pheromone blends in Cameroun by Mahob *et al.* (2011). Attraction by one key component of multiple component pheromones shown in this study is, however, by no means universal in the mirids. This is because Zhang and Aldrich (2008) reported that none of the two key components of pheromone by *Phytocoris calli* Knight (i.e. hexyl acetate and (*E*)-2-octenyl acetate) was attractive on its own, except in a mixture.

Maximisation of attraction by binary blends

The results showing that highest catches were obtained by blends composed of a mixture of diester and monoester are suggestive of positive interaction between the components that enhances attractiveness of the pheromones. Synergism between components of pheromones in mirids has been widely reported. For example enhancement of major component by minor one was reported by Kakizaki and Sugie (2001) in the rice leaf bug *T. caelestialium*. McBrien *et al.* (2002) also reported the attraction of female red-shouldered stink bug, *Thyanta pallidovirens* (Stål), by mixture of the sex pheromones of the male consisting of esters and sesquiterpenes. Lowor *et al.* (2009) reported catches of the green mirid, *C. dilutes,* by all five different ratios of hexyl hexanoate to (*E*)-2 hexenyl hexanoate, major and minor components respectively of the sex pheromone, in the field, but they did not show whether either component on its own was attractive. Zhang and Aldrich (2008) reported that the sex pheromones of the female plant bug, *P. calli* optimally attracted males with the full four components though the absence of one component did not decrease the attraction. The importance of all components of synthetic sex pheromones for attraction of mates has also been reported in the Lepidoptera (Yang *et al.,* 2008).

Lure ageing

The ageing experiment was meant to determine how long the lure could be in traps in the field and still be effective in attracting mirids. From the results, attraction was optimal up to 4 weeks but mirid pheromone remained attractive for at least 12 weeks with attraction reducing with age. However, the suggestion by the results that the optimal time of attraction by synthetic pheromone could be at least 6 weeks and the lure could also stay attractive for at least 16 weeks, show that the interval between 4-week and 8-week lures was too long and the terminal age of 12-weeks was also too short in the present experiment. This suggestion is from the observation that the 2-week and the fresh lures attracted mirids optimally at all times (Fig. 2.4), including when the 2-week old lure was aged 6-weeks old at the end of the 4-week interval of changing the lures. Similarly, by the equal catches of the 8-week and 12-week old lures, the latter was attractive albeit reduced at 16 weeks. Although an unbaited trap was not included in this experiment, in all other experiments no mirids were caught in unbaited traps.

Longevity of lure

The long attractiveness of the lure is probably due to the slow release of the major component of the blend as reported by Padi *et al.* (2002) from the analysis of the diester and monoester residual components of the mirid synthetic pheromone. The long stay of the synthetic pheromone makes it suitable for use in monitoring to detect immigrant species into crops as traps can be left in the field for at least three months without changing the lure. Ayenor (2007) reported attraction by mirid synthetic pheromone for at least three months in a mass trapping experiment, but the present results show that such long trapping periods for the purposes of reducing mirid numbers would be less efficient. Differences between the differently aged lures became significant ($P<0.05$) late in the experiment after catches had accumulated to 224, due to low catches, probably as a result of low populations of mirids.

Attraction and age of lure

The greater attractiveness to less aged pheromones shown by the results concurs with the results of previous aging experiments on the synthetic lure of the cocoa mirids, when pheromone traps baited with fresh lures were changed fortnightly and others not changed for six months (Sarfo and Ackonor, 2007b). The previous results did not determine the optimal attraction of lures but suggested the change of lures every 4 weeks which is supported definitively by the present results. The amount of residual pheromone in polyethylene vials was not estimated in the present experiment neither was it done in the previous one, but according to the report by Padi *et al.* (2002), reduction in attractiveness of mirids also occurred with reduced lure loading. As the loading of pheromone reduced with age the reduced attractiveness observed in this experiment can be attributed to the progressive reduction in loading as the lures aged.

Reduction of attractiveness of pheromone lure with age has been reported in the mirid bugs, *P. relativus*, (Millar *et al.,* 1997) and *P. californicus* (Millar and Rice, 1998) in which attractiveness of males to septa impregnated with the female sex pheromones decreased quickly with age due to volatility of the pheromone components. It has also been demonstrated in other Hemiptera such as the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) by Francis *et al*. (2007), that fewer males were caught in traps as the lure aged. Age associated decline in the attractiveness of pheromone lures has been widely observed in the Lepidoptera (Showler *et al.,* 2005; Leonhardt *et al.,* 1990 and Lopez, 1988).

Monthly catches of mirids

The trend of monthly catches in the blend experiment show that responses of male mirids by way of flight into pheromone traps were at their peak between January and February 2008 at Akwadum. This coincides with reported peak in population at the area (Padi and Acheampong, 2003) though at least a month earlier than the peak in March reported by Ayenor (2007). However, this population peak is different from what pertains in most cocoa growing areas in Ghana and which forms the basis for the nation-wide mirid control regime of monthly foliar application of chemical insecticides, from August to December

omitting November. The implication is that applying insecticides at Akwadum at the recommended period will not control the mirids because they will be missed. The results therefore suggest that the time to control mirids in Ghana should not be universally set, but be determined for specific localities or areas based on monitored population dynamics of the insects. Monthly catches by pheromone traps in the ageing experiment did not show any particular pattern probably because of the short time for the experiment.

Chapter 3
IMPROVEMENT OF PHEROMONE TRAP DESIGN AND POSITIONING

3.1 INTRODUCTION

In Chapter 2 the most attractive blend of pheromone components was selected for sustainable trapping for control of mirids. Other important factors that need considering in pheromone trapping are the design and placement of the trap because these factors affect catches (McBrien *et al.,* 1996; Athanassiou *et al.*, 2004). At CRIG, trials to optimise pheromone traps have been limited to the sticky New Rectangular Trap (NRT) and delta traps. Comparison of full sizes and half sizes of sticky green and white NRT and delta traps on CRIG experimental plots showed the white NRT as the trap with the highest captures of male mirids although no statistical analysis was possible on the data because of low catches (Padi, *et al.,* 2002). Further comparison of catches by the two designs on farmers' farms in Ghana confirmed the superiority of the white NRT over the Delta trap (Sarfo and Ackonor, 2008). Mahob *et al.* (2011) also confirmed the superiority of white NRT over the white delta in the capture of mirids in Cameroun.

The white NRT has therefore been the standard trap used in trapping experiments both in Ghana and Cameroun. Few traps were required for these experiments so there was little or no problem importing the Correx, the main material used to fabricate NRT in Ghana, where it is not available on the market. Research therefore, failed to address the issues of availability and affordability of pheromone traps, but these need to be considered in order to provide adequate traps for large scale captures in control methods such as mass trapping. Furthermore, only one type of trap, sticky trap, was tested. No water trap was tested but water traps provide viable alternatives for mirid captures (Yasuda and Higuchi, 2012). Therefore, it became imperative to obtain substitute trap(s) designed from local materials, at least as efficient as the NRT, readily available

and accessible for use on large scale, as an important contribution to the development of sustainable control of mirids through pheromone trapping.

Trap placement also affect captures by pheromone baited traps (Ishimoto, 2006; McBrien*, et al.,* 1996; Bhardwajt and Chander, 1992). The height of placement of traps used in mirid pheromone trapping experiments in Ghana (1.8 m) was adopted from Sarfo *et al.* (2007). They used virgin female of *S. singularis* as lure and found trap catch of males at 2.7 m to be 3.5 times more than that at 1.8 m, and more than twice as many trapped at 1.8 m than at 0.6 m. However, trap heights derived using virgin females as bait may not be optimal for traps using a synthetic lure, as observed by Yonce *et al*. (1976) in the case of the lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson) (Lepidoptera: Sessiidae).

The objectives of the studies were therefore, firstly to determine the optimal trap designed from local materials and baited with synthetic mirid pheromone, and secondly, to determine the optimal height placement for traps. To achieve these objectives, five experiments were performed at Acherensua, Suhyen, Akwadum and Afosu. The first experiment was used to test the attractiveness of male mirids to local water and sticky traps and the standard NRT. The next experiment was aimed at improving the trap design further. It was used to determine the effect that using the outside surface of a trap for additional trapping had on trap catches. This experiment was conducted in selected mirid pockets to limit plot-to-plot variation due to the patchy distribution of mirids (Bisseleua *et al.,* 2011; Gibbs *et al.,* 1968). The last three experiments evaluated catches by traps placed at different vertical heights. Two experimental designs were used to determine the more efficient one; one design had traps on single cocoa tree or single pole while the other had the traps on different cocoa trees. Results of the first of these experiments, which was carried out at Acherensua with traps on single cocoa trees, suggested that mirids oriented towards cocoa canopy in flight, but the heights did not go high enough to make any conclusions. Subsequently in the two experiments that were conducted afterwards, trapping was extended into the canopy to determine its effects on trap catches. One was conducted at Suhyen

where traps were strung along single poles and the other at Akwadum where the traps were placed on different cocoa trees.

3.2 MATERIALS AND METHODS

3.2.1 Study sites and experimental plots

The studies were conducted on cocoa farms at various locations in Ghana. Plots were selected from CRIG research plantations and also from individual farmers' farms. Among the criteria used in selecting the plots were the presence of mirids, non-application of chemical insecticides, non-interference with the experiments and the willingness of farmers to release parts or whole farms for experiments for long periods.

Effect of trap designs on trap catches

The study was used to test attraction of mirids to different trap designs. The site was at Akwadum, the same as in section 2.2.1 above. The experimental plot was from a different part of the same 200 ha organic cocoa plantation. It was about 7 ha and more weedy than the plot above (section 2.2.1). It also had open canopy in several areas.

Effects of captures by outside surface of trap on total trap catches

The experiment to determine the effects of additional trapping surface on trap catches was carried out on CRIG plantation farm at CRIG substation at Afosu. The substation is located in the semi-equatorial climatic zone (06° 23'N, 01º 00'W) in the New Abirem District of the Eastern Region of Ghana, about 290 m above sea level. Rainfall is poorly distributed rendering the loamy soils dry and marginal for the cultivation of cocoa. The experimental plot was about 30 years old and 10 ha in size. There was a 10 m road around the plot separating it from the surrounding plantations and forest. The cocoa trees were tall averaging about 6.5 m in height. About half of the farm consisted of closed canopy. The remainder had open areas often referred to as 'pockets' where mirid populations tend to be

higher. The trees consisted of mixed hybrid, planted in near straight lines at about 3 m x 3 m intervals. Thus a hectare contained about 1,100 trees interspersed with about six shade trees as recommended by CRIG. The farm was well-managed with most agronomic practices such as pruning, removal of basal chupons and mistletoes etc., undertaken.

Attraction of male mirids to traps at different heights on single cocoa trees

The site was a CRIG research plantation at Acherensua (07° 00' 354" North, 002° 15' 261" West) in the Brong Ahafo region of Ghana. Three continuous experimental plots forming the three sides of a U-shape and each measuring about 70 m x 150 m, were selected from boundaries between mass trapping plots (Chapter 4) for the experiment.

Attraction of male mirids to traps at different heights on single poles

The experiment was carried out on a farmer's farm at Suhyen in the New Juaben District of the Eastern Region of Ghana. Though the farmer was not producing organic cocoa, no chemical insecticide was applied during the period of the experiment. The farm was about 1.5 ha and was about 15 years old. The trees were planted irregularly and they averaged 6.5 m in height. The canopy was closed with few open areas. About a third of the farm was densely shaded with about six trees in an acre. The crop was of mixed hybrid. The farm was bordered on one side by a village. The remaining sides shared boundary with plots cultivated with food crops such as cassava, plantain and cocoyam.

Attraction of male mirids to traps at different heights on different trees

The experiment was conducted on a farmer's farm at Akwadum Brong- Densuso in the Suhum Kraboah Coaltar District of the Eastern Region of Ghana. The farm was used to cultivate organic cocoa so no insecticide was applied on the plot. The size of the farm was about 3 ha. It was about 10 years old. The cocoa trees were between 3.5 m and 6.5 m high and irregularly planted under shade trees. The canopy was mostly closed but there were few open areas. With the exception of one side which was bordered by a village, the farm was bordered on all other

sides by land used for the cultivation of food crops such as cassava, plantain and cocoyam. The crop consisted of mixed hybrid. Good agronomic practices such as the clearing of weeds, removal of basal chupons and pruning were partially done.

3.2.2 Field experiments to optimise trap designs

Lures

In both experiments the synthetic pheromone consisted of the diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate, and the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate in the proportion of 1000:500 µg diester:monoester. The synthetic pheromone was synthesised and supplied by NRI. Each trap was baited with 1.5 mg of the lure dispensed in polyethylene vials (20 x 8 x 1.5 mm thick; Just Plastics, London, UK). In Ghana they were kept in the freezer when not used.

Fabrication of traps

In fabricating the traps a constant aperture size of 280 cm² (10 cm x 14 cm x 2) as in the NRT was used as standard to determine equal aperture sizes for the traps which were calculated from area formulae.

New Rectangular Trap NRT (Figure 3.1).

The new rectangular trap (NRT) was made of corrugated plastic sheets ('Correx'; Sign Trade Supplies, Maidstone, UK). They were cut and folded into open-ended boxes (38 cm long, 10 cm wide and 14 cm high) whose axis was horizontal in normal use. A smaller second sheet (38 cm long, 9.6 cm wide and 12 cm high) was folded and inserted into the trap as liner. The liner was coated with polybutene sticker (Agralan, Ashton Keynes, Wilts, UK) on all the three inner surfaces such that it formed a sticky retentive surface for capturing mirids. The lure was suspended in the middle of the trap on aluminium wire. It was aligned horizontally when in use.

Plastic Plates Trap (Figure 3.2).

This sticky trap was joined from two plastic plates purchased from the local market. The upper plate (18 cm in diameter) and lower plate (16 cm diameter) were joined together at the middle with a piece of wood (5 cm long). The upper surface of the lower plate was coated with polybutene sticker (Agralan) to serve as retention surface. The lure was suspended under the roof of the upper plate on aluminium wire. The upper plate shielded the lure and lower plate from precipitation and direct sunlight.

Plastic Cylinder Trap (Figure 3.3).

This sticky trap was made from two plastic bottles (2.5-litre, diameter 13.4 cm). Both ends of each bottle were cut open and joined to form a hollow cylinder. An insert was cut to fit about 80% of the inner diameter and was coated with polybutene sticker (Agralan) to serve as retention surface. The lure was suspended from the roof in the middle of the trap on aluminium wire. In use it was aligned horizontally.

Big Water Bottle Trap (Figure 3.4).

This water trap was made from an opaque, white plastic water bottle (4.5 litre, 26 cm high and 16 cm in diameter). Two opposite windows (each 7.0 cm x 20 cm) were cut out of the bottle. In use, the trap was hung bottom up and filled with water containing soap and a little ethanol to reduce surface tension and help preserve trapped mirids respectively. The water was filled to the level of the aperture to serve as the retention medium. The lure was suspended just above the water surface.

Small Water Bottle Trap (Figure 3.5).

This water trap was made from a transparent plastic water bottle (1.5 litre, 31 cm high with 8 cm diameter). Four windows (each 5.0 cm x 22 cm) were cut in the sides near the bottom. In use, the trap was hung bottom up and filled with water containing a little ethanol and soap to the level of the aperture to serve as the retention medium. The lure was suspended just above the water surface.

Figure 3.1 New Rectangular Trap (NRT).

Figure 3.2 Plastic Plate Trap.

Figure 3.3 Plastic Cylinder Trap.

Figure 3.4 Big Water Bottle Trap.

Figure 3.5 Small Water Bottle Trap.

Experimental designs and trap set-up

In the first experiment on optimisation of traps designs done at Akwadum, four new trap designs (Figures 3.2–3.5) were tested against the NRT (Figure 3.1) in a randomised complete block design (RCBD) experiment. The experiment was replicated 8-fold from 3 March 2007 to 30 May 2008. Using a measuring tape, inter- trap spacing was measured as 20 m while inter- block spacing was 40 m. Traps were suspended about 1.8 m in cocoa trees arranged in a line in each block. A randomisation plan generated by Genstat was used in placing the traps in the plots. The relative positions of traps were rotated and lures were changed as in section 2.2.1 above.

In the second experiment of trap optimisation done at Afosu, four traps were used: two large water bottle traps (Figure 3.4) and two sticky cylinder traps (Figure 3.3). The two water bottle traps had water as the retention substance but in addition

one had the outside surface covered by polybutene sticky glue (Agralan). The two sticky traps had the usual sticky surface inside and in addition one of them had its outer surface also covered with polybutene sticky glue.

The four traps were tested in a randomised complete block design RCBD experiment. It was replicated 8-fold in mirid "pockets" (i.e. areas of high mirid concentration) from 10 May to 14 June 2011. The mirid pockets measuring about 30 m x 30 m were selected at least 40 m apart. Traps baited with synthetic pheromone were placed 20 m apart in mirid pockets. A randomisation plan generated by Genstat was used in placing the traps in four quarters of the pocket. Baited traps were suspended in the canopy or as near to it as possible (following the results from the trap height experiments at Acherensua, Suhyen and Akwadum. See section 3.2.2). Traps were rotated and lures renewed as in section 2.2.1 above. The heights, about 5 m, were reached by climbing a ladder and the distances were measured with measuring tape. The experiment was visited weekly to record trap catches.

3.2.3 Attraction of male mirids to traps at different heights

Lures and traps

In all the experiments the large water bottle trap (Figure 3.4) was used. The lures contained a blend of the diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate, and the corresponding monoester, hexyl (*R*)-3-hydroxybutyrate, in the proportion of 1000:500 µg diester: monoester as described above (3.2.2) and were renewed every month.

Attraction of male mirids to traps at different heights on a single cocoa tree

In the first experiment, three large water bottle traps baited with synthetic pheromones were suspended at 0.6 m, 1.8 m and 2.7 m respectively on the same cocoa tree from 28 January to 20 July 2009 in a completely randomised design at Acherensua. The experiment was replicated 15-fold with the trees in a line, at least 20 m apart, and at least 30 m from any other adjacent trapping experiment.

Measurements were done using a measuring tape. Traps were visited every two weeks for recording of catches and traps were cleaned every month.

Attraction of male mirids to traps at different heights on single pole

This second experiment extended trapping into the canopy and beyond at Suhyen. Traps at four heights were tested in a completely randomised design experiment replicated 10-fold. Four large water bottle traps were pulled by ropes and suspended on a single pole placed near a cocoa tree about 6.3 m in height. The traps were placed one each at the following four heights; 1.8 m, 2.7 m, inside canopy above 2.7 m and about 0.3 m above the canopy (Figure 3.6). Poles were placed at least 30 m apart. Traps were visited weekly for recording of catches during March 2010 - August 2010. Replacement of lures and cleaning of traps were done every month.

Attraction of male mirids to traps at different heights on different cocoa trees

This third experiment also extended trapping into the canopy and beyond with treatments on different trees. There were five different treatments in a randomised complete block design (RCBD) experiment replicated 6-fold from 12 March to 3 August 2010. Three large water bottle traps were suspended at the following heights; 1.8 m, 2.7 m, and inside canopy above 2.7 m on three different tall cocoa trees of average height 6.3 m. A fourth trap was placed on a pole and placed by a tall cocoa tree such that the trap was suspended 0.3 m above the canopy. The fifth trap was placed below 1.8 m inside the canopy of a short cocoa tree average height 3.5 m. Traps were at least 30 m apart and inter-block spacing was at least 40 m. Traps were baited with synthetic pheromone and visited weekly for recording of catches. Lures were replaced and traps cleaned every month.

Figure 3.6 Four water traps arranged on a pole beside a cocoa tree at Suhyen.

3.2.4 Analysis of data

The data were analysed using Genstat package (9th Edition). ` Total trap-catch data per treatment per replicate were transformed to √(x+ 0.5) to stabilize error variances. The raw and transformed data were subjected to analysis of variance by using the factors of replicate block and treatment. Also catches inside both bottle and cylinder traps without glue on the outside (normal traps) were compared with catches inside the same traps with glue on the outside (glued traps) in a linear contrast as 'inglue v innoglue'. Where ANOVA indicated significant differences ($P < 0.05$), differences between means were tested for significance by an LSD test.

3.3 RESULTS

3.3.1 Field experiments to optimise trap design

Trap design

All the traps caught male mirids and no females. *Sahlbergella singularis* dominated the catches. *Distantiella theobroma* was absent but another pod feeding mirid*, B. laticollis* which was identified by experts from CRIG, was also caught. In the study on the effect of trap designs on catches at Akwadum, out of a total of 309 male mirids trapped, 308 were *S. singularis* with one being *B. laticollis.* The highest number of 23 *S. singularis* was recorded by a single trap of the sticky disc design in January 2008. The trend of total monthly catches (Figure 3.7) which showed that catches were low from March 2007 to December 2007 before increasing to a peak in February and then declining in March 2009, portrayed the population dynamics of mirids reported previously in that area (Padi and Acheampong, 2003; Ayenor, 2007).

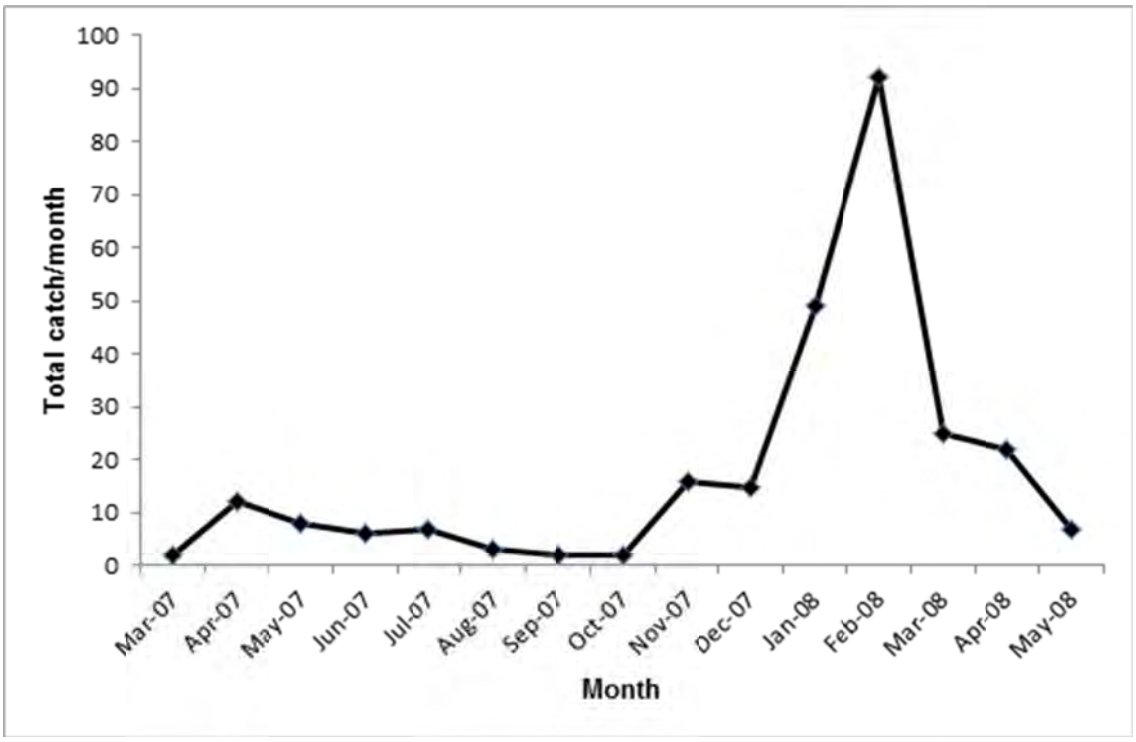

Figure 3.7 Total monthly catches of male *Sahlbergella singularis* in first trap design (40 traps) experiment at Akwadum; 3 March 2007- 30 May 2008.

Data on male *S. singularis* separately and also on mirids only (i.e. combined *S. singularis* and *B. laticollis*) were analysed because a negligible number of *D. theobroma* was caught and also *B. laticollis* caught were few. Analysis of variance was carried on raw as well as transformed data for the whole period March 2007 - May 2008, and also from November 2007 to May 2008, a period of highest total catches. Results of untransformed data are presented for ease of interpretation because they are the same as the untransformed.

No single trap was outstanding in performance. ANOVA showed no significant differences ($P>0.05$) in catches by the big bottle, cylinder, discs, small bottle and the standard NRT (Figure 3.8) ($F = 0.10$, df 4,28, $P = 0.986$). Thus, both sticky and water traps were equally effective and traps made from locally available materials were as effective as the NRT standard. These results were the same for both raw and transformed data for the two periods analysed.

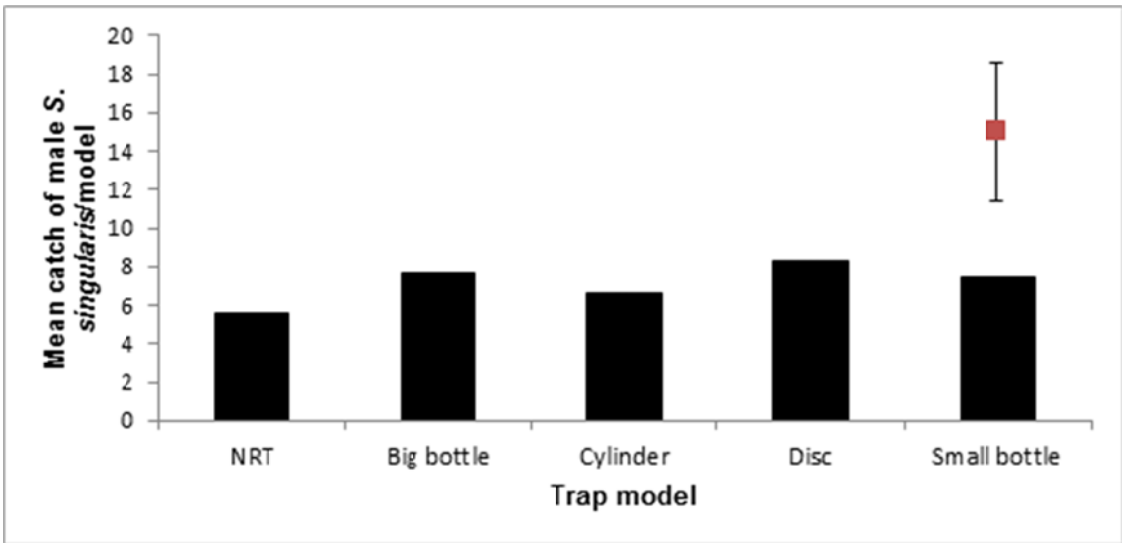

Figure 3.8 Mean trap catches of male *Sahlbergella singularis* in different traps models at Akwadum (3 March 2007- 30 May 2008; 8 replicates). Error bar shows standard error of difference between means.

Additional surface for trap captures

In the study of the effects of sticky on the outside surfaces on catches at Afosu, all the traps caught male mirids and no females. *S. singularis* dominated the catches with few *B. laticollis* and *D. theobroma* virtually absent. Of the 172 male mirids caught, 135 were *S. singularis* with 36 *B. laticollis* and one *D. theobroma*. Catches were high throughout the experiment and highest in the first week of June (Figure 3.9) but the period was too short to show any obvious trend.

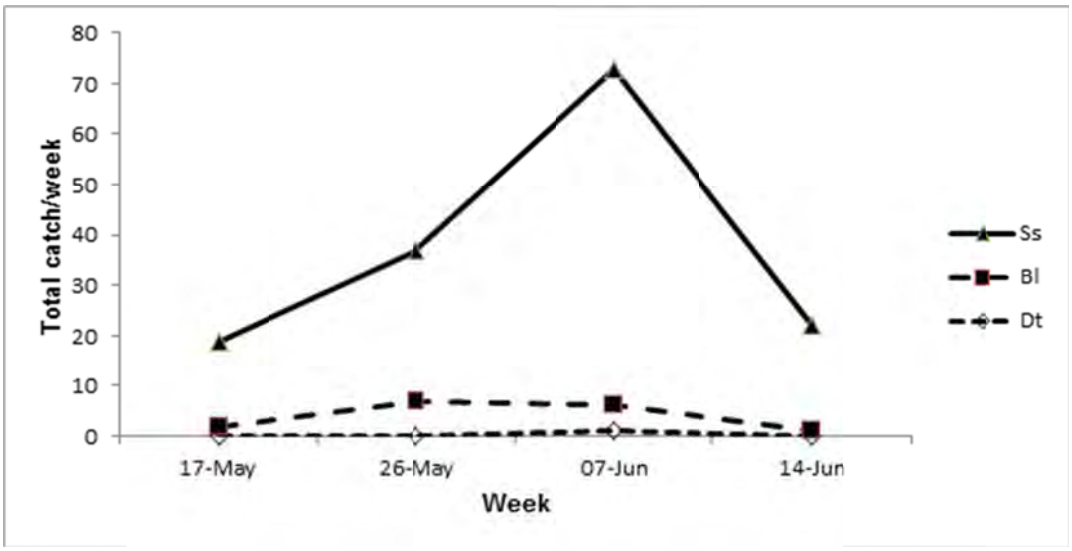

Figure 3.9 Total weekly catches of male *Sahlbergella singularis* (Ss), *Distantiella theobroma* (Dt) and *Bryocoropsis laticollis* (Bl) in second trap improvement experiment at Afosu (10 May – 14 June 2011; 32 traps).

Untransformed mean catches of male *S. singularis* ranged from 1.25 by outside catches in cylinder traps to 5.62 by outside catches in bottle traps (Figure 3.10). The results showed that the addition of the outside surface for trapping increased total catch of the trap. ANOVA on transformed data showed catches on the outside of the bottle trap to be significantly higher than all the treatments (F = 5.99 *df* 5, 35, P = 0.001) except the inside catches of the same trap with the glue outside (Figure 3.10). The inside of the normal bottle caught only about 38% of the outside catch and 23% of the total catch (inside and outside) by the bottle with glue. This indicated that not all mirids attracted to the normal trap were caught and that catches can be optimised with the inclusion of outer surface for trapping.

The results also confirmed the parity of performance by the normal bottle trap and the cylinder in the results on trap designs above. ANOVA showed no significant differences (P >0.05) in the inside catches of all the traps. Results for the total mirids were the same as for *S. singularis* as catches were dominated by the latter.

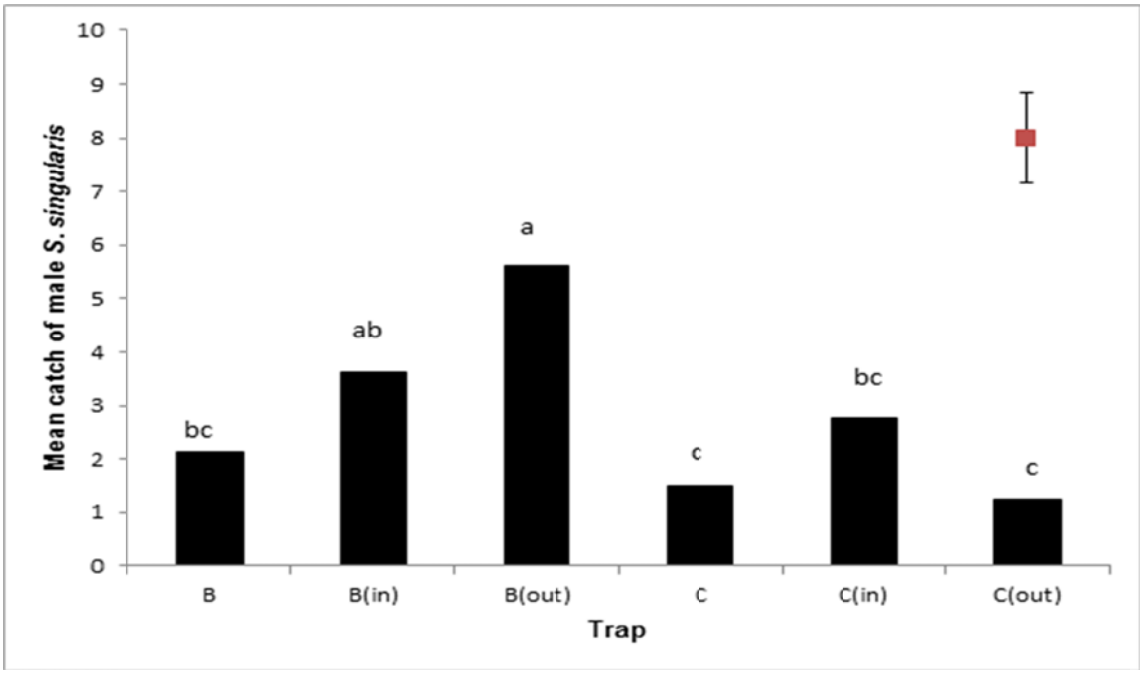

Figure 3.10 Mean catch per trap of male *Sahlbergella singularis* inside and outside of traps from 17 May to 14 June, 2011. (Bars with different letters are significantly different ($P < 0.05$) by LSD test after transformation of data to $\sqrt{(x+0.05)}$ and analysis of variance. Error bar shows standard error of difference between means. B = catches inside water bottle trap; B(in) = catches inside water bottle trap with glue outside; B(out) = catches outside water bottle trap; C = catches inside cylinder sticky trap; C(in) = catches inside cylinder trap with glue outside; C(out) = catches outside cylinder trap).

Figure 3.11 shows untransformed mean catches inside normal bottle and cylinder traps and glued traps of the same trap models. The addition of sticky polybutene on the outside surface appeared to have boosted catches inside. From linear contrasts of the transformed data, mean catch inside a trap with glue on the outside was significantly higher ($F = 4.57$,df 1,35, $P = 0.039$) than the mean catch inside the same trap without glue on the outside (normal), showing that the application of glue on the outside of the trap increased the inside catches of the same trap. These results of the study on inside and outside captures, however, cannot be extended to the traps that were not tested since they do not show a general trend.

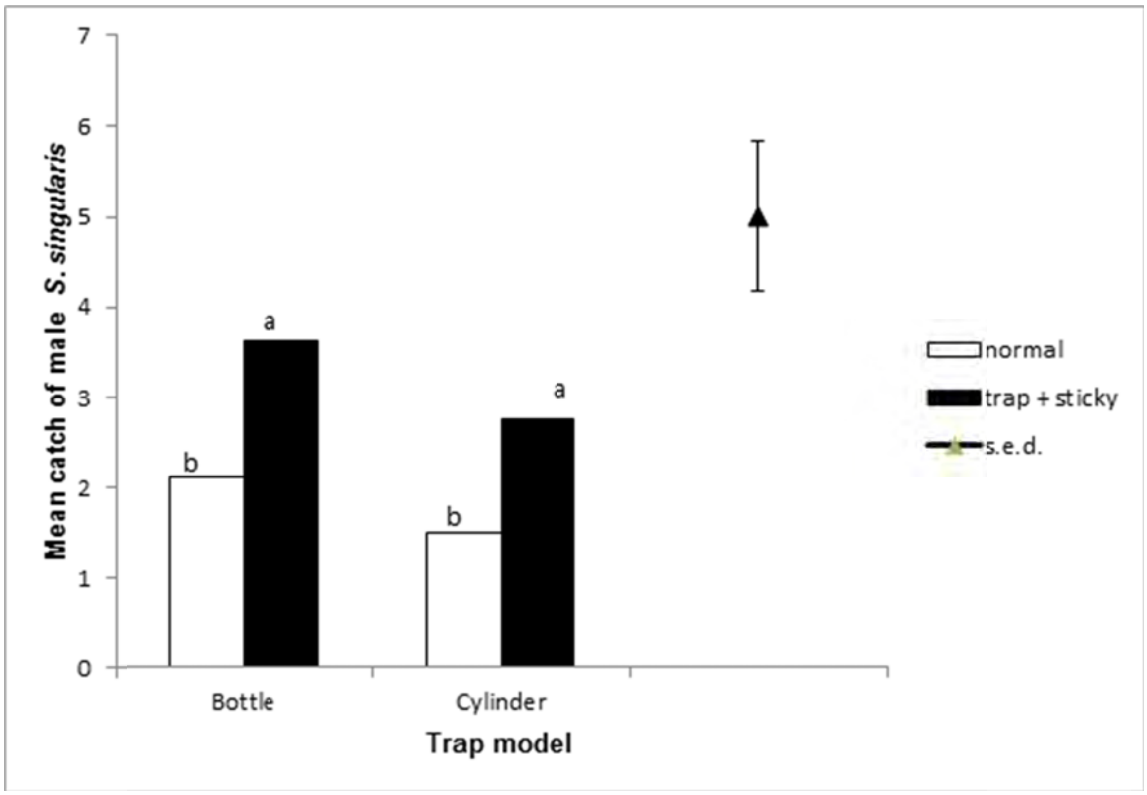

Figure 3.11 Untransformed mean inside catches of male *Sahlbergella singularis* by normal and glued traps of the same model from 17 May to 14 June 2011. Bars with different letters are significantly different ($P < 0.05$) by LSD test after transformation of data to $\sqrt{(x+0.05)}$ and analysis of variance. Error bar shows standard error of difference between means.

3.3.2 Attraction of male mirids to traps at different heights

Male mirids only were attracted to all heights in all the three experiments. Of the two species caught, there was clear dominance of *S. singularis* over *D. theobroma*. Results of untransformed data are presented if not different from the transformed data for ease of interpretation. However, significance of differences was always calculated with transformed data.

Traps on single cocoa tree

In the first experiment with traps on a single cocoa tree at Acherensua, a total of 97 *S. singularis* and 5 *D. theobroma* were caught at the different heights. Total

monthly catches of *S. singularis* rose from January to the highest peak in March before declining continuously to the lowest in July (Figure 3.12).

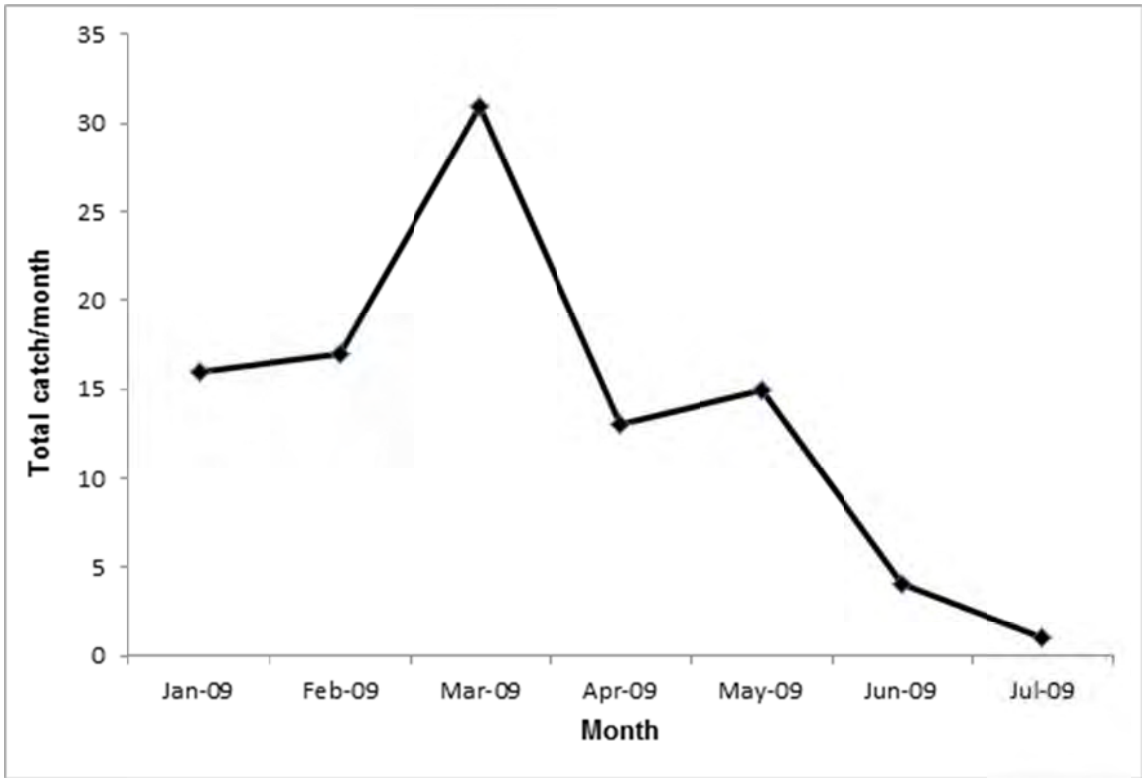

Figure 3.12 Total monthly catches of male *Sahlbergella singularis* in bottle trap baited with synthetic pheromone at different heights on one cocoa tree at Acherensua; 28 January 2009- 20 July 2009.

The highest number of mirids was caught at the highest point of 2.7 m contrary to, and higher than, the 1.8 m reported by Sarfo *et al*. (2007) (Figure 3.13). ANOVA on both transformed and raw data showed that mean catch at 2.7 m was significantly greater than mean catch at either 1.8 m or 0.6 m (F=18.79, *df* 2,28, P = 0.001), but those at 1.8 m and 0.6 m were not significantly different (P>0.05). The results appeared to suggest orientation towards the canopy in mirid flights because the traps at 2.7 m were very close to the canopy. Apparently the heights considered did not go high enough in the experiment.

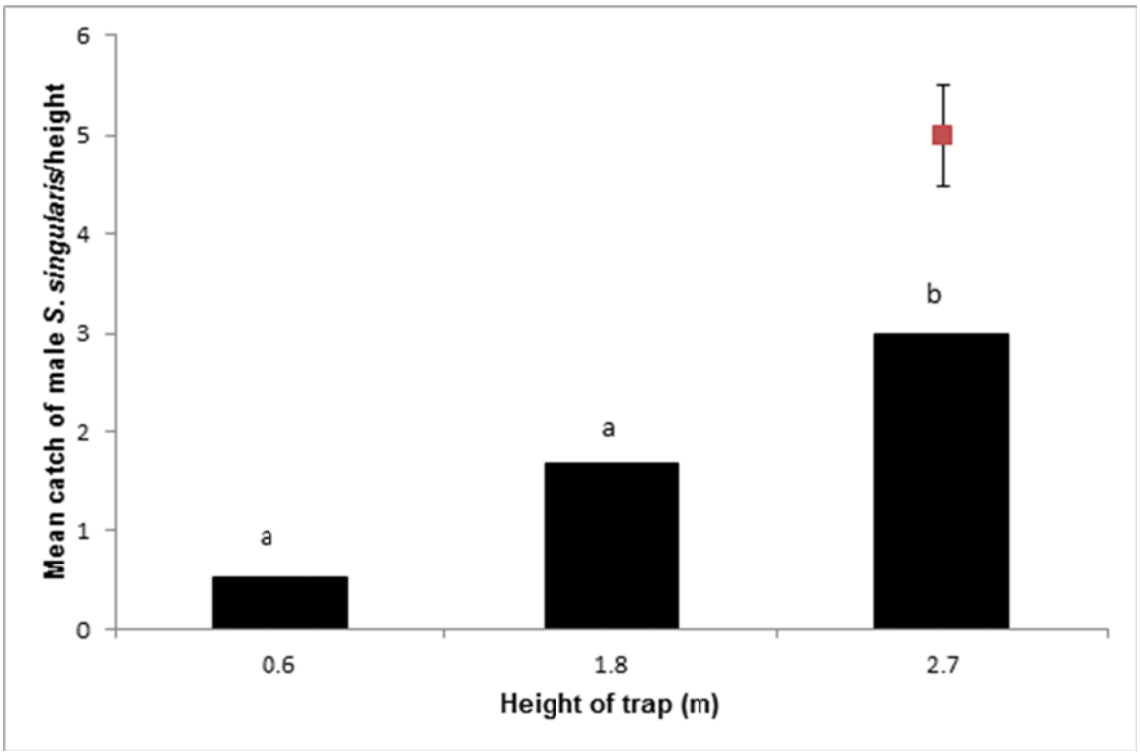

Figure 3.13 Mean catch per height of male *Sahlbergella singularis* in water bottle trap baited with synthetic pheromone at different heights on one cocoa tree at Acherensua (28 January 2009 - 20 July 2009; 15 replicates). Bars with different letters are significantly different (P<0.05) by LSD test after transformation of data to $\sqrt{(x+0.05)}$ and analysis of variance. Error bar shows standard error of difference between means.

Traps on single pole

In the second experiment with traps on a single pole at Suhyen, traps at the four different heights attracted male mirids totalling 157, of which 148 were *S. singularis* and 9 *D. theobroma*. Catches of male *S. singularis* were high throughout the study period. They rose from May to the highest peak in July before declining in August (Figure 3.14).

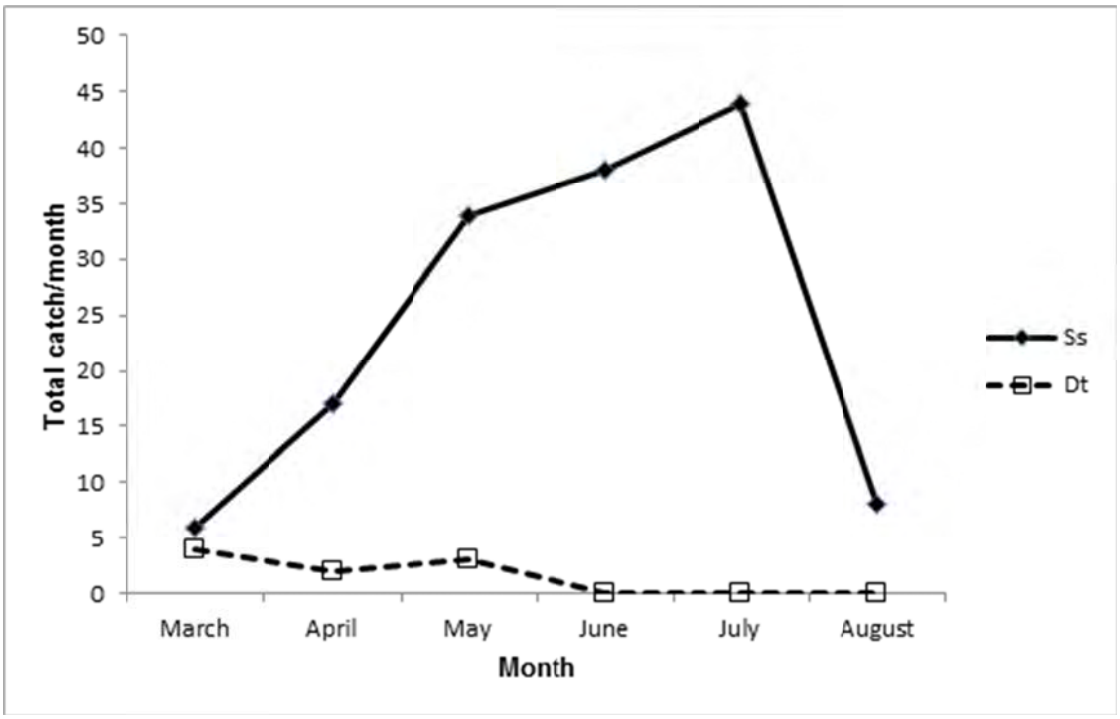

Figure 3.14 Total monthly catches of male *Sahlbergella singularis* (Ss) and *Distantiella theobroma* (Dt) in water bottle traps (40 traps) baited with synthetic pheromone at different heights on one pole at Suhyen; 12 March - 3 August 2010.

Results of the experiment clearly showed the orientation of mirids to the canopy. ANOVA on both transformed and untransformed data showed that mean catch by traps inside the canopy was significantly greater (F=33.98, df 3, 27, P = 0.001) than mean catches at 2.7 m and 1.8 m though significantly lower than that above canopy (Figure 3.15) (F = 33.98, df 3, 27, P = 0.001).

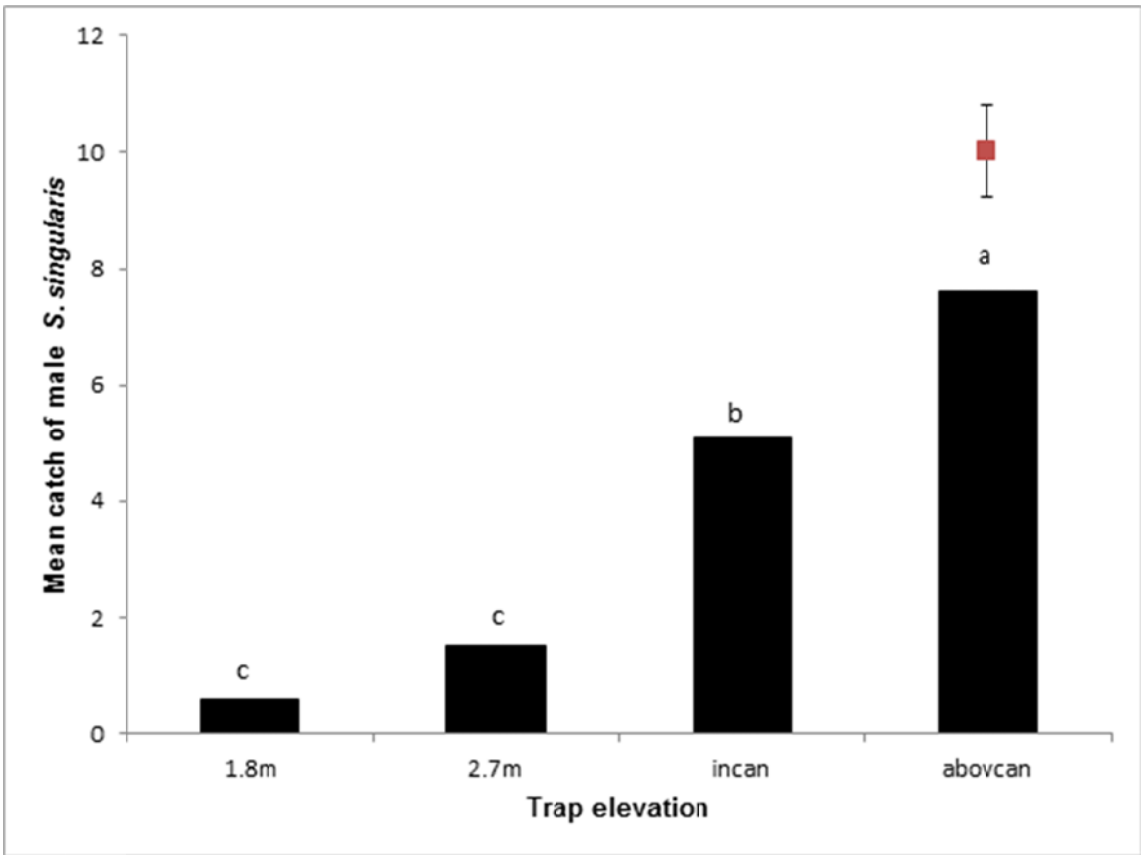

Figure 3.15 Mean catch per height of male *S. singularis* in water bottle traps baited with synthetic pheromone at different heights on a single pole at Suhyen (12 March 2010 - 3 August, 2010; 10 replicates). Bars with different letters are significantly different (p<0.05) by LSD test after transformation of data to √(x+0.5) and analysis of variance. Error bar shows standard error of difference between means; incan = in canopy above 2.7m; abovcan = above canopy.

Traps on different cocoa trees

In the third experiment with traps on different cocoa trees at Akwadum, traps at the five different heights caught a total of 116 male mirids consisting of 108 *S. singularis* and only 8 *D. theobroma.* The initial rise in catches of *S. singularis* in April declined in May before rising to the highest peak in July and declining again in August (Figure 3.16). The trend of catches in the experiments at Suhyen and Akwadum did not reflect the incidence of mirids in these locations in spite of being in the same vicinity as the lure and trap design experiments (Sections 2.3.1 and 3.3.1) where catches mirrored reported mirid incidence.

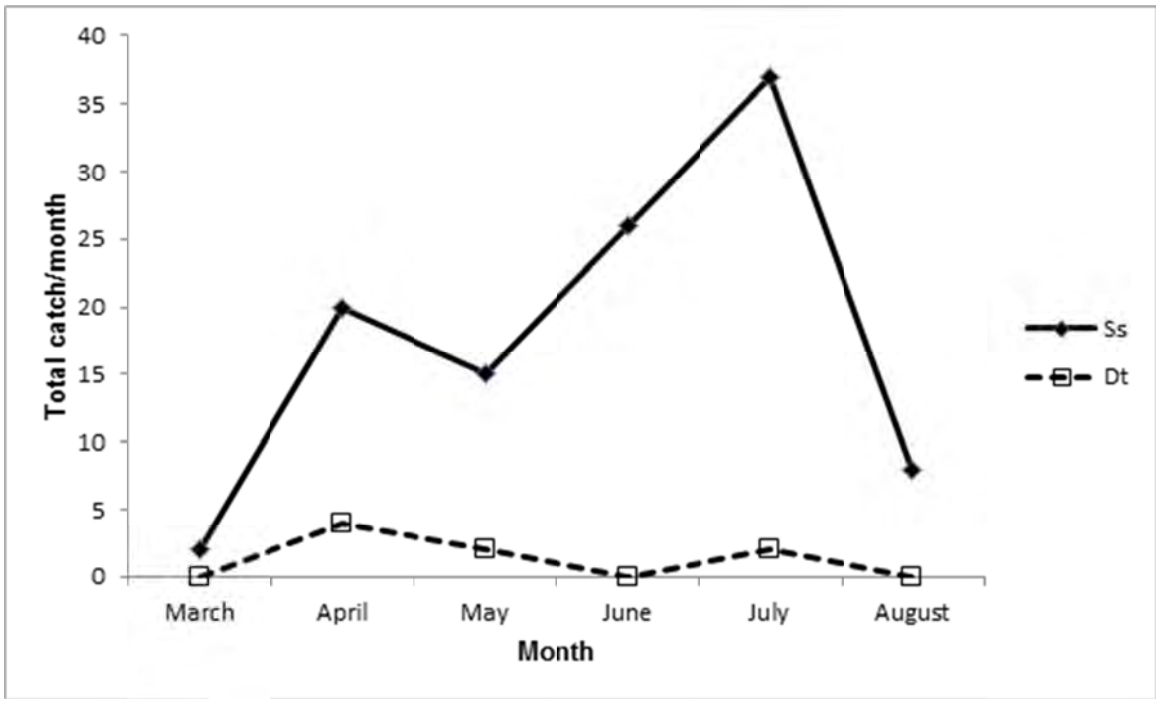

Figure 3.16 Total monthly catches of male *Sahlbergella singularis* (Ss) and *Distantiella theobroma* (Dt) in water bottle trap (30 traps) baited with synthetic pheromone at different heights on different cocoa trees at Akwadum; 20 March- 8 August 2010.

The highest number of mirids was caught by traps inside the canopy though not significantly different ($P > 0.05$) from catches above the canopy and at 2.7 m in tall trees (Figure 3.17). Mean catches in and above canopy were all significantly higher than mean catches at 1.8 m or in the canopy of short trees ($F = 2.74$, df, 5,20, $P = 0.05$). Mean catch in the canopy of short trees was about 2.5 times as much as that at 1.8 m although the difference was not significant ($P>0.05$) statistically. Thus these results confirmed greater male mirid flight activity around the canopy, viz.: just beneath it at 2.7 m, inside and above it than lower down the tree from 1.8 m downwards and further suggested that placement of traps in canopy or nearest to it is key in maximising catches.

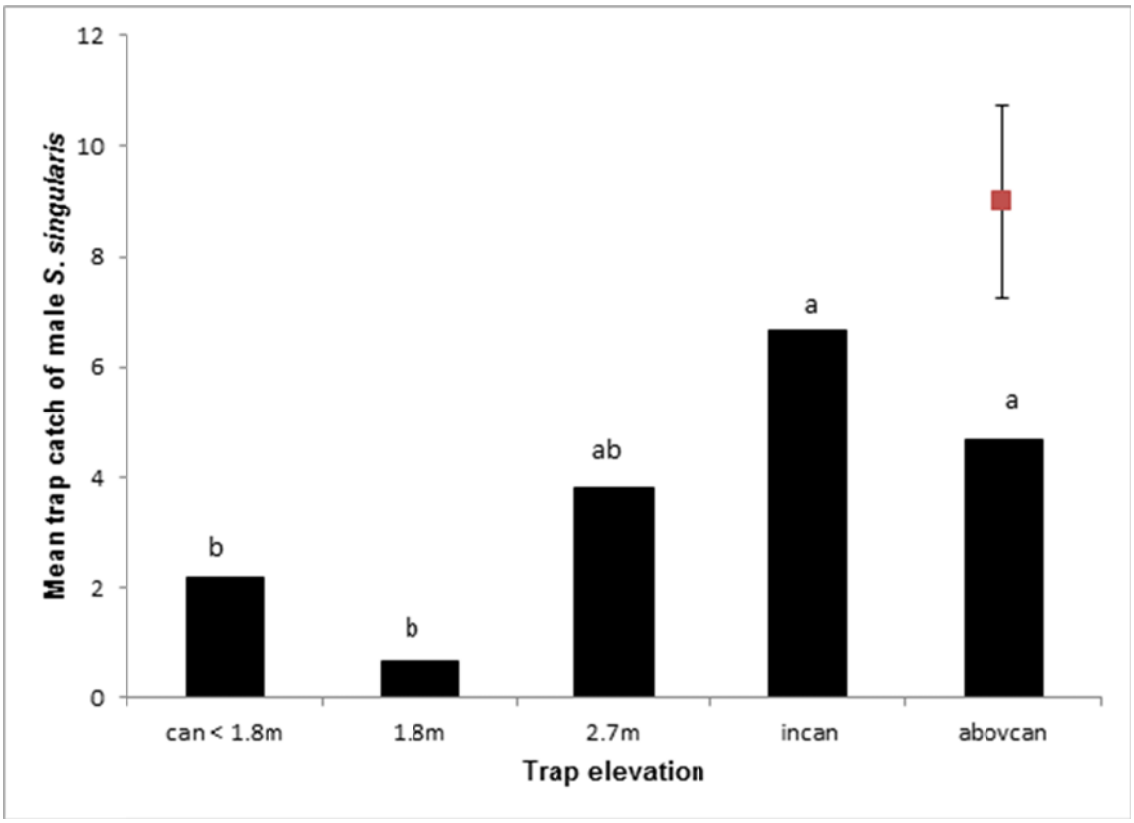

Figure 3.17 Mean trap per height catches of male *S. singularis* in water bottle traps baited with synthetic pheromone at different heights on different trees at Akwadum (20 March 2010 - 8 August, 2010; 6 replicates; bars with different letters are significantly different (p<0.05) by LSD test after transformation of data to √(x+0.5) and analysis of variance; 'incan' = in canopy above 2.7 m; 'abvcan' = above canopy. Error bar shows standard error of difference between means.

The points (heights) of highest catches in the three experiments showed clearly that fewer mirids were trapped in previous trials at 1.8 m. From the highest catches of experiments with traps on single cocoa tree, single pole and different cocoa trees, mirids caught at 1.8 m were 2.9x, 12.7x and 9.9x less optimal respectively.

The results suggest that the two experimental designs, i.e. having several traps on a single pole or cocoa tree and having them on different cocoa trees produced similar distribution patterns. However the larger experiment with the traps on different trees was less efficient. This is because it had a higher standard error of difference (SED) which was 3.6x and 2.1x those in the other experiments with

traps on single cocoa tree and single pole respectively, indicating greater plot to plot variation arising from the patchy spatial distribution of mirids (Bisseleua, *et al.,* 2011; Gibbs *et. al.,* 1968).

3.4 DISCUSSION

3.4.1 Trap design

The objective of the trap design experiment was to identify the best performing local trap(s) for use in pheromone trapping particularly in methods that will require large numbers of traps. Results of the experiment showed that all the traps were equally efficient. The design and type (water and sticky) of trap did not affect captures of male *S. singularis* and *D. theobroma*. However, the ability of the disc plates to collect the numerically highest single catch is worthy of note. It may have been possible because of the omni-directional attraction capability of the design and this needs to be taken into consideration in future improvement of the traps.

Since any of the local designs was as efficient as the standard NRT, factors of cost, availability, ease of fabrication and convenience of usage among others should inform the determination of choice of design for any method of mirid trapping. However, some functional characteristics of the traps make different designs suitable for either control or monitoring.

The water traps were made wholly from local materials which are available and accessible because they are cheap. They were easy to fabricate too. However, catches would decompose, break and become messy, making identification and counting difficult if they stayed for about three weeks or more even in alcohol, as was observed in a different experiment. Also the traps needed frequent refilling with water due to fast evaporation and/or drinking by birds and other animals. The water traps would therefore, be suitable for use in methods of trapping in which counts of insects removed is not of priority such as mass trapping and lure and kill.

The sticky traps were easy to obtain and fabricate and cheap to buy. Catches by these traps remained identifiable even after three weeks and, therefore, would be suitable for scientific monitoring of insect numbers where counts are of essence. Their efficiencies could however, be reduced with the accumulation of debris, dust and dead insects and would therefore need frequent maintenance. Their sustainability will also be challenged by the availability of the polybutene sticker which currently needs to be imported.

Previous trap optimization trials at CRIG have all involved testing of different sizes of green and white sticky traps models NRT and Delta. The standard white NRT was superior (Padi *et al*., 2002) even though colour was not critical. White NRT also proved better than the Delta trap for mirid captures in optimization trials in Cameroun, in which no water trap was tested (Mahob, *et al*., 2011). The present study is the first time water traps have been tested for capture of cocoa mirids but in other mirids, pheromone baited water traps have been tested. For example, Yasuda and Higuchi (2012) tested double-sided sticky traps and water pan traps for capturing male *S. rubrovittatus* and *T. caelestialium* and reported that the former was more effective in capturing male *S. rubrovittatus* but found the traps to be equally effective in trapping male *T. caelestialium*.

Results of the further optimisation experiment showed improvement in the efficiency of the large water bottle and the maximisation of its catches with the addition of sticky outside surface for trapping. Putting the sticky on the surface is labour intensive, tedious and cumbersome and the sticky polybutene needs to be imported which defeats one of the reasons for testing the water trap. Moreover, presence of dust, dead insects and debris on the exposed sticky requires regular clearing and repeated application of sticky. As an alternative, the outside surface could be impregnated with safe contact insecticide to knock down mirids on contact in a lure-and-kill strategy. Alternatively, the outside surface of the trap can be reduced by increasing the sizes and number of the windows. This will make the trap multi-directional in attraction and also reduce available space for landing on the trap. Maximisation of pheromone trap catches with additional surface for trapping has been reported in the Coleoptera. Bakke *et al*. (1983) reported increase in the catches of the spruce bark beetle by more than 90% by attaching

an exterior funnel to pipe traps for additional collection of beetles. The precursor to that study by Regnander and Solbrek (1981) had found that pipe traps without funnels caught about 50% of those with funnels.

The higher catches of combined inner and outer collecting surfaces than the inner surface alone of the mirid pheromone traps, is obviously the result of the increment in surface area with the combination of the two surfaces. The greater efficiency shown by outer surface of the bottle in capturing mirids than the sticky cylinder traps may have resulted from the possible formation of pheromone cloud around the whole trap due to the shortness of the bridge separating the windows. This cloud would attract mirids from all directions unlike the cylinder which would attract from two sides only because of the possible separation of the cloud because of the long bridge. The capturing of more insects on the outside than inside which indicated that not all insects attracted to the trap were captured shows that the design is not optimal. Optimal designs ensure that a large proportion of attracted insects are caught (Mottus *et. al*., 1996). Therefore the design needs to be improved further.

The increment of inside catches of traps with the administration of glue on the outside might be attributed to the presence of dead mirids caught on the outer surface of the trap serving to deflect incoming mirids probably as a result of residual warning signals released before dying. Trematerra *et. al.* (1996) reported reduction in trap catches of *Tribolium castaneum* whether baited with pheromone or not as a result of the presence of the dead species and suggested the probable presence of residual alarm pheromone released by the insect before dying as the reason.

McBrien and Millar (1999) suggested the possible flight of mirids straight into traps. The captures of male mirids by both traps on their outer surfaces suggest that in cocoa mirids entry into traps is both by direct flight into the trap and also by walking from the outer surface of the trap. Male mirids were encountered walking into normal bottle traps from the outer surface in the field.

3.4.2 Trap placement

In the trap placement experiments trap catches of male mirids showed close association with the cocoa canopy rather than absolute height. The 22 *D. theobroma* males that were caught represented <6% of the total mirid catch and all but one was trapped either inside the canopy (10 individuals) or just above it (7 individuals). The numbers of *S. singularis* caught also increased with trap height at Acherensua. At Suhyen and Akwadum this increase was up to points inside the canopy. Trap catch inside the canopy at Suhyen was 240% above that at 2.7 m, a point just beneath the canopy, but 49% less than above the canopy, demonstrating that the increase in catch with height was slowing, while at Akwadum the highest numbers were caught inside the canopy, but not significantly more than were caught above the canopy. The lower catches of mirids inside the canopy of short trees than tall trees may be a reflection of low populations in short canopy and the presence of surrounding taller trees. Nevertheless, catches in traps in the canopy of short trees were numerically greater than catches in traps at similar heights of 1.8 m on tall trees.

Catches around canopy height (2.7 m, inside and above canopy) removed 10 times more *S. singularis* males than those at the fixed height of 1.8 m, at both Suhyen and Akwadum, despite the presence of four traps at each trap-point at the former and a single trap at each trap-point in the latter. This suggests that there is little vertical displacement by *S. singularis* males when tracking to pheromone lures. Highest male mirid catches inside canopy, above it and at 2.7 m at Akwadum, Suhyen and Acherensua respectively, are all higher than and contrary to the reported optimal height of 1.8 m observed with virgin female as lure (Sarfo *et al.,* 2007).

Highest catches above the canopy may have been aided by attraction of mirids to light (Williams, 1953b) but it also shows that mirids are capable of flying at higher elevations probably in search for food and mates. Highest catches at canopy level also depict high male flight activity probably for mating and feeding in the canopy which is supported by reported results of earlier workers. Williams (1953a) reported that *S. singularis* extended feeding into the fans in the canopy but in later

work, Entwistle (1957) showed that *S. singularis* and to some extent *D. theobroma,* breed on the branches in the canopy. This finding also supports the observation by King (1971) that young adult *D. theobroma* favour exposed parts of the cocoa canopy. Maximisation of trap catches around canopy has also been reported in other mirids. Ishimoto *et al.* (2006) estimated the most effective trap height in the monitoring of adult rice leaf bug, *T. caelestialium* (Heteroptera: Miridae), to be near the canopy of the rice plant. In apple orchards McBrien *et al.* (1996) reported catches of significantly more males of the mullein bug, *C. verbasci* on non-baited cards placed at 2.5 m high in the canopy than those placed 1.5 m in the canopy in pheromone treated plots, and suggested incidental catches of males following false trails to pheromone dispensers hung in the upper canopy to be responsible. In other insects such as the Lepidoptera. Bhardwajt and Chander (1992) reported maximisation of trap catches around canopy in the apple leaf roller, *Archips pomivora* Meyrick.

The two different experimental designs used in the trap height experiments were meant to identify the more reliable one for future use. The results showed that the closer experiment of having several traps on a single pole in a plot, was more efficient than the larger one of having several traps on different cocoa trees and therefore more preferable for future work. With the treatments at a single point the plot-to-plot variation usually experienced in mirid field experiments resulting from the patchy spatial distribution of the insect, is reduced to the minimum to reduce the uncertainties in the results. Therefore, reliable and significant results in mirid field bioassays will be achieved in smaller units, e.g. pockets, where populations are generally uniform. Practically, though, it is very difficult finding sufficient areas of such units for enough replication of experiments that will achieve statistical integrity.

3.4.3 Monthly trap catches

The trend of catches in the trap design experiment at Akwadum reflected the seasonal incidence of mirids in the area, but it is out of step with the general one for the country (Gibbs *et al.,* 1968; Collingwood and Marchart, 1971; Owusu-Manu

and Somuah, 1989), which is the basis of a national mirid control programme. Work by these authors established that numbers are low from February to June but rise consistently from July to reach a peak between October and December. In the present results catches were low from March and started to rise in November and reached a peak in February but dropped in March.

3.4.4 Low trap catches of *D. theobroma*

Though the synthetic pheromone was identified from female *D. theobroma,* low numbers of the pest were caught throughout all the experiments giving credence to the observation by Owusu-Manu (1994) about the reducing numbers of the pest in Ghana. No reasons were assigned for this decline but it could be as a result of the increased adoption of good agronomic practices by farmers, especially chupon removal and early and regular harvesting of pods (Baah *et al.,* 2009; Baah, 2010, 2011). As chupons are removed constantly and pods are regularly harvested by farmers in adherence to advice by experts, as was done in the experimental plots, the feeding and breeding sites of *D. theobroma* (Entwistle, 1957; King, 1971), are greatly reduced which would result in dwindling populations.

Chapter 4
INVESTIGATION OF MASS TRAPPING AS A METHOD OF CONTROL OF MIRIDS AT ACHERENSUA (2008-2009)

4.1 INTRODUCTION

The main method of control of mirids on cocoa in Ghana is by foliar application of chemical insecticides four times in the year at monthly intervals, from August to December omitting November, and using motorized mist blower machines recommended by CRIG. This recommendation, which was made by the International Capsid Team in 1957 (Collingwood, 1971b), has been adhered to religiously to the neglect of alternative pest management methods. However, there are problems and risks associated with insecticidal use, such as toxicity of chemicals which threatens the lives of farmers and applicators, adverse effects on the environment, high costs and difficulties of application (Davis, *et al*., 2001) and taint and residues in beans. These problems, coupled with the sophistication of consumers who demand cocoa with little or no insecticides, have generated great interest in the use of safe, non-chemical methods of disinfestations of cocoa.

Mass trapping with pheromone-baited traps aims to capture enough insects in a treated area so that reproductive potential of the population become so lowered as to reduce their population and the damage they can do (Johnson, 2008). As an insect management strategy, trapping with sex pheromone traps removes mainly the target pest and it is environmentally friendly because a limited amount of insecticides, if any at all, is used (reviewed by Witzgall *et al.,* 2010). Traps may also be easy to put out to remove the pests directly and reduce infestation, but there is the risk of failure if the relevant biology and ecology of the pest are not integrated. Also, in insect species such as mirids where only males are trapped, high proportions of those available may need to be trapped to reduce mating for any meaningful impact to be made.

In Chapter 3 suitable traps were suggested for mass trapping the cocoa mirids after the selection of a lure blend in Chapter 2. These parameters provided necessary inputs for carrying out mass trapping of cocoa mirids in Ghana as an alternative pest management method. Mass trapping of cocoa mirids has been demonstrated in an unreplicated plot by Sarfo *et al*. (2007) but its efficacy as a control method has not been tested. Therefore, in order to test a complete mass trapping experiment within the life of the project, suitable blends and traps were selected from the encouraging preliminary results in the optimization of pheromone blends and trap design experiments in Chapters 2 and 3, for mass trapping of cocoa mirids. The trial was conducted with dual objectives of determining (1) whether mass trapping would reduce the mirid numbers and damage and (2) the optimal trapping density of traps needed to trap each species.

In the experiment, the effectiveness of mass trapping was tested with mass trapping traps on treatment plots. The experimental design used was different from the routine method of trapping whole plots as a block against whole untrapped plots. The method was adapted from Stelinski *et al*. (2005). It was a split plot design with trapping done in subplots and it also involved two types of methods in monitoring the effectiveness of mass trapping with traps. One type involved the use of different densities of monitoring traps while the other had single traps in the subplots.

The subplots provided space for the different density monitoring traps to be used dually to monitor the effectiveness of mass trapping and also help in determining the optimal density of traps for trapping the two species simultaneously on the same plot. The assumption was that the smaller units would increase the likelihood of trapping the same populations in subplots and reduce variation in trap catches because of the spatial aggregated distribution of the mirids. The different densities provided the information required for the determination of the optimal density/densities. In addition to the monitoring traps, insecticide knockdown and visual counting were also used to assess the effectiveness of mass trapping.

4.2 MATERIALS AND METHODS

4.2.1 Study site and experimental plots

The experimental site was selected from plantation farms at CRIG sub-station at Acherensua (07° 00' 354" North, 002° 15' 261" West) in the Brong Ahafo region of Ghana. The farms were inherited from the defunct Plantations Division of Ghana Cocoa Board (COCOBOD). The trees were tall, averaging 6.5 m in height, and formed a closed canopy for the greater part with few open areas where mirid populations tended to be higher. The trees were about 25 years old and consisted of mixed hybrid, which were planted at 3 m x 3 m intervals. Thus a hectare which had about six shade trees carried approximately 1,100 cocoa trees, well-arranged in near straight lines and well-managed with most agronomic practices undertaken. The experimental site consisted of two adjacent areas of 10.5 ha, separated by a road approximately 10 m wide. The contiguous experimental areas were surrounded by insecticide-treated cocoa plantings on three sides and by a secondary forest of trees and cultivated foodstuffs such as cassava and plantain on the fourth side. No chemical insecticide was applied on the experimental plot during the trial period other than a knock-down assessment on selected plots.

4.2.2 Lures and traps

Lures were polyethylene vials (0.5 ml, 22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK) impregnated with a blend of the diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate, and the monoester, hexyl (*R*)-3-hydroxy butyrate, prepared at NRI. From January-August 2008 a blend of 1000µg : 1000 µg was used. From September 2008-June 2009 a 1000µg : 500µg blend was used and lures were renewed each month.

The trap model used was the large 4.5 L plastic water bottle fabricated into traps (see section 3.2.1) based on initial results in a selection experiment.

4.2.3 Experimental design and trap set-up

The experimental design was a split-plot, randomised complete block with eight replicates. Each of the two experimental sites above was divided into four blocks and each block was divided into two whole plots of 50 m x 100 m (Figure 4.1). Each 0.5 ha whole plot was separated from adjacent whole plots and from peripheral cocoa plantings by a 50 m wide guard of untreated cocoa trees. A randomisation plan generated with GenStat was used to assign treatments to the two plots in each block. The two treatments were either 75 large water bottle traps (Chapter 3) baited with pheromone (equivalent to 150 pheromone traps per hectare) ('treated plot') or 75 unbaited, large bottle water traps ('untreated control'). Each whole plot was split into five subplots 0.1 ha in size, each thus having 15 baited or unbaited water bottle traps and spaced about 10 m equidistantly apart on a 15 row x 5 column grid with no intervening internal guards between the subplots. Traps were suspended about 1.8 m high in trees (Figures 4.2 and 4.3). The experiment was maintained from January 2008 to July 2009 and trap catches were recorded twice per month at approximately three-week intervals.

Whole plot treatments were allowed to run for three months after which time pheromone-baited monitoring traps were added to all subplots such that subplots **a, b, c, d** and **e** had 0, 2, 4, 8, and 15 monitoring traps added respectively (Figure 4.1). Treatment subplots thus had totals of 15, 17, 19, 23 and 30 traps which is equivalent to 150, 170, 190, 230 and 300 traps/ha respectively, whereas formerly untreated control plots now had the equivalent of 0, 20, 40, 80, and 150 traps/ha respectively. Monitoring traps were placed symmetrically in vacant cocoa trees in the subplot such that no single tree housed both mass trapping and monitoring traps.

In July and August 2008, all monitoring traps were removed from the subplots. However, in September 2008, one was re-introduced in each subplot to resume monitoring of mirid numbers. Traps were inspected and catches recorded fortnightly (Figure 4.4).

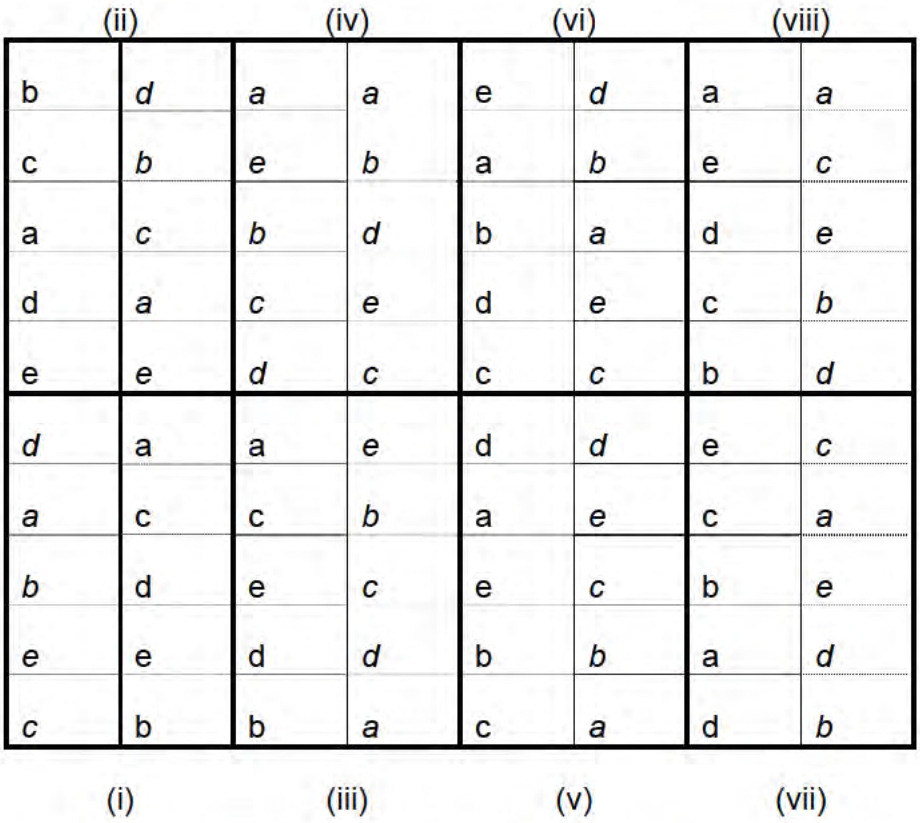

Figure 4.1 Layout of mirid mass trapping experiment at Acherensua, Ghana, showing randomised block, split-plot design with 0.5 ha whole plots (i)-(viii); sub-plot treatments are a = 0, b = 2, c = 4, d = 8 and e = 15 monitoring traps; treatment and control plots are in plain and italic fonts respectively.

Figure 4.2 Pheromone traps in pristine cocoa

Figure 4.3 Pheromone traps in 'mirid pocket'

Figure 4.4 Examining the catch in the mass trapping experiment at Acherensua

4.2.4 Assessment by insecticide knockdown

In July/August 2008, mirid numbers were assessed in all plots by the insecticide knockdown method. Using a motorized knapsack spraying machine, Confidor (imidacloprid) was applied at 30 g a.i/ha to the trunk and canopy of five cocoa trees selected randomly, one from each row in a subplot. Knocked-down insects were collected on white calico sheets spread under the trees before the insecticide application. After one hour, mirids were sorted into species and stages and counted into 70% alcohol in plastic vials and recorded.

4.2.5 Visual assessment of mirid numbers and damage

Insect numbers and fresh mirid injury to pods in both treatment and control were counted up to hand height, i.e. the height reached with a raised hand when standing on the ground, along trunks of 15 cocoa trees selected randomly from each subplot, while damaged branches and ‘chupons’ (shoots) were counted along the entire height of the tree. Pods and chupons damaged by mirids as well

as numbers of adult and nymph mirids were recorded for analysis. Measurements were made at approximately three-week intervals on the same dates as trap catches were counted/

4.2.6 Analysis of data

The data were analysed using Genstat package (9th Edition). Total trap catch of mass trapping traps and multiple monitoring traps as well as visual count data per treatment per replicate were summarised and transformed to $\sqrt{(x+ 0.5)}$ to normalise the data. The raw and transformed data were subjected to analysis of variance (ANOVA) by using the factors of replicate block and treatment. Where ANOVA indicated significant differences ($P<0.05$), differences between means were tested for significance by a Least Significant Difference (LSD) test. Total trap catch of solitary monitoring traps and count data of visual and knockdown assessments were summarised and analysed by unpaired two sample *t*-test for differences between treatment and control. The nature of relationships between trap catches and number of traps were ascertained by regression. Also Chi-square test of independence was performed on total trap catches to determine differences where necessary.

4.3 RESULTS

4.3.1 Mass trapping of mirids

A total of 2,288 male mirids was caught in pheromone baited traps between January 2008 and July 2009. Out of this number, 1,776 (78%) were *S. singularis* while 512 (22%) were *D. theobroma*. None was caught in untreated control traps. Total monthly catches summarized in Figure 4.5 show that mirids were captured in all months of the study with two main peaks in March-June and October-December in 2008. Catches were lower and varied less in 2009. At all times numerically more *S. singularis* than *D. theobroma* were caught.

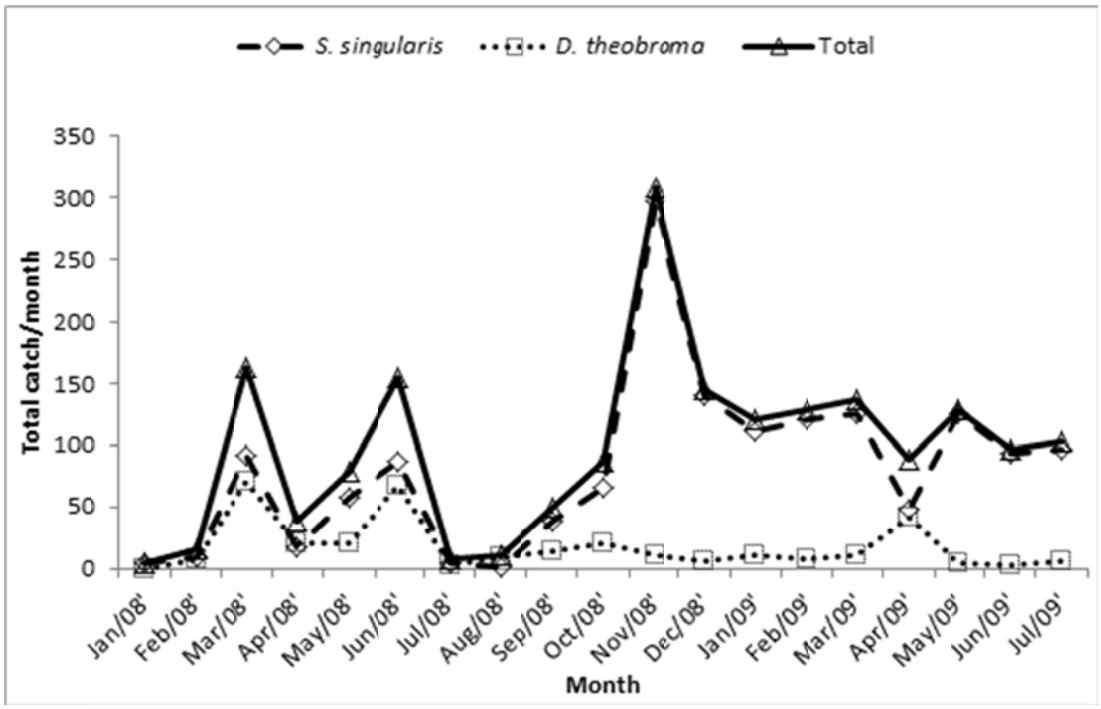

Figure 4.5 Monthly catches of *Sahlbergella singularis* and *Distantiella theobroma* in mass trapping traps at Acherensua (January 2008-July 2009).

ANOVA on total catches in treated plots before putting in the initial different numbers of monitoring traps i.e. from January 2008 to March 2008, analysed as *S. singularis* or *D. theobroma* only showed no significant differences between mean catches in subplots with raw data or $\sqrt{(x+0.5)}$ transformation (Figure 4.6 and Figure 4.7 respectively) (F = 0.09, df 4, 28, P = 0.986, and F = 1.10, df 4, 28, P = 0.375 for the respective species). This showed that subplots were homogenous and suitable to test effects of the different densities of monitoring traps.

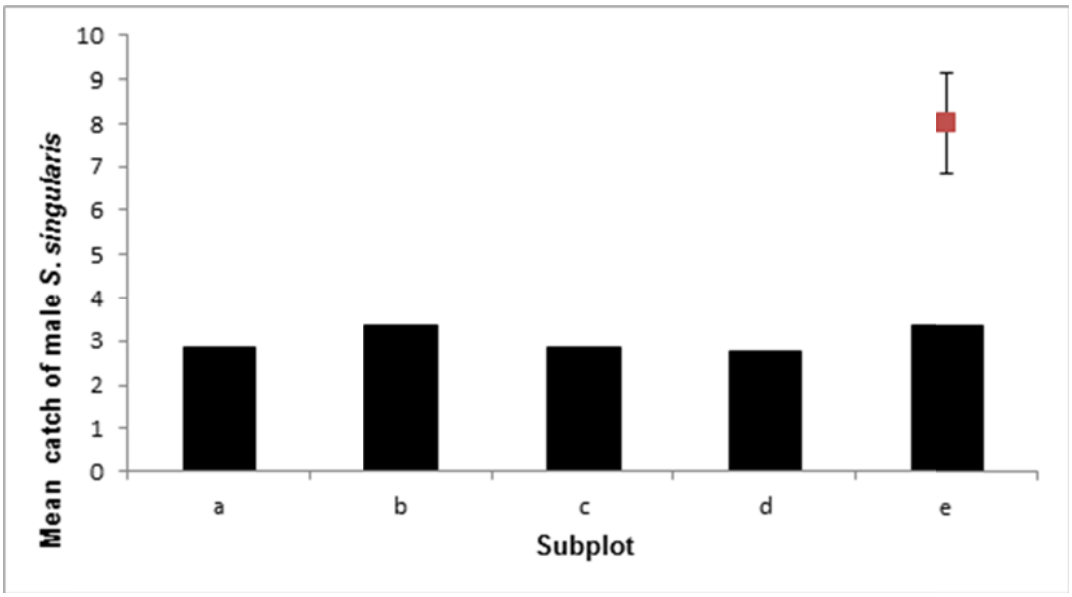

Figure 4.6 Mean catch per subplot of male *S. singularis* in mass trapping plots (15 traps/subplot; 8 replicates) prior to deployment of monitoring traps, January 2008-March 2008. Error bar shows standard error of difference between means.

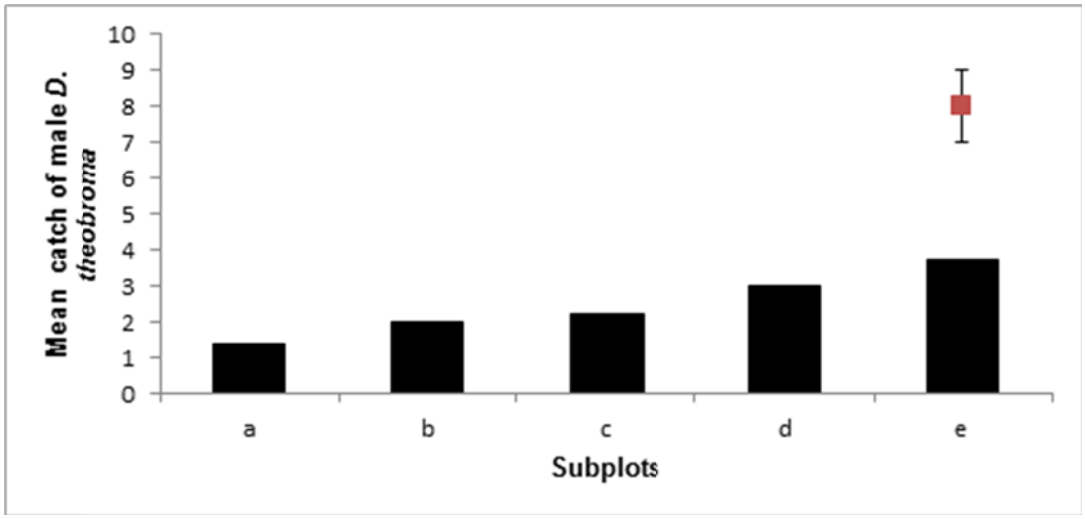

Figure 4.7 Mean catch per subplot of male *D. theobroma* in mass trapping plots (15 traps/subplot; 8 replicates) prior to deployment of monitoring traps, January 2008-March 2008. Error bar shows standard error of difference between means.

ANOVA on total catches by mass trapping traps only after deploying the different monitoring traps, analysed as *S. singularis* (Figure 4.8), *D. theobroma* (Figure 4.9) only or combined also showed no significant differences between mean catches in subplots with raw data or $\sqrt{(x+0.5)}$ transformation (F = 0.26, df 4, 28, P = 0.901; and F = 0.72, df 4, 28, P = 0.588, for the respective species). Thus monitoring trap catches were independent of mass trapping catches and the former in treated and control plots can be compared.

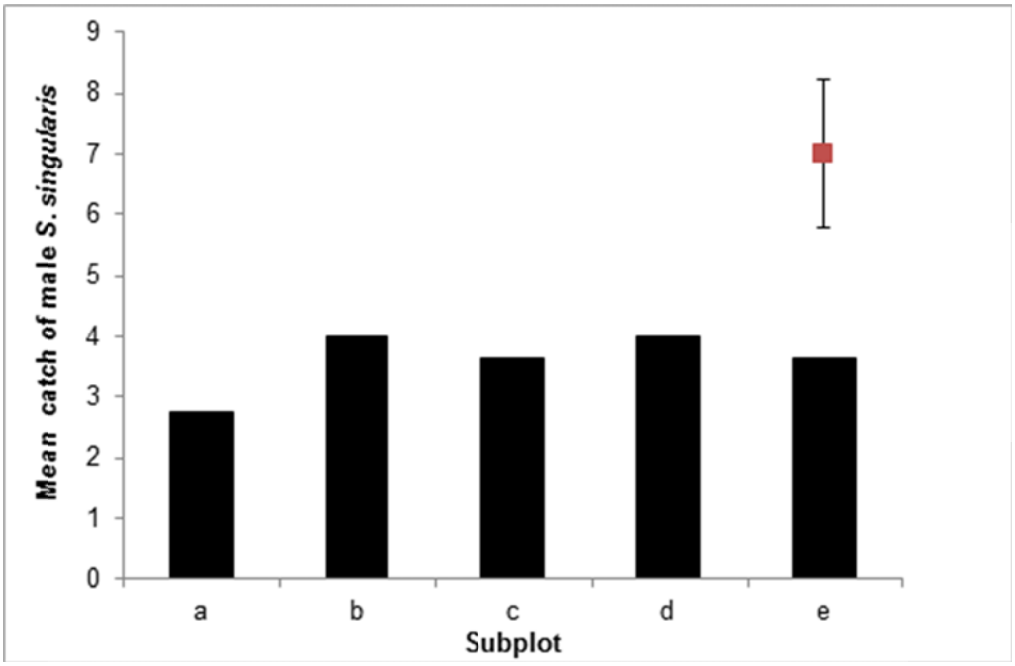

Figure 4.8 Mean catch per subplot of male *Sahlbergella singularis* by mass trapping traps only (15 traps/subplot; 8 replicates) after deployment of monitoring traps, May 2008-June 2008. Error bar shows standard error of difference between means.

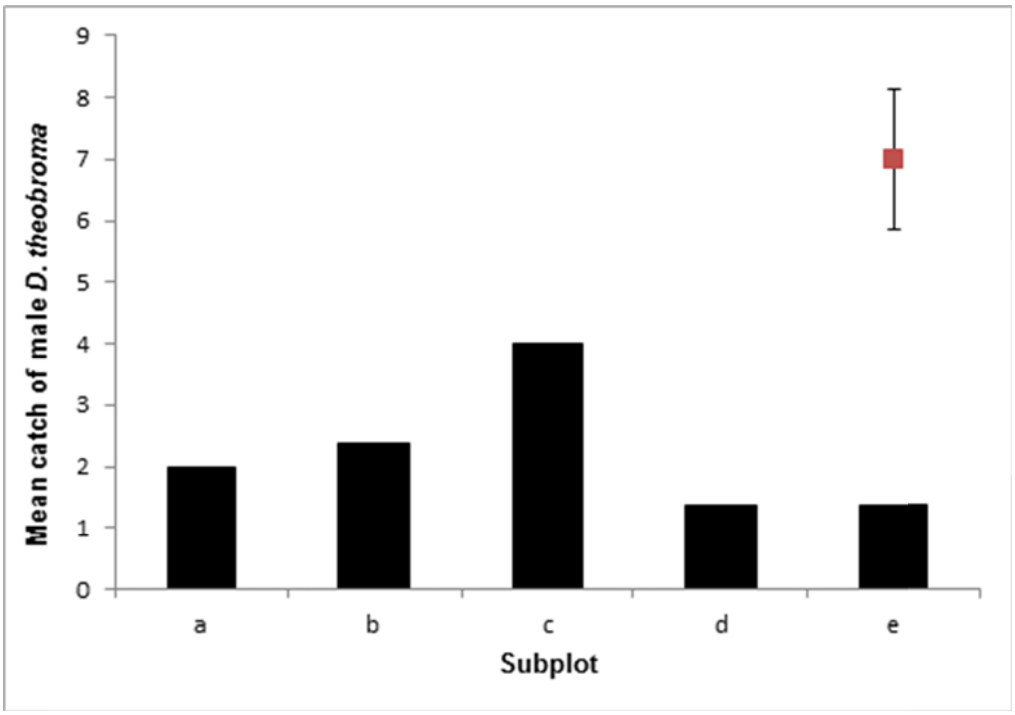

Figure 4.9 Mean catch per subplot of male *Distantiella theobroma* by mass trapping traps only (15 traps/subplot; 8 replicates) after deployment of monitoring traps, May 2008-June 2008. Error bar shows standard error of difference between means.

4.3.2 Monitoring with different densities of traps

A total of 267 male mirids, 216 of them *S. singularis* (81%) and 51 *D. theobroma* (19%), was caught in monitoring traps from 12 May to 24 June 2008, before the insecticide knockdown in July/August, 2008.

Male S. singularis

ANOVA on total catches for the period analysed as *S. singularis* only with raw data or transformed to $\sqrt{(x+0.5)}$ showed that mean total catch by all monitoring traps in control plot was significantly greater than that in treatment (F = 8.82, df 1, 42, P = 0.021). This showed that mass trapping has reduced male *S. singularis* numbers.

Mean total catches of male *S. singularis* by monitoring traps in treatment and control, summarised in Figure 4.10, showed that more insects were trapped with

increasing numbers of monitoring traps; mean catch in eight monitoring traps and 15 monitoring traps were significantly greater than two monitoring traps and four monitoring traps (F = 11.73, df 3,42, P = 0.001), though neither eight and 15 nor two and four differed significantly (P>0.05).

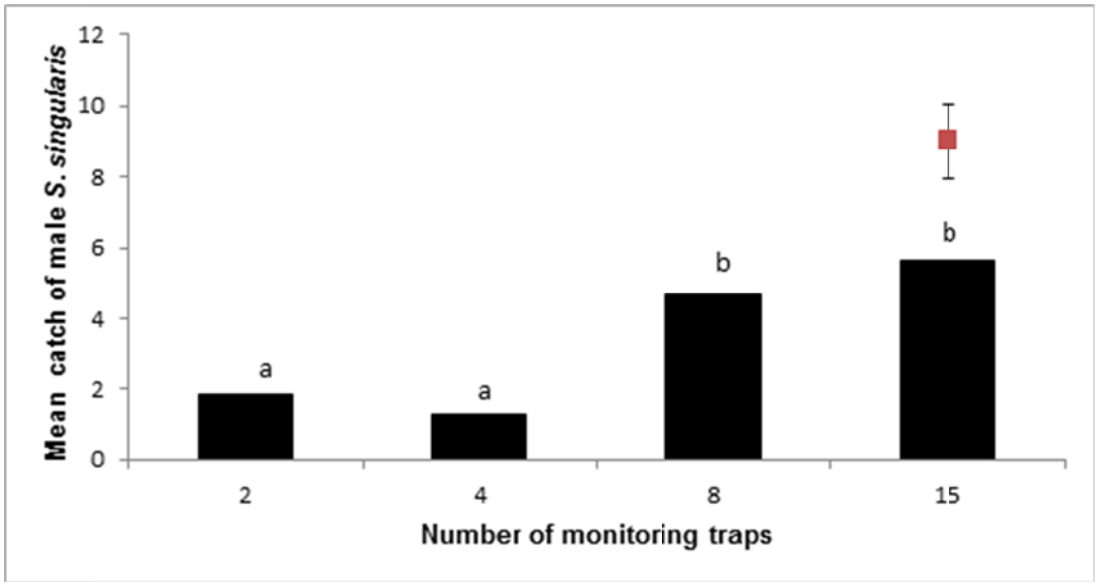

Figure 4.10 Average mean catch of male *S. singularis* by monitoring traps in treatment and control at Acherensua during the period 12 May-24 June 2008. Bars with different letters are significantly different (p<0.05) by LSD test after transformation of data to √(x+0.5) and analysis of variance. Error bar shows standard error of difference between means.

There was significant linear relationship between mean total catches and the number of monitoring traps of treatment and control (F = 30.26, df 1, 42, P = 0.001), which was expressed by the equation, y = 0.3265x+1.0057; where x is the number of monitoring traps and y the mean catch. From the equation, a unit increase in traps resulted in an increase mean catch of 0.33. Expression of the equation in a line on a graph drawn in Excel is shown in Figure 4.11. The intercept is 1.0 and the gradient, 0.33. The correlation coefficient is very high and the model accounts for 87% of the variance in data counts.

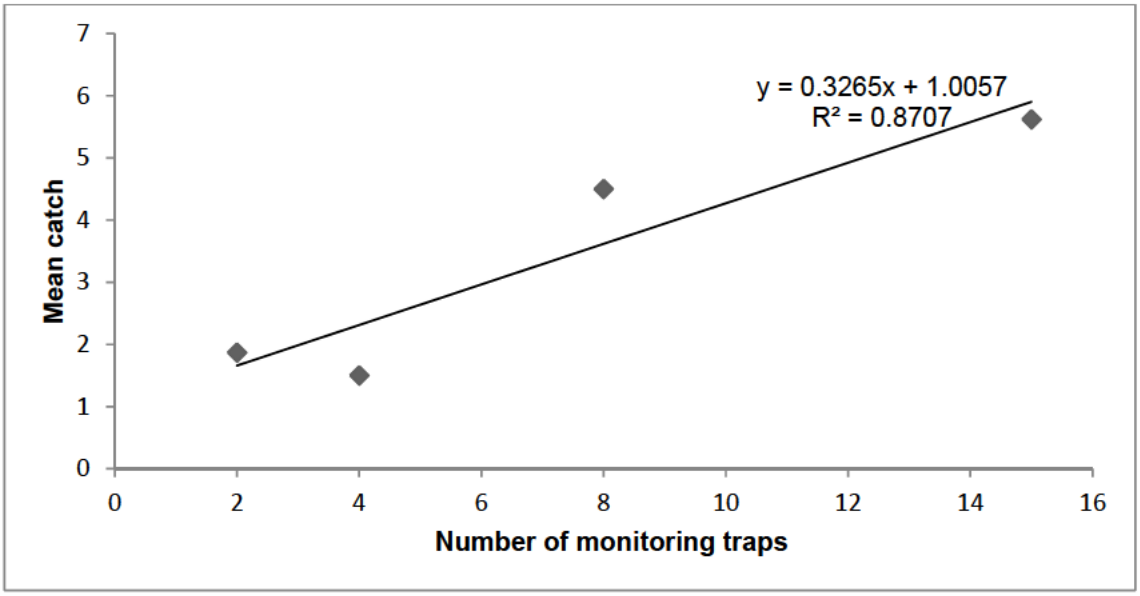

Figure 4.11 Relationship between average mean catches of male *S. singularis* and monitoring traps in treatment and control at Acherensua during the period 12 May-24 June 2008.

However, catches in monitoring traps of treatment plots had no significant relationship with the mass trapping traps (F = 0.027, df 3, 42, P = 0.848) showing that monitoring traps were independent of the mass trapping traps and thus catches in the monitoring traps in treatment and control were comparable. This is consistent with the results of catches by the mass trapping traps above.

Male D. theobroma

ANOVA on total trap catches analysed as *D. theobroma* only with raw data or transformed to $\sqrt{(x+0.5)}$ showed no significant difference in mean catches by monitoring traps of control and treatment plots (F = 0.05, df 1, 42, P = 0.838). However, mean total catches of male *D. theobroma* by monitoring traps in treatment and control summarised in Figure 4.12 showed that more insects were trapped with increasing numbers of monitoring traps; the mean catch in 15 monitoring traps was significantly greater than mean catches in two traps and four traps but not eight traps (F = 5.66, df 3,42, P = 0.002).

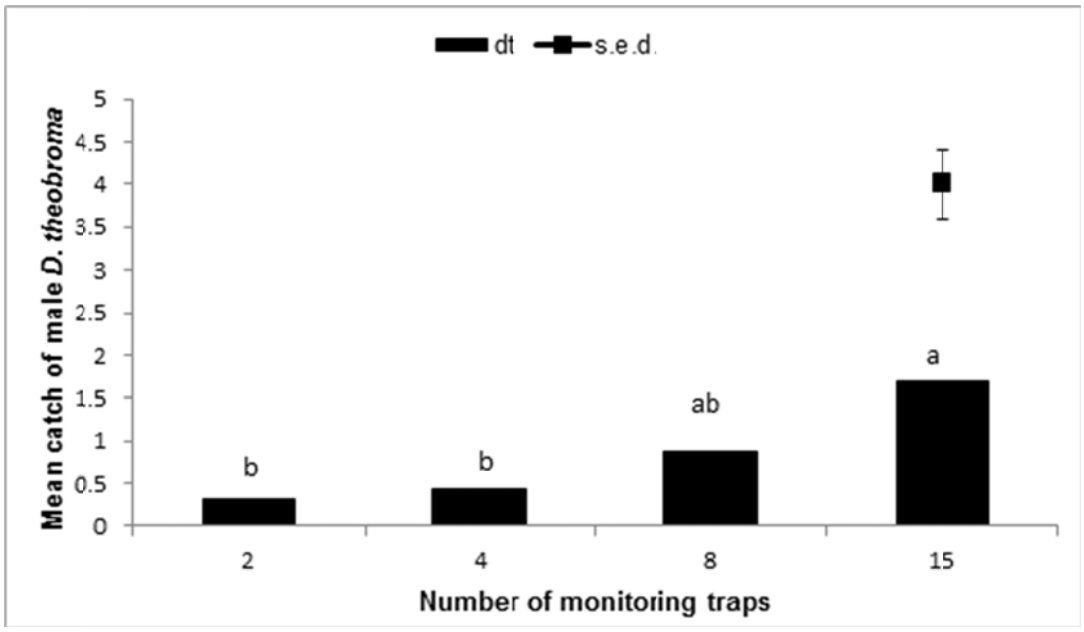

Figure 4.12 Average mean of male *D. theobroma* by monitoring traps in treatment and control at Acherensua during the period 12 May-24 June 2008. Bars with different letters are significantly different (p<0.05) by LSD test after transformation of data to $\sqrt{(x+0.5)}$ and analysis of variance. Error bar shows standard error of difference between means.

There was a significant linear relationship (P<0.05) between mean total catches and the number of monitoring traps of treatment or control (F = 16.89, df 1,42, P = 0.001) expressed by the equation y = 0.1126x−0.0191; where x is monitoring traps and y is the mean trap catch. The line expressing the equation is shown in Figure 4.13. The intercept of the line on a graph is -0.12 and the gradient is 0.11 which showed that a unit increase in monitoring traps resulted in a mean increase of 0.11 in trap catch. The coefficient of correlation was very high and the model accounted for approximately 98% of the variance in data counts.

Catches in monitoring traps in treatment had no significant relationship with mass trapping traps (F = 1.02, df 3,42, P = 0.395), showing that catches in monitoring traps can be compared between treatment and control, as with male *S. singularis* above.

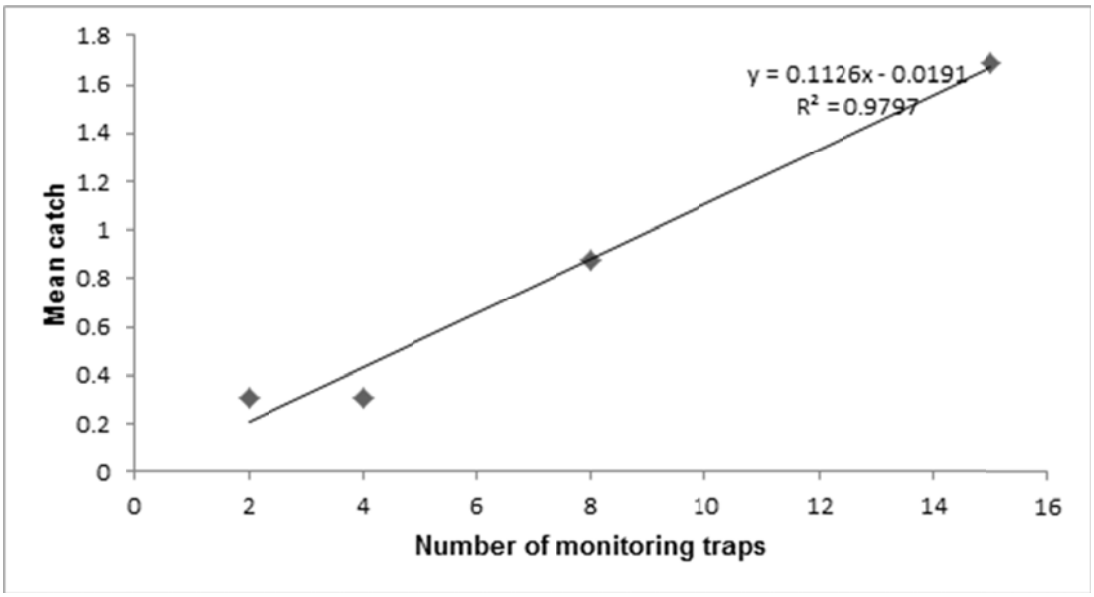

Figure 4.13 Relationship between average mean catches of *D. theobroma* and monitoring traps in treatment and control at Acherensua during the period 12 May-24 June 2008.

4.3.3 Regression relationships between trap densities and catches of male mirids

Catches by different densities of monitoring traps did not show any saturation indicating that more traps might be needed to trap the mirids than the density used. Trap catches of the mirids were therefore regressed on total numbers of monitoring traps (14 April-24 June) and mass trapping traps (May-June) together in the respective subplots to determine any saturation point.

Male S. singularis

Results of the analysis showed that there was significant (P<0.05) parallel regression relationship between the number of traps and trap catches of male *S. singularis* among the blocks. The regression model which had number of traps and blocks as explanatory variates accounted for 16.7% of variance in trap catch records (F = 8.13, *df* 2,69, P = 0.001) while the blocks term (F = 2.23, *df* 7,62, P = 0.01) accounted for 26.0%.

Figure 4.14. shows the fitted and observed values of the relationship. The X is the observed values and the line is the average fitted values. The line shows curvature and the best fit model curve is $\mathbf{y = a+br^x}$ where **x** is the number of traps and **br** is the coefficient of regression. The term **a** is the intercept of the curve on the y axis of a graph. The curve flattens at 15 traps. This showed that the optimum mass trapping rate was 15 traps per subplot of 0.1 ha or 150 per hectare for *S. singularis*.

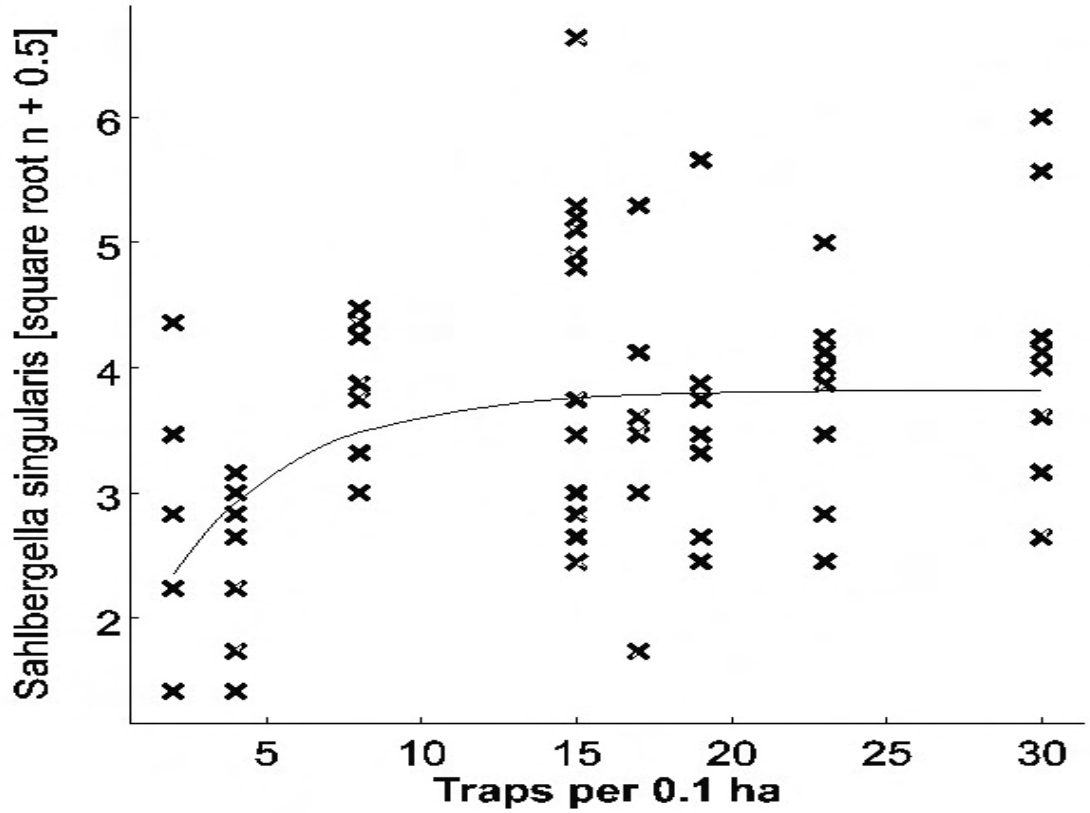

Figure 4.14 Relationship of total trap catches of male *S. singularis* regressed on different densities of traps, start April to end 24 June 2008.

Male D. theobroma

Results of the analysis showed that there was significant (P<0.05) parallel regression relationship between the number of traps and trap catches of male *D.*

theobroma among the blocks. The regression model which had number of traps and blocks as explanatory variates accounted for 25.5% of variance in trap catch records (F = 13.18, *df* 2,69, P = 0.001) with the blocks term (F = 3.82, *df* 7,62, P = 0.01) also accounting for 42.1%.

Fitted and observed values of the relationship are shown in Figure 4.15. In the *D. theobroma* also the line shows curvature and the same model curve is fitted. However, for *D. theobroma* the curve flattened at 23 traps showing that the optimum mass trapping rate was 23 traps per subplot of 0.1 ha or 230 per hectare.

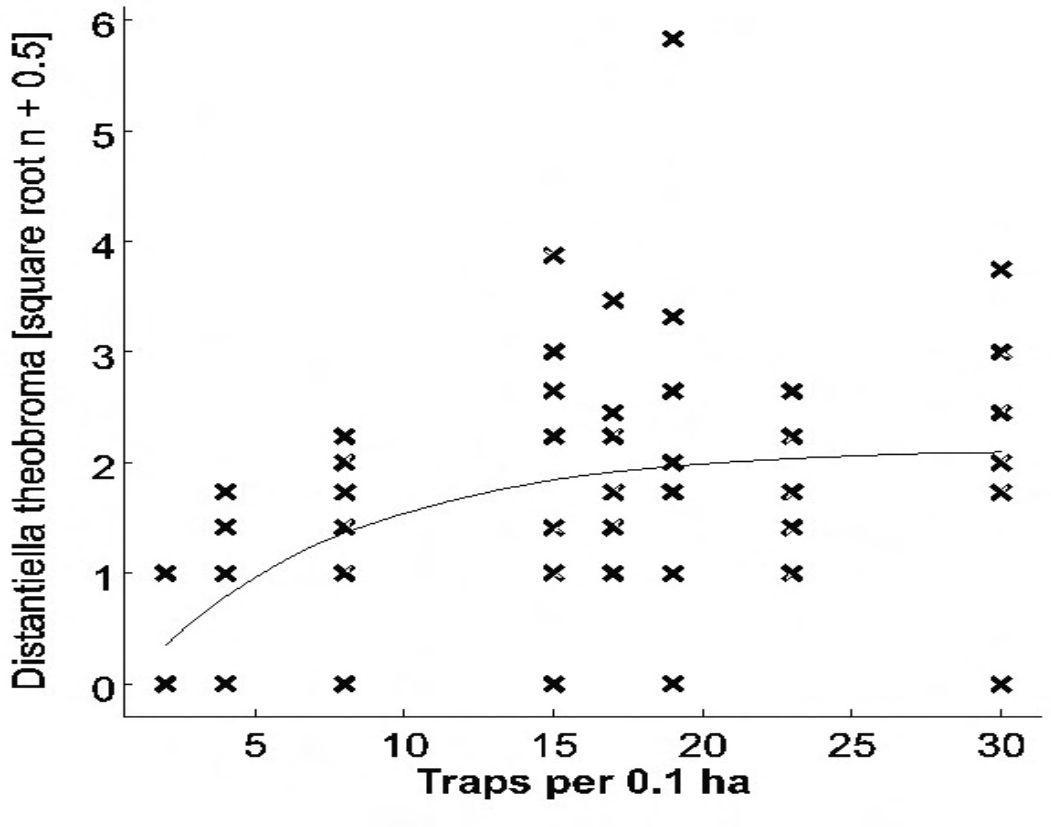

Figure 4.15 Relationship of total trap catches of male *D. theobroma* regressed on different densities of traps, start April to end 24 June 2008.

4.3.4 Assessment by insecticide knockdown

A total of 117 mirids consisting of 19 adult and 13 nymph *S. singularis* (27%) and 13 adult and 72 nymph *D. theobroma* (73%), were recorded from insecticide knockdown. Results of two unpaired sample *t*-test analyses on the data showed that trapping did not result in significant decrease in the total populations of the mirids indicating that it had not been effective. Mean numbers of the adult and nymph mirids knocked down did not differ significantly between control and treatment (Adult *S. singularis*; $t = 1.05$, df 78, $P = 0.298$: Nymph *S. singularis*; $t = 1.15$, df 78, $P = 0.257$: Adult *D. theobroma*; $t = 1.09$, df 78, $P = 0.280$: Nymph *D. theobroma*; $t = 0.89$, df 78, $P = 0.378$). However, as shown in Figure 4.16 there was some numerical decrease in both adult and nymphal populations of *S. singularis* in the treated plots.

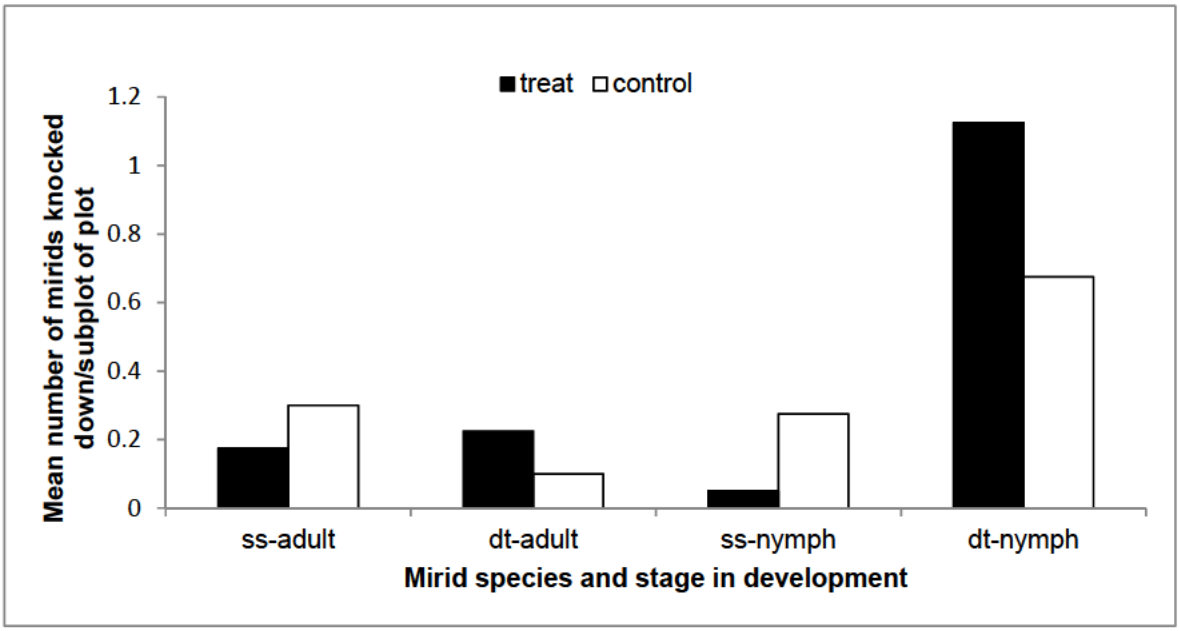

Figure 4.16 Mean knockdown count per plot of adults and nymphs of *S. singularis* and *D. theobroma* in treatment and control, 5 August 2008.

Results of comparisons of total knockdown counts of nymphs of the two species in treatment and control by 2x2 Chi-square test of independence, showed significant differences between total counts of the species in both treatment and control

(*P*<0.05). There were 22.5x as many *D. theobroma* nymphs as *S. singularis* nymphs in the pheromone-treated plots, and 2.5x as many *D. theobroma* nymphs as *S. singularis* nymphs in the control plots (X^2 = 9.20 *df* 1, *P* = 0.001; Yates correction). This showed with very high probability that pheromone trapping changed the balance between the two species with a higher proportion of *S. singularis* males entering the traps than *D. theobroma* males. These results are consistent with those of the monitoring by different densities of traps.

4.3.5 Monitoring by solitary traps

A total of 277 male mirids consisting of 270 *S. singularis* (97%) and 7 *D. theobroma* (3%) were caught in single monitoring traps from September 2008 to July 2009. Unpaired two sample *t*-test analysis on data of *S. singularis* only or *D. theobroma* only, showed significant differences (*P* < 0.05) between plots. Mean catch of male *S. singularis* in control plots was over 8-fold that in treatment plot (*t* = 4.97, *df* 78, *P*=0.001). Thus trapping was effective in decreasing male *S. singularis.* This result is similar to the results of the multiple traps. Similar analysis on data on male *D. theobroma* also showed significant differences between control and treatment plots (*t* = 2.48, *df* 78, *P* = 0.015). However, while none was caught in treatment, only 7 were caught in control and so definitive conclusions could not be made.

ANOVA on raw or transformed data to √(x+0.05) on *S. singularis* for the period, showed no significant differences (*P* > 0.05) between mean catches in individual subplots which once had two, four, eight and 15 monitoring traps deployed on them (Figure 4.17) (*F* = 0.83, *df* 4,56, *P* = 0.510) showing that there was no long-term or carry-over effect of the previous deployment of the monitoring traps.

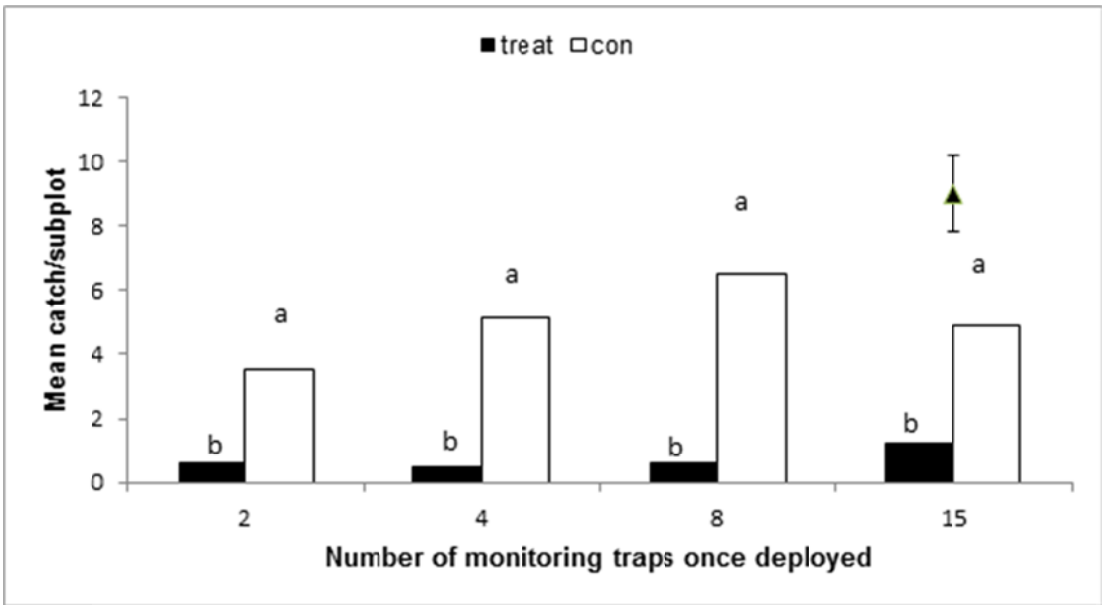

Figure 4.17 Untransformed mean catch per subplot of male *S singularis* by single monitoring traps, showing similarities in catches in control or treated subplots previously trapped with varying numbers of monitoring traps at Acherensua (24 September 2008-9 July 2009; 8 replicates). Bars with different letters are significantly different (P<0.05) by LSD test after transformation of data to √(x+0.05) and analysis of variance. Error bar shows standard error of difference between means.

4.3.6 Visual assessment of mirid numbers

Untransformed mean numbers of male and female adult *D. theobroma, S. singularis* and combined nymphs of the two species visually assessed from September 2008 to July 2009 in the plots, are shown in Figure 4.18. Results showed that trapping did not reduce the overall population of mirids. Results of unpaired two sample *t*-test analysis on mean numbers of adults and nymphs of mirids showed no significant differences between the treated and untreated plots (*S. singularis*; t = 0.70, *df* approximately 70.62, P = 0.486: *D. theobroma*; t = 0.99, *df* 78, P = 0.325: nymphs; t= 0.73, df 78, P = 0.465). The results of *S. singularis* are surprisingly not reflective of the significant (P < 0.05) reduction in males shown by the monitoring traps. They are however, consistent with the knockdown results on the overall populations of adults and nymphs.

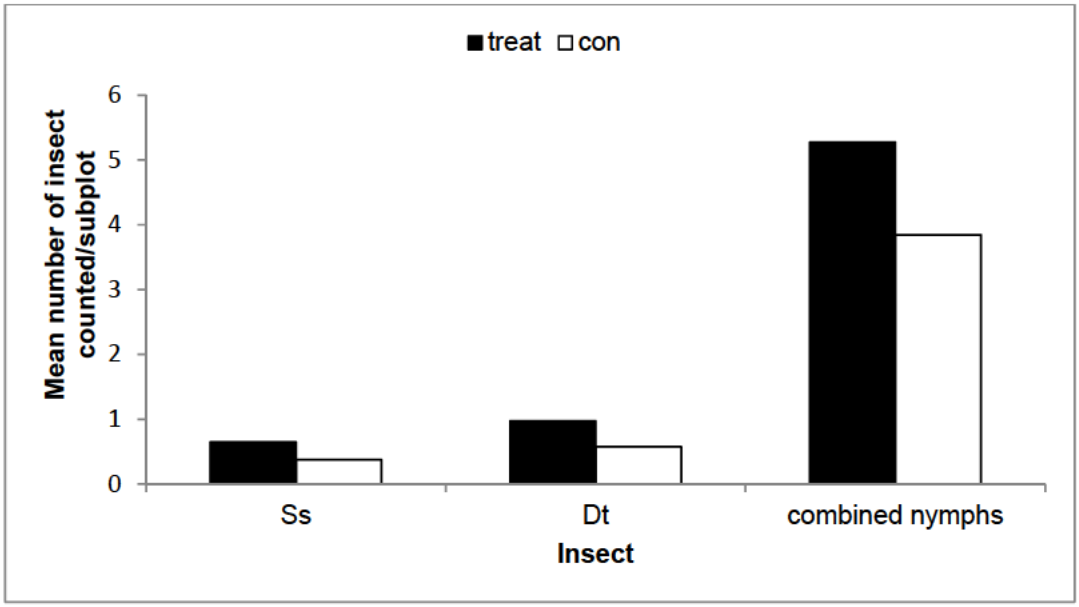

Figure 4.18 Untransformed mean count per subplot of adult *S. singularis, D. theobroma* and nymphs of both species from treatment and control plots (15 trees/subplot) in 8 replicates of mass trapping experiment at Acherensua, from 24 September 2008 to 9 July 2009.

4.3.7 Visual assessment of mirid damage

Untransformed mean numbers of damaged pods and shoots visually assessed from September 2008 to July 2009 in the plots are shown in Figure 4.19. Analyses by *t*-tests on mean numbers of pod and shoot damage, showed no significant differences ($P > 0.05$) between the treated and untreated plots (pod; $t = 0.32$, df 78, $P = 0.752$: shoot; t = 0.35, df 78, $P = 0.729$) showing that mirid damage was not reduced by pheromone trapping. The results also reflected the presence of the mirids as assessed both visually and by knockdown.

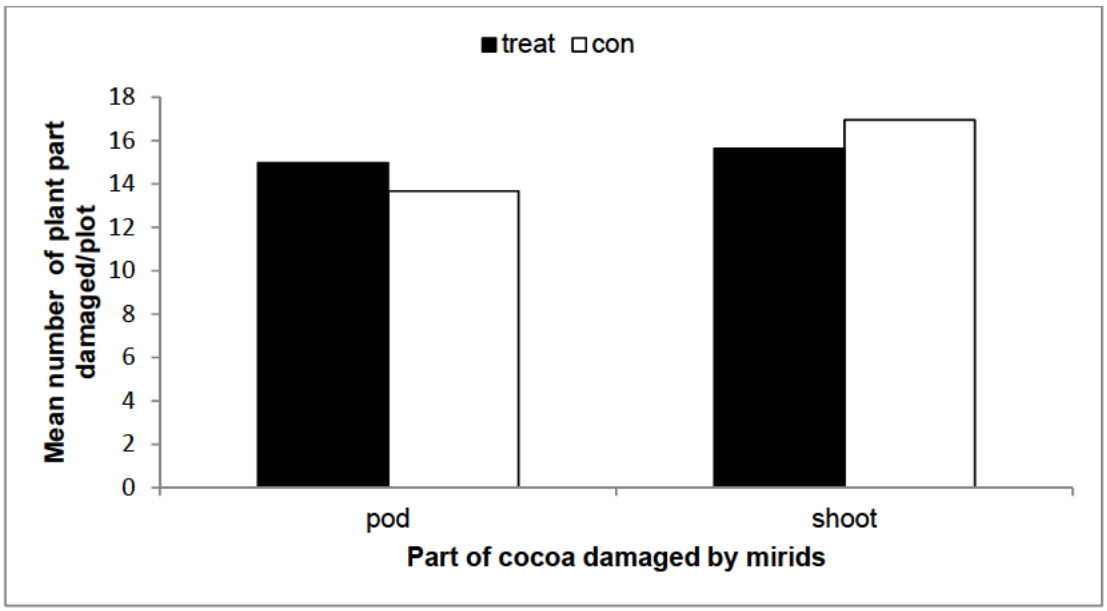

Figure 4.19 Untransformed mean count per plot of pods and shoots from treatment and control plots (75 trees/plot) in 8 replicates of mass trapping experiment at Acherensua, from 24 September 2008 to 9 July 2009.

4.4 DISCUSSION

4.4.1 Effectiveness of mass trapping as a method of mirid control

Mass trapping was undertaken to find out if the method could be used as an alternative to the application of insecticides in the control of mirid infestation of cocoa. From the results of the study, mass trapping did not demonstrate the ability to significantly reduce the total numbers of mirids, neither did it protect the crop. Results of assessments of the effectiveness or otherwise of mass trapping by monitoring traps consistently showed significant reduction ($P < 0.05$) in numbers of male *S. singularis* and *D. theobroma* trapped. Insecticide knockdown and visual assessments, however, showed definitively that not enough reduction had occurred in population numbers of the mirids to halt their damage to pods and shoots, indicating that the reproduction potential of the mirids was not lowered. The ineffectiveness of trapping against the mirids was manifested in subplots by

the high infestation levels particularly in the peak season despite the continuous trapping.

Possible reasons for ineffectiveness of mass trapping

Several reasons including re-infestation from immigration, high density of mirids, low trap density for *D. theobroma* and non-optimal trapping height may be assigned for the failure of the mass trapping. Since the plots were not isolated there could have been immigration of mirids from surrounding cocoa to re-infest the trapped areas which were small also. Re-infestation by immigrant pests into trapped areas has been reported by several workers as being the main cause of failure of the method. For example, Madsen and Carty (1979) evaluated mass trapping for removal of the codling moth in three orchards in the Okanagan valley and showed that one critical reason for the success of mass trapping was the isolation of the moth population to prevent re-infestation of trapped plots. Huber *et al.* (1979) also concluded from their investigation on the possible control of the pink bollworm by mass trapping, that one of three pre-requites for successful control by mass trapping of the insect was the isolation of the trapping area. The failure of mass trapping to reduce the incidence of Dutch elm disease of the American elm tree despite the successful trapping of the disease vectors, the European elm bark tree beetles, was attributed to immigration of the beetles from outside the treated area (see review by El-Sayed *et al*., 2006).

Sex attractant traps may be less effective when populations of pests are high (e.g. Madsen, 1967). The period of peak catches (October-December) showed periods of high density of the mirids by inference and this might have contributed to the failure of mass trapping. Roelofs *et al.* (1970) demonstrated that sheer large numbers of the pests would increase attraction by virgin females more than the trap. They showed both practically with data from field pheromone trapping of the red banded leaf roller moth *Argyrotaenia velutinana* (Walker), and theoretically with a programme for calculating the control of mating by sex pheromone trapping that control of flights (hence population) was 99% with 0% damage in low density but only 48% with 32% damage in high density.

The number of traps used in the experiment which was adopted from Sarfo *et al.* (2007) showed that while the adequate density of pheromone traps were deployed to trap *S. singularis*, that for *D. theobroma* was inadequate and fell short by about 23%, and this could have contributed to the failure. In the Lepidoptera, Sternlicht *et al.* (1990) revealed a clear tendency to control the *Prays citri* numbers and damage to flower by larvae only when the required minimum density of 120 traps per hectare were deployed and also maintained through the entire year

Pheromone traps were placed at 1.8 m vertically on the tree as an adoption from Sarfo *et. al*. (2007), who using a virgin female as lure, found 3.5x as many mirids trapped at 1.8 m as at 2.7 m and more than 2x as many at 1.8 m as at 0.6 m. However, trapping at 1.8 m missed a greater proportion of the male mirid flights because traps collected between 8.5-10x lower than the catches at optimal height in canopy (see section 3.4). Therefore, mass trapping at 1.8 m in the study removed less mirids which also helps to explain the failure to control mirid populations and their damage. Possible reasons for the failure of mass trapping of mirids in this study are discussed further in Chapter 8.

4.4.2 The present study in relation to previous studies

This is the first time extensive and detailed mass trapping of cocoa mirids has been done on a large scale. The only reports available on pheromone mass trapping of cocoa mirids are the less replicated studies by Sarfo *et al.* (2007) and Ayenor *et al.* (2007). Sarfo *et al.* (2007) carried out mass trapping in an unreplicated plot experiment to determine the optimal density of traps for trapping cocoa mirids. No assessment of trapping to suppress mirid numbers or their damage was made and therefore the success of their trapping to control the mirids was not known. Ayenor *et al.* (2007) compared control of mirids by pheromone mass trapping and two other methods, viz; organic insecticide using aqueous neem extract and biological control using predation by the weaver ant *Oecophylla longinoda*. They conducted the experiment on farmers' farms used for organic cocoa production at in the Brong Densuso area of Akwadum in the

Eastern region of Ghana. Using NRT sticky traps baited with standard lure at density of 32 traps /ha separated at 6 m intervals and replicated three times with lures changed once every three months, they reported that synthetic pheromone controlled mirid numbers and damage as well as neem and *Oecophylla longinoda*. The results of this present study are at variance with those by Ayenor et al. (2007). However, the results by Ayenor *et al.* (2007) ought to be interpreted with caution because not only was the experiment small, but also field and laboratory trials by Adu-Acheampong (1977) in Tafo, Ghana, clearly showed that aqueous neem extract caused mortality in mirids that were below acceptable levels.

Attempts at using pheromone mass tapping for control abound in other insect groups particularly Lepidoptera where the record of successes appear to be matched by those of failures. For example, Huber *et al.* (1979) reported failure to control the pink bollworm despite reduction in numbers so also did Wilson and Trammel (1980) in the codling moth and Pasqualini *et al.* (1997) in the leopard moth *Zeuzera pyrina* L and *Cossus cossus*. Hagley (1978) and Yamanaka *et al.* (2001) neither achieved reduction nor control in the codling moth and fall webworm, *Hyphantria cunea* (Drury) respectively. In the light of the failure of mass trapping to control mirids, which appears not to be an exception, it might be worthwhile considering the more successes achieved with mating disruption and lure and kill (reviewed by El-Sayed *et al*., 2006 and Witzgall *et al*., 2010).

4.4.3 Trap density and captures of male mirids

The results of the regression analysis showed that the optimal densities for trapping males of *S. singularis* and *D. theobroma* were 150/ha and 230/ha respectively. The density of traps for the capture of male *S. singularis* is exactly the same as reported by Sarfo *et al.* (2007), who trapped male cocoa mirids dominated by *S. singularis* with trap densities equivalent to 20, 40, 80 and 150 traps /ha on research plantations at Tafo, and found the highest catch by 150 traps/ha. They observed that catches increased with the number of traps which is also confirmed by the results of the present study which generally showed

increasing mirid catches with increasing trap density. Indeed the number of mass trapping traps used in the present experiment was adopted from their report.

At optimal densities the traps would have captured all available males and additional traps would not increase catches. In reality, however, this interpretation would be too optimistic because knockdown and visual assessments showed no decline in the populations of the mirids. It could be that the optimal densities were too high, giving out great amounts of pheromone within the plot which might have rendered the male mirids less capable of orienting to the traps. In the earlier CRIG/NRI/CABI studies on the pheromone trapping of mirids, none of the species was captured when the dosage of the lure was multiplied in traps (Padi *et al.,* 2002). Also a study by Larraín *et al*. (2009) in Valle del Elqui, in the Coquimbo Region of Chile to determine the effect of trap density on captures of the potato tuber moth*, Phthorimaea operculella* (Zeller) showed similar results. Using 5 L capacity plastic drums containing 2 L of water for capture Larraín *et al*. (2009) showed that catches increased with densities from 20 to 40 traps/ha, but decreased drastically when density was increased to 84 traps/ha in another study (Ortu and Floris, 1989). Sexual confusion of the insect at that density was suggested to be responsible.

However, the high numbers of traps needed to reach saturation point in the capture of the mirids may be due to their patchy distribution (Bisseleua *et al.,* 2011; Squire, 1947; Williams, 1953b) which requires the deployment of more traps overall to cover larger areas to trap them. The higher number needed to trap *D. theobroma* could be because they are more aggregated than *S. singularis* (Gibbs *et al.,* 1968).

4.4.4 Low trap catches of *D. theobroma*

Despite the fact that the synthetic pheromone used in this trial was based on results from females of *D. theobroma*, surprisingly this species appears to be inefficiently trapped by the pheromone traps. This concurs with the observation by King (1973). In an experiment to attract male *D. theobroma* into a trap using virgin

female as lure, he recorded low capture of wild males in the trap; only 73 wild males were caught in 20 traps in 70-day trapping period. The reason he suggested for this apparent weak response by the males was the possible confinement of mating within local aggregations of the species, which would make lures outside the aggregated species have small influence, and incapable of competing with attraction by virgin females within. This suggestion may hold true for the similar observation made in this study. Perhaps saturating the field with higher amount of the pheromone might give it greater influence to disrupt mating between the mirids.

4.4.5 Trap catches and population dynamics of mirids

Results of total monthly catches in 2008 reflect the bimodal incidence of mirids at Acherensua, the first maximum of which is between February and March and the second from July to December. This trend reflects the population dynamics of the mirids in Ghana as reported first by Gibbs *et al.* (1968). The authors employed sampling methods such as visual counts of adult and nymph mirids to hand height levels in uncoppiced trees and on whole trees in coppiced crop, as well as knockdown spraying of insecticides and collection of mirids on cotton sheets. They showed that on average low numbers are recorded from February to July and high numbers from August to January. This fluctuation in population was subsequently confirmed by work on population dynamics of the mirids (Owusu-Manu and Somuah, 1989)

The reflection of the population dynamics of mirids by trap catches indicates the potential of pheromone trapping to monitor the incidence of cocoa mirids, as already suggested by results of the blend and trap design experiments in Chapters 2 and 3 respectively. The dip in September observed in the second year followed the insecticide application carried out in July and August in the knockdown assessment.

The homogeneity of the mass trapping plots as shown by results of trapping before the introduction of monitoring traps engendered comparison of trapping

between treatment and control plots. The independence of catches by the mass trapping and monitoring traps showed non-interference of synthetic pheromone plumes from adjacent traps and also provided the environment for valid assessment of the effectiveness of mass trapping by the monitoring traps. These results are, therefore, a vindication of the method used.

4.4.6 Novelty of experimental approach

The method used was novel to mass trapping though adapted from one originally used in mating disruption experiment (Stelinski *et al*., 2005). It had the advantage of circumventing the setting up different experiments in several plots to test mass trapping and also determine optimal densities for trapping the mirids. Considering the fact that pheromone trapping is slow acting, limiting the space for experiments helps to reduce the apprehension and concern generated as a result of the apparent persistence of damage in plots during trapping, as opposed to the quick results in conventional insecticide application.

Chapter 5
MASS TRAPPING AS A METHOD OF CONTROL OF MIRIDS ON SMALLHOLDERS' FARMS (2009-2010)

5.1 INTRODUCTION

Chapter 4 described a trial of mass trapping to control cocoa mirids on a research plantation at Acherensua. Although catches of mirids in monitoring traps were reduced in the treated plots, there were no differences between treated and untreated plots in terms of numbers of mirids assessed by knockdown or damage caused by mirids. Several factors were suggested as being possible reasons for the failure of the mass trapping. It was suggested that the small sizes of the plots (0.5 ha) might not have precluded treated plots from re-infestation from surrounding cocoa. The results further suggested that perhaps trapping larger plots than those selected at Acherensua might reduce immigration and re-infestation and improve the efficacy of the method. This had to be tested before the end of the Acherensua trial in order to get sufficient time for the trial within the life of the project. Advantage was therefore, taken of an opportunity offered by funding from the World Cocoa Foundation (WCF).

Mass trapping at Acherensua was done on a research plantation where agronomic practices such as weed clearing, pruning, thinning, planting distances, mistletoe removal etc. were near optimal. However, in Ghana and West Africa generally, cocoa is produced by peasant farmers whose agronomic practices are less optimal than in research plantations. This might affect the behavioural responses of the mirids to the traps since the trial environments are different.

This Chapter describes a mass trapping trial done on farmers' farms with the dual objectives of examining the effects trapping larger plots would have on the results and also determining the effects of mirid response to pheromone trapping in farms where agronomic practices are less than optimal.

For the achievement of these objectives trapping was done on farmers' farms used for organic cocoa production. Six farms mapped out to be at least 1.5 ha each were selected. However, after setting up the traps only three had areas of cocoa larger than the Acherensua plot size (0.5ha).

5.2 MATERIALS AND METHODS

5.2.1 Study sites and plots

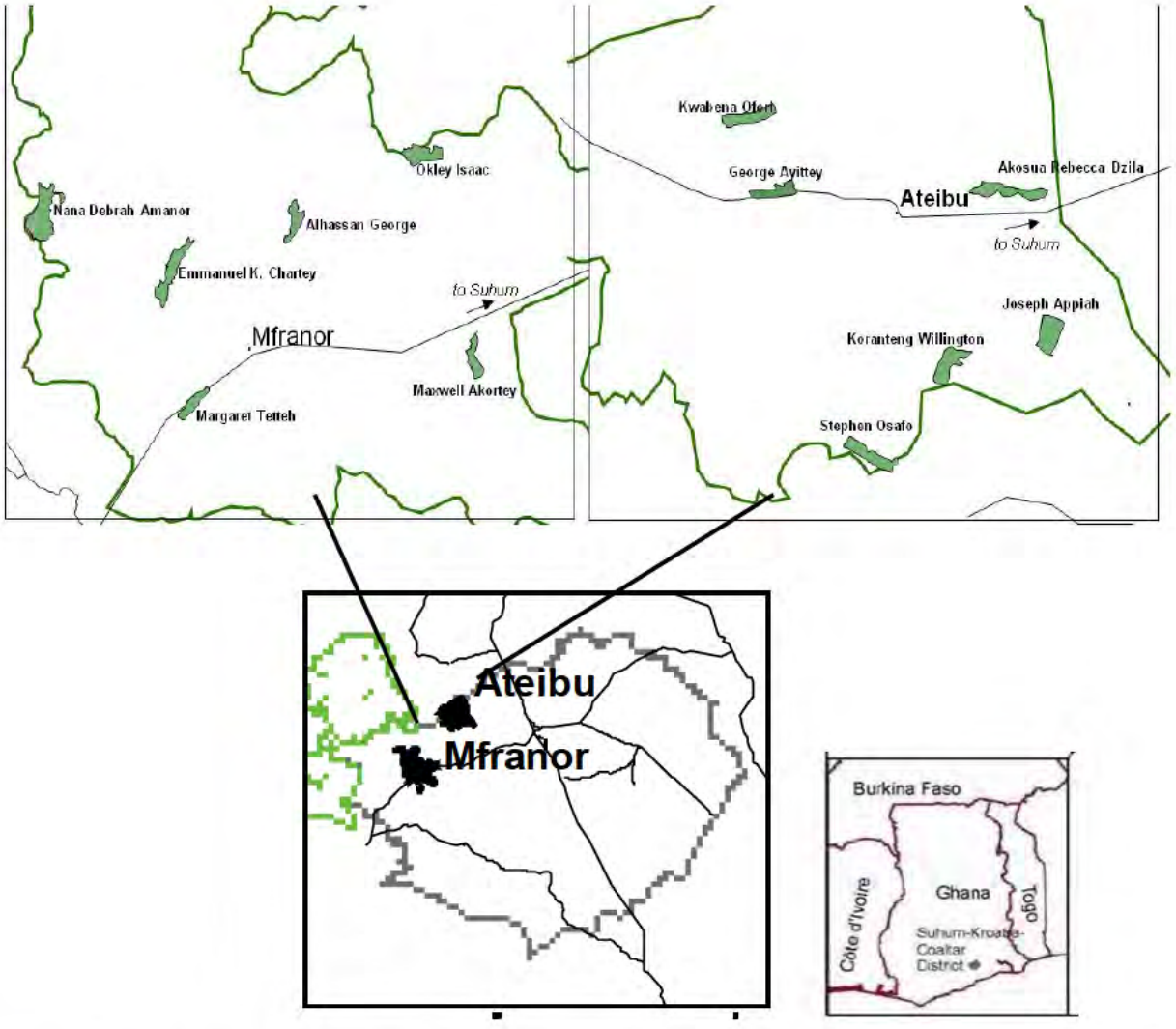

Figure 5.1 Farmers' plot at Mfranor and Ateibu in the Suhum/Kraboa/Coaltar District of the Eastern Region of Ghana.

The study was carried out on experimental plots selected at Mfranor and Ateibu (Figure 5.1) in the Suhum Kraboa Coaltar district of the Eastern Region of Ghana. The farmers were members of Yahyra Company, a group licensed to facilitate production, purchase and export of organic cocoa in Ghana. The farms were selected for the trial for three reasons: the presence of mirids, the non-application of insecticides and the enthusiasm of farmers borne out of the expectation of an efficacious method to replace the ineffective neem extract they were applying. The farmers agreed and expressed their happiness to see their names mentioned in the thesis.

Mfranor study site and experimental plots

Mfranor is located in tropical forest zone (05° 59' 874'' North, 000° 34' 001'' West) in the Suhum Kraboa Coaltar District, about 220m above sea level. The climate is tropical with two rainy seasons and two dry seasons. Annual rainfall varies between 1,270 mm and 1,651 mm with relative humidities of between 50% and 89%. The mean temperature is relatively constant around 26.5°C (Sourced from CRIG Meteorological station, Tafo). The soils are loamy and suitable for the cultivation of cocoa. Three farms belonging to Margaret Tetteh, Maxwell Akote and Alhassan George were selected as treatment plots and another three belonging to Emmanuel Chartey, Nana Debrah Amanor and Isaac Okley were selected for untreated controls at Mfranor (Figure 5.1).

Margaret's farm. This was about 1.5 ha in size. It was bordered on all sides by cocoa except one side which is a vehicular road. The farm had a lot of open areas without cocoa. The cocoa trees were about 30 years old and estimated about 6.5 m in height. The crop was of mixed hybrid and was planted irregularly on a gentle slope. The farm had many shade trees, more than the CRIG recommended six trees per hectare. Agronomic practices such as chupon removal, thinning and mistletoe removal were rarely followed by the farmer.

Akote's farm. This was a large farm covering an area of about 2.0 ha. It was bordered on all sides by cocoa except one side which was a vehicular road. About a third of the farm was about 15 years old, the rest was about 25 years old. The trees were tall, estimates ranging from an average of 5 m tall in the young crop to

about 7 m in the old one. They consisted of mixed hybrid, irregularly planted close together. The farm had an undulating topography of slopes and valleys. There were some open areas without cocoa which remained weedy during most of the trapping period. Thinning of trees, removal of basal chupons and pruning of the canopy were not practiced.

Alhassan's farm. This was about 2.0 ha in size. The farm was contiguous with other farms on all sides. There were few open areas. The trees which were about 25 years old, were estimated to average about 7 m in height. The canopy was closed for most part of the farm. The crop consisted of mixed hybrid planted irregularly on two slopes. There were more than six shade trees in one hectare which provided dense shade to the farm. Most of the farm was without weed and basal chupons were regularly removed. However, thinning and pruning of trees were rarely done.

Chartey's farm. This farm covered an area of about 2.0 ha. It was contiguous with cocoa farms on all sides. The farm was about 15 years old and it was cultivated on a flat land. The trees were estimated at about 5 m high in height. They consisted of mixed hybrid and were planted irregularly and close together. The canopy was closed except for few open areas. There were more shade trees than the CRIG recommended density. Basal chupon removal, thinning and pruning were sparingly practiced.

Amanor's farm. This was the largest farm covering over 4.0 ha. It was contiguous with cocoa farms on all sides, some of which were not used for organic production. The farm was about 15 years old. The trees were estimated to be about 5 m high in height, consisting of irregularly planted but well-spaced mixed hybrid. The canopy was generally closed except for few open areas. The number of shade trees conformed to the CRIG recommended density of six trees per hectare. Agronomic practices such as basal chupon removal, thinning, pruning weeding and general farm sanitation were satisfactorily practiced.

Okley's farm. The size was about 2.0 ha. and was bordered on two sides by cocoa farms and the remaining sides by food crop farms. The farm was about 15 years old and was cultivated on slopes. The trees were young but tall and

estimated to be about 6 m in height. They consisted of mixed hybrid and which were planted irregularly but well-spaced about 3 m apart. The canopy was closed. The number of shade trees was about six per hectare. The farm had no weedy undergrowth and basal chupon removal, thinning, and pruning were done to some degree.

Ateibu study site and experimental plots

Ateibu is located in the tropical forest (06° 03' 251'' North, 000° 31' 454'' West), also in the Suhum Kraboa Coaltar District, about 210 m above sea level. The climate is tropical with two rainy seasons and two dry seasons. Annual rainfall varies between 1,270 mm and 1,651 mm with relative humidities between 50% and 89%. Temperatures also range between 24°C and 29°C (Sourced from CRIG Meteorological station, Tafo). The soils were loamy and suitable for the cultivation of cocoa. Three treatment plots and equal number of control plots were selected at Ateibu . The treatment farms belonged to George Ayittey, Stephen Osafo and Joseph Appiah and the untreated control belonged to Rebecca Dzita, Kwabena Ofori and Willington Koranteng (Figure 5.1).

Ayitey's farm. This was about 1.5 ha in size. It was bordered on three sides by cocoa farms and on one side by a road. The farm was about 15 years old with virtually no open areas. The cocoa trees were of mixed hybrid and were planted irregularly though well-spaced. The average height of the trees was estimated at about 5 m in height and the canopy was closed for most of the farm. Shade was dense as a result of the several shade trees present. Pruning and basal chupon removal were not regularly carried out on the farm.

Osafo's farm. This farm covered an area of about 2.0 ha. It was contiguous with cocoa farms on all sides. The farm was about 15 years old and it was cultivated on a flat land. The trees were estimated to be about 5 m high in height. They consisted of mixed hybrid and were planted irregularly and close together. The canopy was closed except for few open areas. There were more shade trees than the CRIG recommended density. Basal chupon removal, thinning and pruning were sparingly practiced.

Appiah's farm. This was about 2.0 ha but only about a hectare was populated by cocoa. The farm was surrounded by forests and secondary forests. The trees were about 20 years old and estimated to average about 6.5 m in height. The crop was of mixed hybrid with the trees planted irregularly. The farm was patchy. Cocoa was dense in some parts and solitary in open areas. The number of shade trees far exceeded the CRIG recommendation. As a result, shade was very dense allowing very little sun penetration. It was poorly managed with the greater portion remaining weedy for most part of the trapping experiment. Basal chupons were not removed and the dense areas were also not thinned out or pruned.

Dzita's farm. This farm size was about 2.5 ha. It was bordered on one side only by a cocoa farm with the remaining sides sharing borders with food crop farms. The age of the crop varied from about 15 to 20 years with the trees ranging from an estimated 5 m to 6.5 m in height. They consisted of mixed hybrid and were planted irregularly and close together. About a third of the farm had closed canopy and the rest open. The number of shade trees was in conformity with the CRIG recommended density. Basal chupon removal and weeding were sparingly practiced.

Ofori's farm. The farm was about 2.0 ha. and shared borders with secondary forest on all sides. It was over 25 years old with very tall trees estimated to be over 6.5 m in height. The crop consisted of mixed hybrid planted irregularly and close together. The canopy was closed except for few open areas. The farm was heavily shaded far and above the CRIG recommended density. Basal chupon removal, thinning, pruning and farm sanitation were not practiced.

Koranteng's farm. The size was about 2.0 ha. and was bordered on three sides by cocoa farms and on one side by a food crop farm. The farm was about 15 years old and was cultivated along the bank of a stream. The trees averaged about 5 m high in height and consisted of mixed hybrid planted irregularly but spaced out. The canopy was closed for most of the farm. The number of shade trees conformed to the CRIG recommended density of 6 trees per hectare. Agronomic practices such as basal chupon removal, thinning and pruning and weeding were satisfactorily practiced.

5.2.2 Lures and traps

Lures were polyethylene vials (0.5 ml, 22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK) impregnated with a blend of diester, hexyl (*R*)-3-((*E*)-2-butenoyl)-butyrate and monoester, hexyl (*R*)-3-hydroxy-butyrate. The blend of 1000µg : 500 µg was used.

Traps were the small water bottle trap (Chapter 3). Renewal of lures and cleaning of traps were done monthly.

5.2.3 Experimental design

The experiment was set up to determine whether mass trapping will control mirids and their damage in farmers' farms. It was a randomised complete block design replicated 5-6 fold at Mfranor and Ateibu. The experiment started in February 2009 and ended in September 2010. Each farm was a replicate. From March 2009 to September 2009 five replicates, two at Mfranor and three at Ateibu, were used. However, in the following year, i.e. from February 2010 to September 2010, Margaret's farm was added at Mfranor. The total area of each farm populated by cocoa was trapped at an equivalence of 150 traps/ha. Thus 150, 124, 84, 75, 55 and 55 traps were deployed in Akote, Osafo, Alhassan, Appiah, Ayittey and Margaret's farm respectively. The traps were spaced about 10 m equidistantly apart in straight lines as much as possible. Baited traps were suspended about 1.8 m above ground in trees. The 10^{th} trap in each farm was used to monitor male mirid numbers in the treated farms. In July 2009, five different farms were selected for untreated controls. This number was increased to six in February 2010. In these plots traps were deployed at one trap/0.4 ha to monitor male mirid numbers. The control farms were selected late because of the reluctance of farmers to allow their farms to be used for untreated controls instead of mass trapping. The treatment and control were visited twice a month to record trap catches.

5.2.4 Visual assessment of mirid numbers and damage

Thirty- five cocoa trees were randomly selected in both treatment and control plots for visual assessment of mirids and their damage. Starting from July 2009, mirid numbers and their fresh injury to pods were counted up to hand height along the trunk of each sampled tree, i.e. the height reached with a raised hand when standing on the ground. The entire height of the tree was inspected for branches and chupons of the shoot showing fresh damage. Mirid numbers and damaged pods and shoot were counted and recorded. Mirid identification was done by trained personnel from CRIG.

5.2.5 Analysis of data

The data were analysed using Genstat (9^{th} Edition).` Total trap-catch and visual count data per treatment per replicate were transformed to $\sqrt{(x+ 0.5)}$ to normalise the data. The raw and transformed data were subjected to analysis of variance by using the factors of replicate block and treatment. Where ANOVA indicated significant differences ($P < 0.05$), differences between means were tested for significance by an LSD test. Also Chi-square test of independence was performed on total trap catches to determine differences where necessary.

5.3 RESULTS

5.3.1 Pheromone trap catches

Data recording started a month late in 2009 at Ateibu because challenges of irregular planting and poor adherence to good agronomic practices delayed the deployment of traps in farmers' farms. From March 2009 to September 2010, a total of 583 mirids, made up of 571 *S. singularis* (98%) and 12 *D. theobroma* (2%) were captured in the mass trapping pheromone traps in treatment farms at Mfranor and Ateibu. In 2009, 170 *S. singularis* and one *D. theobroma* were caught at Mfranor while 216 *S. singularis* and 8 *D. theobroma* were caught at Ateibu. In

2010, 113 *S. singularis* and two *D. theobroma* were trapped at Mfranor and 72 *S. singularis* and one *D. theobroma* were trapped at Ateibu. A total of 21 mirids were collected by the monitoring traps; 12 in 2009 and 9 in 2010.

Male *S. singularis* captured per trap per month for the years 2009 and 2010 are summarised in Figures 5.2 and 5.3 respectively. In 2009, there were higher trap catches at Ateibu than at Mfranor (Figure 5.2). At both locations, however, trap catches declined continuously from March/April to June before starting to rise in July. Catches were at their peak at Ateibu in July before dropping drastically in August and September. In a reverse situation, catches at Mfranor rose from July continuously to a peak in September. Combined catches per trap followed the trend at Ateibu because of the influence of the higher catches at that location.

In 2010 catches per trap per month were higher at Mfranor than Ateibu in a reversal of the previous year's trend (Figure 5.3). However, trap catches varied similarly at both stations. Catches were low in April but started to rise in May until they peaked in June at Ateibu, and a month later in at Mfranor. From the peaks, catches dropped drastically in August at both Mfranor and Ateibu. However, while catches at Ateibu rose again in September, those at Mfranor dropped further in September. The combined catches generally followed catches at the two locations because none appeared dominant.

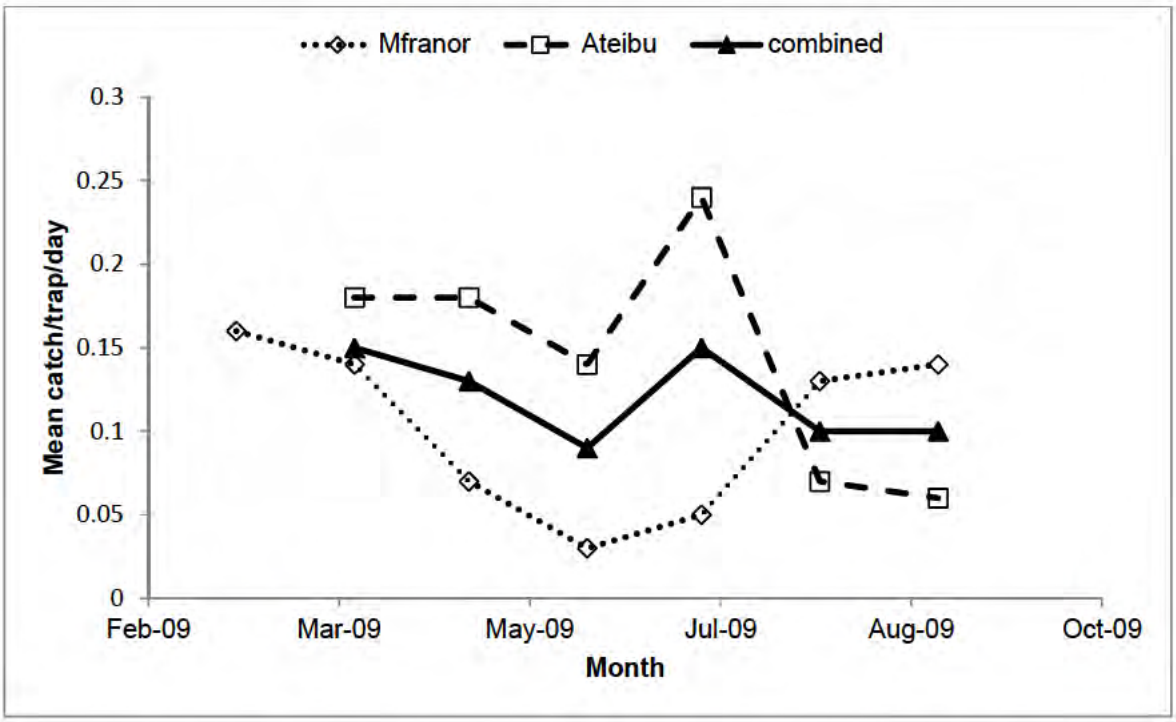

Figure 5.2 Mean catches per trap per per day monthly of male *S. singularis* by pheromone traps in farmers' farms at Mfranor (234 traps) and Ateibu (254 traps) in 2009. Combined is the overall mean from both sites.

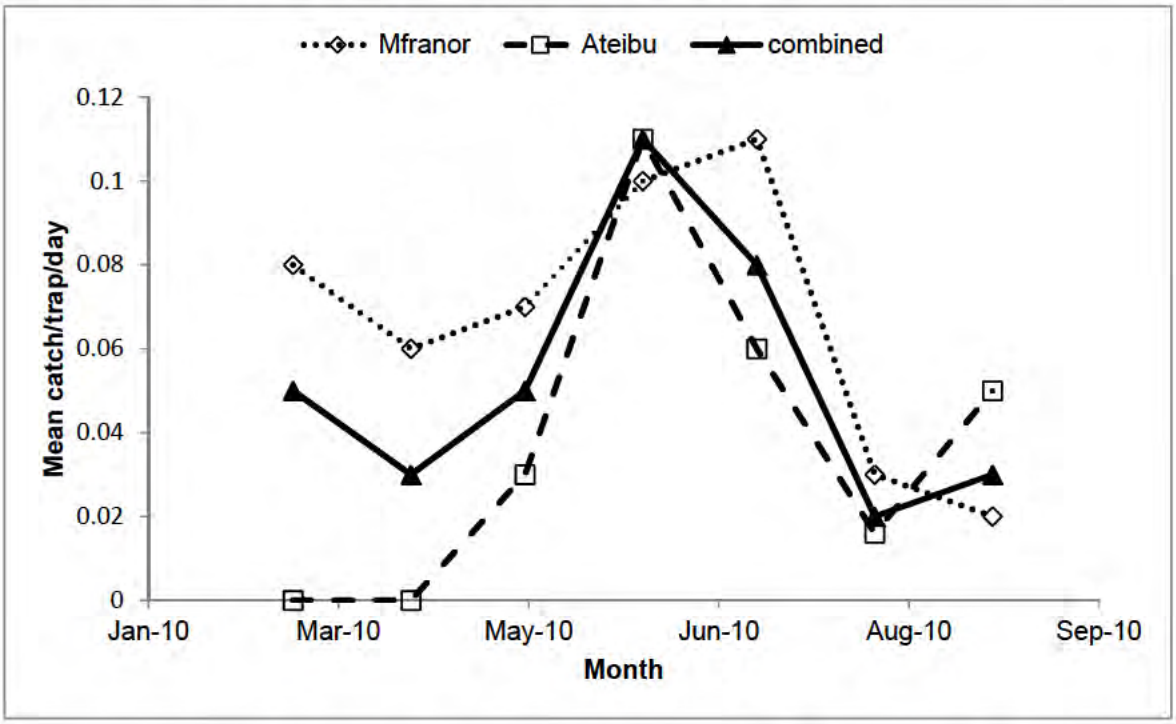

Figure 5.3 Mean catches per trap per day of male *S. singularis* by pheromone traps in farmers' farms at Mfranor (289 traps) and Ateibu (254 traps) in 2010. Combined is the overall mean from both sites.

Too few *D. theobroma* were trapped so total male *S. singularis* and *D. theobroma* combined per hectare (corrected to 150 traps /ha) for 2009 and 2010 are presented in Figures 5.4 and 5.5 respectively. Trap catches in the various plots in 2009 after the introduction of monitoring traps were used. In 2009 numerically highest total catch per hectare occurred in Alhassan's farm, followed by Ayittey's with the lowest in Appiah's and Osarfo's farms.

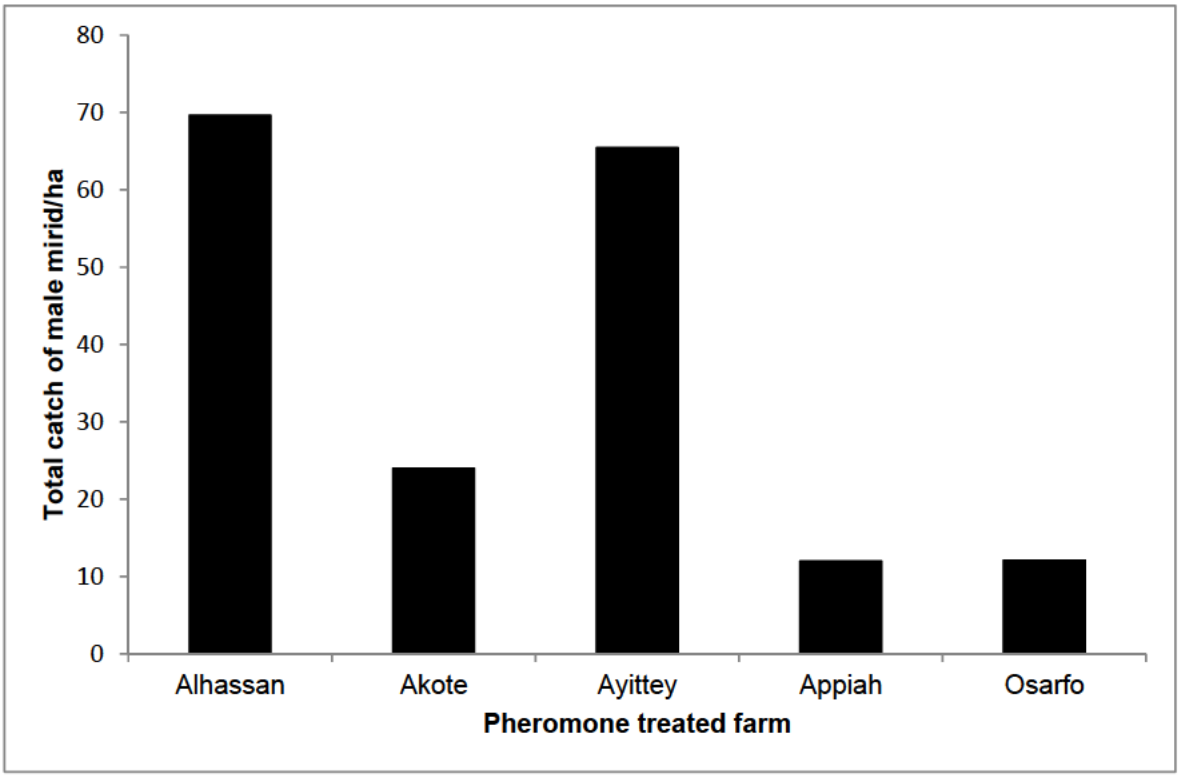

Figure 5.4 Total catches per hectare of combined males of *S. singularis* and *D. theobroma* in pheromone treated farmers' organic farms at Mfranor and Ateibu in 2009. Catches were normalised at 150 traps per hectare

In 2010 (Figure 5.5) the highest catch per hectare was observed on Margaret's farm. Catches in Alhassan's and Ayittey's farms remained high as was observed in the previous year. More catches were recorded in Appiah's and Osarfo's farms in 2010 than 2009 but not much change occurred in the catches at Akote's farm in both years. These results from the pheromone trap catches of male mirids in the treatment plots showed the presence of mirids in the farms selected for the

pheromone trapping experiment and therefore, suitable to evaluate the effect of mass trapping of mirids on farmers farms.

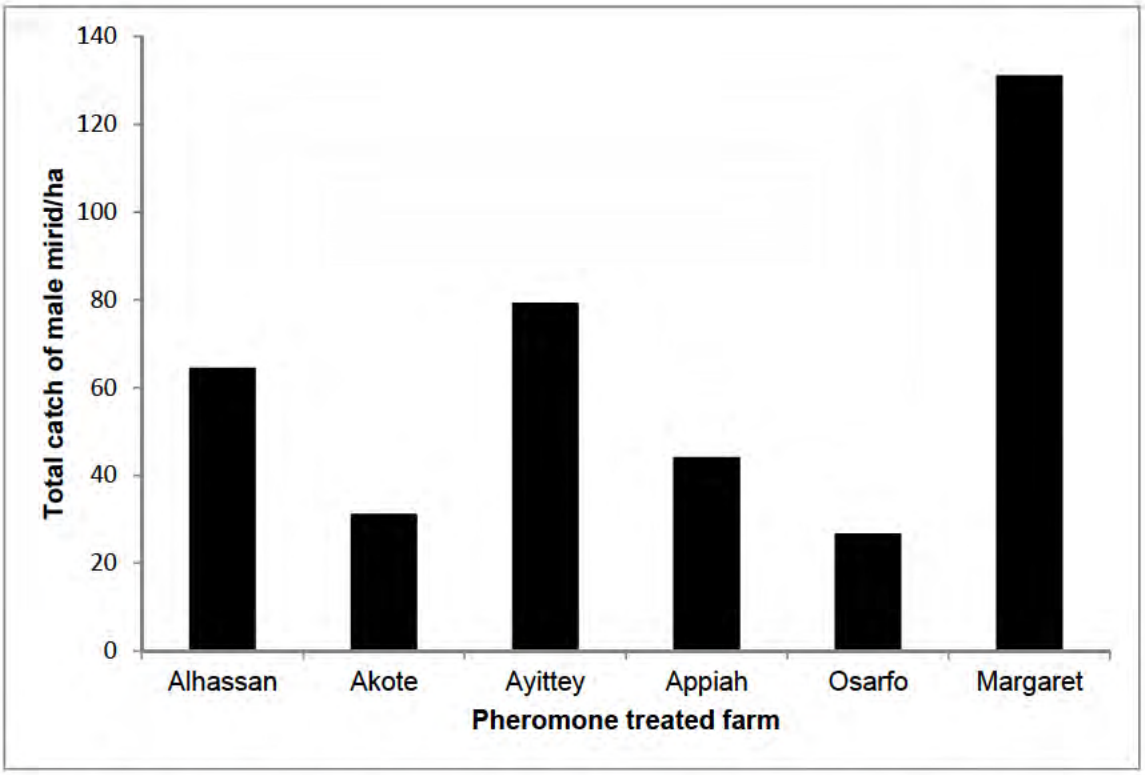

Figure 5.5 Total catches per hectare of combined males of *S. singularis* and *D. theobroma* in pheromone treated farmers' organic farms at Mfranor and Ateibu in 2010. Catches were normalised at 150 traps per hectare.

5.3.2 Visual assessment of mirid numbers and damage

Data for 2009 and 2010 were analysed separately and are referred to as first and second year analyses. In the first year control traps were introduced late after trapping on treated plots had gone on for a while. First year analysis therefore, refers to that carried on the data after the introduction of monitoring traps in all plots.

Visual assessment of mirid numbers

Data on male *S. singularis* as well as *S. singularis* and *D. theobroma* combined were analysed because too few *D. theobroma* were counted. Analysis of variance was carried on untransformed data and also on data transformed to $\sqrt{(x+0.05)}$. Transformation of the data neither improved precision nor affected the results and so results of analysed untransformed data are presented. Mean counts of *S. singularis* are shown in Figure 5.6. Mean counts in the treated farms and control were not significantly different in both the first and second years (2009: $F = 1.38$, df 1, 4, $P = 0.305$; 2010: $F = 2.71$, df 1, 5, $P = 0.161$). This was in spite of the fact that in the second year *S. singularis* counted in trees in pheromone treated farms were more than the control by as many as 6-fold. The results showed that pheromone trapping did not reduce *S. singularis* numbers in the field. These results were consistent with the visual assessment in the previous mass trapping experiment at Acherensua.

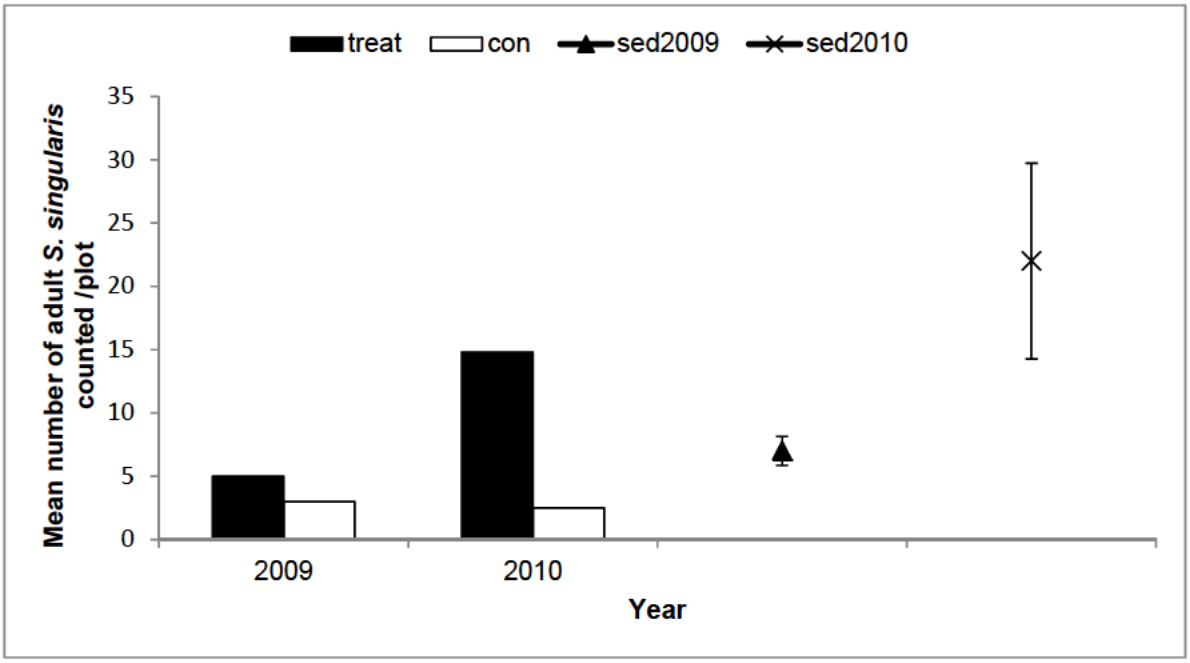

Figure 5.6 Mean count per plot of male *S. singularis* visually assessed in pheromone trapped farmers' organic farms ('treat') and control ('con') at Mfranor and Ateibu in 2009 and 2010. sed = standard error of difference between means.

Mean counts of *S. singularis* and *D. theobroma* combined are summarised in Figure 5.7. Mean counts of the two species combined also showed no significant differences in the treated farms and control in both years (2009: $F = 4.22$, df 1,4, $P = 0.109$; 2010: $F = 3.56$, df 1, 5, $P = 0.118$). It was however, observed that the inclusion of *D. theobroma* in the analysis raised the mean counts in treated farms (21.0 ± 11.43) and increased it about 8x that of the control (2.5 ± 11.43), showing higher counts of *D. theobroma* in the treatment farms. Counts of *D. theobroma* may appear substantial but they were too few to allow a comparison by ANOVA. The results again showed that pheromone trapping did not result in significant reduction in the numbers of mirids in the field which was consistent with the observations at Acherensua.

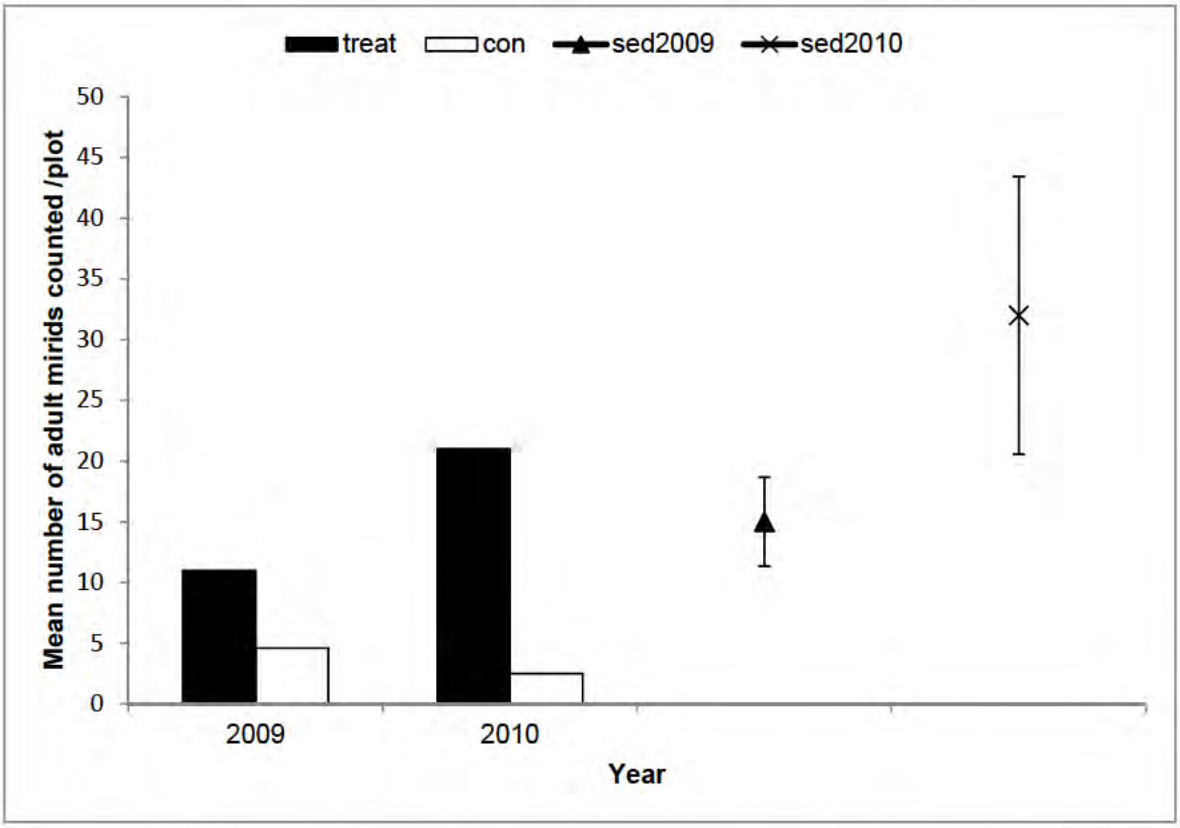

Figure 5.7 Mean count per plot of male *S. singularis* and *D. theobroma* visually assessed in pheromone trapped farmers' organic farms ('treat') and control ('con') at Mfranor and Ateibu in 2009 and 2010. sed = standard error of difference between means.

Results of comparison of trap catches of the two species and visual assessment in years one and two by 2 x 2 Chi-square test of independence, showed significant differences between total catches of males of the species and numbers of mirid species counted in visual assessment in both years ($P < 0.05$). In year one, significantly more *D. theobroma* were counted on trees in the visual assessment than were caught in pheromone traps ($X_1^2 = 178.75$, *df* 1, P = 0.001). This result was repeated in the second year ($X_1^2 = 20.41$, *df* 1, P = 0.001). On the contrary more *S. singularis* were caught in traps than were recorded visually. The results showed that *S. singularis* were more attracted to the traps than *D. theobroma* which concurs with the results in the previous mass trapping in Chapter 4.

Visual assessment of pod damage

Analysis of variance was carried on the raw as well as data transformed to √(x+0.05). Analysed untransformed data is presented because transformation neither improved precision nor affected results. Mean counts of damaged pods in treated and control farms are summarised in Figure 5.8. Mean mirid damaged pods recorded in treated farms and control were not significantly different in both years (2009: $F = 0.24$, *df* 1,4, $P = 0.653$; 2010: $F = 4.44$, *df* 1, 5, $P = 0.089$). However, like the mirid numbers, more pod damage was observed in the treatment than control which was very high in the second year. The damage was about 3.5x more than the control and the difference was significant at $P < 0.09$. The results showed that pheromone trapping did not reduce mirid attack on pods as was the case at Achernsua.

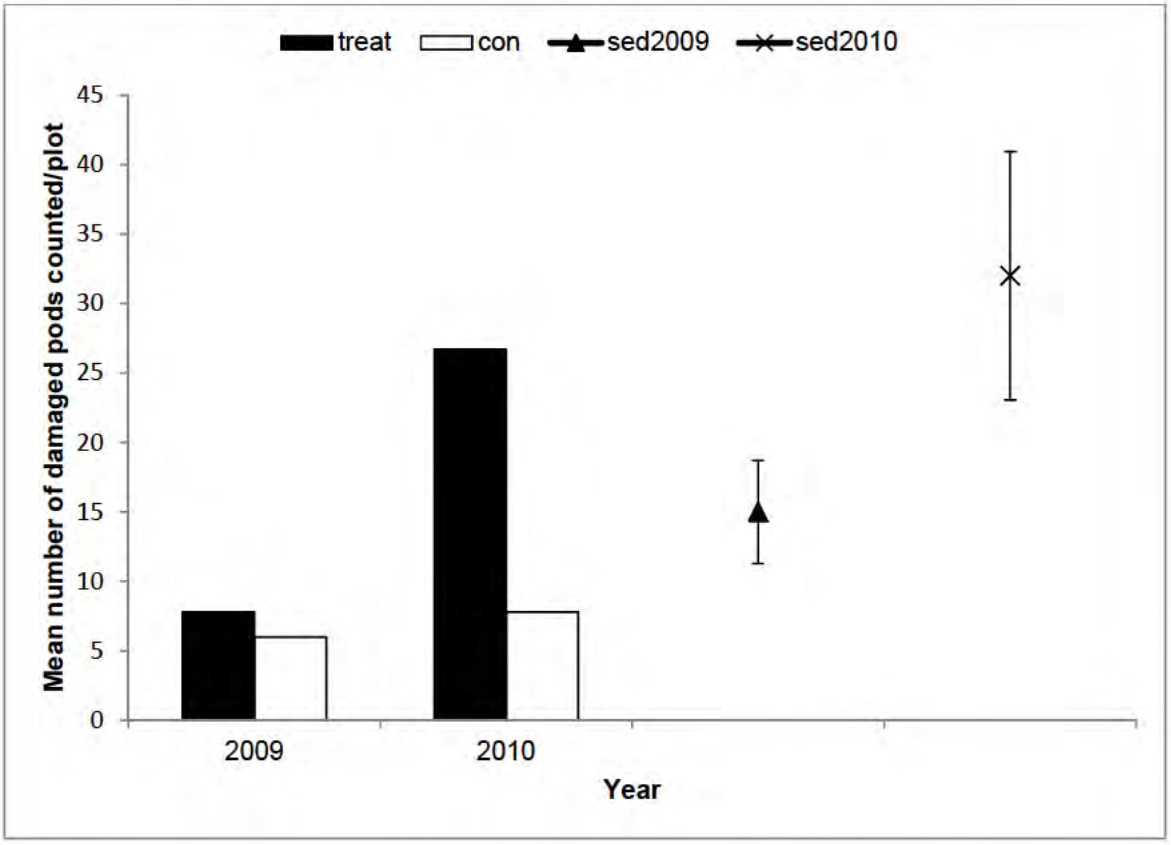

Figure 5.8 Mean count per plot of pods damaged by *S. singularis* and *D. theobroma* in pheromone treated ('treat') and untreated ('con') farmers' organic cocoa farms at Mfranor and Ateibu in 2009 and 2010. sed = standard error of difference between means.

Visual assessment of shoot damage

Analysis of variance was carried on the untransformed and transformed data to √(x+0.05). Analysed untransformed data is presented for the same reason as above. Mean damaged shoot counted in treated and control farms are summarised in Figure 5.9. Mean damaged shoot recorded in treated farms and control were not significantly different in both years (2009: F = 1.85, df 1,4, P = 0.245; 2010: F = 1.46, df 1, 5, P = 0.281). However, in both years more damaged shoot were recorded in the treated farms than control as was the case for the mirids and pods. The results show that pheromone trapping of mirids did not reduce mirid attack on the cocoa trees.

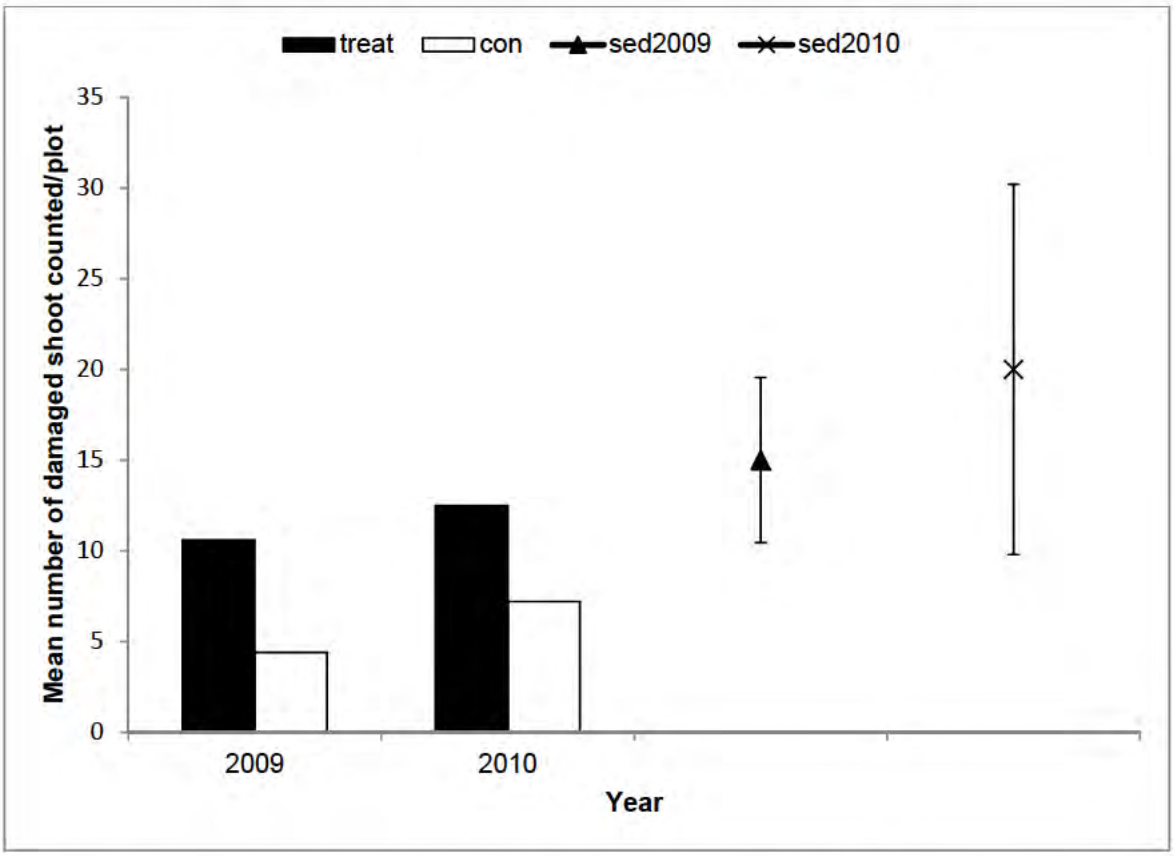

Figure 5.9 Mean count per plot of shoots damaged by *S. singularis* and *D. theobroma* in pheromone treated ('treat') and untreated ('con') farmers' organic cocoa farms at Mfranor and Ateibu in 2009 and 2010. sed = standard error of difference between means.

5.4 DISCUSSION

Results of trap catches and visual assessments of the mass trapping conducted on farmers' farms showed that the method controlled neither the mirids nor their damage on cocoa though numbers of male mirids on trapped plots were numerically reduced. The results support those from mass trapping on the research plantation at Acherensua in the previous chapter. The two results show that the two different conditions of well managed research plots and sub-optimally managed farmers' plots did not affect the behavioural responses of the mirids to the pheromone traps.

It is not surprising that similar results were obtained in the two experiments because they were conducted with essentially the same parameters. Due to time constraints the present experiment had to be started while the Acherensua one was in progress and some of the factors that were suspected to have militated against the success of the Acherensua trial were carried into this one. For example, the same density of traps suspended at the same height were used, factors which might have contributed to failure at Acherensua. A revision of these factors might have resulted in different results.

Results showed that significantly more *S. singularis* males entered the trap than *D. theobroma* males as was observed in the mass trapping at Acherensua in the previous chapter. However, the results that significantly less *D. theobroma* males entered the traps than were counted in both years of the present study, would tend support the suggestion made by King (1973) and reiterated in the previous chapter, that *D. theobroma* males respond weakly to pheromones

Visual assessments for the two years showed that mirid numbers, pod and shoot damage were all numerically higher in treatment than control. The differences were not significant statistically but the observed pattern, nonetheless, may be explained by the inability of the traps to capture all the mirids that were attracted to them, as was established by the results of catches by sticky traps in Chapter 3. Attracted mirids that escaped capture might have remained in the treatment plots to mate with virgin females to increase numbers and consequently their damage to pods and shoot from their feeding. Results of the visual assessments could not be compared with knockdown assessment in this trial because synthetic insecticide could not be applied in the organic farms.

Monthly catches by traps did not show any particular trend in both years, although they showed the dominance of *S. singularis* over *D. theobroma* in terms of numbers which is consistent with trapping in all the previous chapters. In the experiment, trapping was done within the same periods in 2009 and 2010, nevertheless raw figures showed that 2.1x as many mirids were trapped in 2009 (395) as in 2010 (188) despite the fact that an additional farm was trapped in 2010. This may suggest a reduction in the incidence due to differences in

environmental conditions between the two years, particularly climatic (Babin *et al.,* 2011) or differences in yield (Owusu-manu, 1971) or physiological state of trees (Collingwood *et al.,* 1971) other than pheromone trapping.

Differences observed in the normalised total trap captures per hectare in the farms may reflect the spatial aggregated distribution of mirids (Gibbs *et al.,* 1968; Babin *et al.,* 2010; Bisseleua *et al.,* 2011). The big yet non-significant differences in the mean counts of mirid populations between treatment and control plots may also be due to the high number of zeros recorded in counts as a result of the localisation of the damage as a result of the aggregated spatial distribution of the mirids in the farms. For example, in this study adult *S. singularis* counted in 2010 (S.E.D. = 7.74) was 6x in treatment as in control while total adult mirids counted in 2009 (S.E.D. = 3.66) and in 2010 (S.E.D. = 11.43) were 2x and 6x higher in treatment than control respectively but were not different significantly at $P < 0.05$.

The aggregated nature of mirid distribution may be one of the main problems with the trapping of cocoa mirids. It is believed aggregation results in high variation (Laughlin and Allen, 1976; Desjardins and Marble, 1999) in trap catches or counts on trees because two adjacent sampling points in a plot may sample different populations. This would tend to introduce high uncertainties in means resulting in greater uncertainties in differences between means exemplified by higher S.E.D values (see examples above) which make the attainment of significance most unlikely statistically. As suggested in Chapter 3, variation in catches/counts can be reduced if enough areas of mirid aggregations can be obtained for adequate replications for experiments.

Trapping was done on farmers' farms partly to find out if trapping larger plots would improve the effectiveness of the method. However, the actual portions of farms planted with cocoa were smaller than anticipated and not much different from the sizes of plots trapped in the previous experiment at Acherensua. This did not help in the investigation of the effect of larger plots on immigration of mirids into trapped areas suspected in the previous experiment, which was one of the reasons for setting up this experiment.

Though the plots were not as big as expected, it is worth noting that more time and labour were spent in setting up the traps in the farmers' farms because of challenges posed by irregular planting, weeds and un-pruned stands, an experience that should inform future planning.

Chapter 6
MASS TRAPPING AS A METHOD OF CONTROL OF MIRIDS AT AFOSU AND BUNSO (2011-2012)

6.1 INTRODUCTION

In previous Chapters 4 and 5, mass trapping failed to control mirid numbers and infestation of cocoa and it was found out that trapping parameters such as density of traps for trapping *D. theobroma*, and height of suspension of traps were not optimal (Chapter 3). It was also postulated that treated plots might have been re-infested by immigrating mirids from untreated areas surrounding the plots because the treated plots were not isolated to preclude re-infestation. Consequently, a further mass trapping carried out to test these suggestions and is described in this chapter. It was assumed that by deploying the requisite number of traps at optimal height, and by trapping male mirids around treated plots, low populations of mirids could be obtained to prevent the movement of significant numbers of mirids into treated plots.

Mass trapping has also been combined with other approaches such as insecticide application to control pests (Huber *et al.,* 1979; Yamanaka *et al.,* 2001) when high pest populations are suspected to be contributing to failure of the method, as was suspected in the previous mass trapping trials in Chapters 4 and 5. As part of the treatment in this study, mass trapping was combined with the application of the synthetic insecticide imidacloprid. As the experiment was set up during the period of high mirid populations (Gibbs, *et al*., 1968; Owusu-Manu and Somuah, 1989), it was assumed that the insecticide application would reduce the populations to enhance the success of the pheromone trapping (Beroza and Knipling, 1972).

The objectives of this study were therefore twofold. The first was to determine the effect of trapping of isolated plots on the outcome of mass trapping of mirids. To achieve this, data were taken from treated plots isolated by trapping the surrounding cocoa and compared with data from non-isolated control plots. The

same number of upgraded traps was deployed in canopy to improve catches in all plots. The second objective was to find the effect of combination of one application of imidacloprid with mass trapping on the success of the approach on isolated plots. One application only of insecticide was done taking into cognizance the overall aim of the project to eliminate or reduce the application of insecticides in the disinfestation of cocoa. The application was done by the plantation management to obtain results based on the usual way of insecticide application.

6.2 MATERIALS AND METHODS

6.2.1 Study sites

The studies were conducted in two locations, Afosu and Bunso. Afosu is in the New Abirem District of the Eastern Region and the location has already been described above (section 3.2.1). Bunso is located in the tropical forest (6° 16' 25'' North, 000° 34' 001'' West) in the East Akim District of the Eastern Region, about 198 m above sea level. The climate is tropical with two rainy seasons and two dry seasons. The rainy seasons are long with well distributed annual rainfall varying between 145 mm and 195 mm. The mean relative humidity is about 67% with temperatures ranging between 16°C and 28°C. (Meteorological data collected from CRIG, Tafo). The soils are deep and loamy holding enough water for the cultivation of cocoa

Mass trapping plots at Afosu

The study was conducted on two blocks at CRIG substation at Afosu. The study sites were chosen because preliminary monitoring carried out with pheromone traps on the sites showed the presence of mirids and their damage. Each of the blocks was about 10 ha in size. They were rectangular in shape allowing all the three treatment plots in a replicate to be aligned in a line. The first block contained two replicates, 1 and 2, and the second also contained two, 3 and 4. The first block was bordered in the east by a kola plantation and the west by a coffee plantation. It shared boundary with a cultivated plantation of shade trees,

Terminalia spp., in the north. It was bordered in the south by a similar cocoa plantation. However, all the surrounding plantations were separated from the block by a 10 m road. The crop was of hybrid cocoa planted in near straight lines at about 3 m x 3 m intervals (Figure 6.1). Part of the crop in the plot was lost to bush fire in the early 1980's. There had been re-planting albeit not quite successful. The trees which were of mixed hybrid therefore consisted of a mixture of old and young ones about 30 and 15 years old respectively. The old trees were estimated to average about 7 m in height and the young ones were about 6 m. There were several areas without cocoa otherwise the canopy was closed. The number of shade trees averaged about 6 per hectare as recommended by CRIG. Good agronomic practices such as weeding, insecticide application and early harvesting of ripe pods were not optimum because of lack of labour. The second block was bordered by forests and cocoa plantations. A 10 m road separate the block from the plantations and forests. The crop was about 25 years old. The trees were of mixed hybrid and were planted in near straight lines at intervals of about 3 m x 3 m. There were few open areas but the greater part of the block had closed canopy. The number of shade trees conformed to CRIG recommendation.

Figure 6.1 A mass trapping plot at Afosu showing traps high up in the canopy.

Mass trapping plot at Bunso

The experimental plot was about four hectares. It was demarcated from a bigger CRIG experimental farm at Bunso in the East Akim District of the Eastern Region. It was bordered by cocoa farm, forest and land used for the cultivation of plantain, cassava and cocoyam. The crop was about 25 years old. The trees consisted of mixed hybrids and were planted in near straight lines at about 3 m x 3 m intervals. Average height of the trees was estimated to be about 5 m and the canopy was generally closed. The CRIG recommendation of having six shade trees in a hectare was adhered to, but pruning was rarely done.

6.2.2 Field experiments

Demarcation of plots

Each of the two experimental blocks at Afosu was divided into 6 equal plots with a measuring tape. The Bunso block was also divided into 3 similar plots. The size of each plot at both locations was about 1.2 ha and the plots were separated from each other by boundary lines of about 5 m in width. A central portion of about 0.2 hectare was also demarcated out of each plot and marked with red and white coloured polyethylene warning tape.

Lures and traps

In all the experiments the lure was polyethylene vial (0.5 ml, 22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK) impregnated with a blend of diester, hexyl (*R*)-3-(*E*)-2-butenoyl-butyrate and monoester, hexyl (*R*)-3-hydroxybutyrate. The blend of 1000µg : 500 µg was used.

The big water bottle trap (Chapter 3) was used, and renewal of lures and cleaning of traps were done monthly.

Monitoring of mirid populations on demarcated plots before mass trapping experiment

Two baited traps were placed in the canopy about 40 m apart in each half of all demarcated plots at Afosu from June to August 2011. This was to monitor mirid populations to determine the suitability of plots for mass trapping. The experiment was visited weekly to record trap catches.

Experimental design for mass trapping

The experiment was started from the beginning of September 2011 and was a randomised complete block design (RCBD) replicated 5 fold with four replicates at Afosu and one at Bunso due to the inadequate space at one location. Each replicate had three 1.2 ha plots. Three treatments were applied to the plots. In one plot an equivalent of 230 traps/ha, the optimum density to trap *D. theobroma* as indicated from the results in the mass trapping experiment at Acherensua (Chapter 4), were deployed in the whole plot. The traps were spaced about 6.5 m equidistantly apart. Each baited trap was placed in the canopy or about 2.7 m high in trees following the results of the trap height experiments (Chapter 3). This served as the treatment plot. In another plot 35 similarly baited traps were deployed at the centre only at the same density to serve as untreated control. In the third plot also, an equivalent of 230 traps/ha were deployed in the whole plot like the first plot but in addition, imidacloprid was applied once, 6-8 September 2011, at 150 ml/ha. This plot served as the chemically treated control. Data were taken from 35 traps at the centre of each plot. Traps were visited fortnightly for recording of catches.

Monitoring by pheromone traps

After trapping for about a month, two traps were selected; one from the central point of each half of the plot and designated as monitoring traps in order to test the success or otherwise of the mass trapping.

Visual assessment of mirid numbers and damage

Thirty-five cocoa trees were randomly selected in the trapped central portion of each plot for visual assessment of mirids and their damage at two-week intervals, from October 2011 to February 2012. Mirid numbers and their fresh injury to pods were counted up to hand height along the trunk of each sampled tree, i.e. the height reached with a raised hand when standing on the ground. The entire height of the tree was also inspected for branches and chupons of the shoot showing fresh damage. Mirid species and stages were identified by trained personnel from CRIG, and their numbers and damaged pods and shoot were recorded.

Assessment by insecticide knockdown

From September 2011 to end January 2012 monthly assessment of mirid numbers was done in all plots by the insecticide knockdown method. Using a motorized knapsack spraying machine, Confidor (imidacloprid) was applied at 30 g a.i/ha to the trunk and canopy of ten cocoa trees selected randomly from the trapped central portion of each plot. Knocked-down insects were collected on white calico sheets spread under the trees before the insecticide application. After one hour, mirids were sorted into species and stages by trained personnel from CRIG and counted into 70% alcohol in plastic vials and recorded.

6.2.3 Analysis of data

The data were analysed using Genstat (9th Edition). Total trap-catch, visual count and knockdown count data per treatment per replicate were transformed to $\sqrt{(x+0.5)}$ to normalise the data. The raw and transformed data were subjected to analysis of variance by using the factors of replicate block and treatment. Where ANOVA indicated significant differences ($P < 0.05$), differences between means were tested for significance by an LSD test.

6.3 RESULTS

6.3.1 Monitoring of mirid numbers before the deployment of mass trapping traps.

Plots in this section refer to those that were later used for the respective mass trapping treatments at Afosu. Before the mass trapping traps were deployed, 167 male *S. singularis* were trapped by 24 pheromone baited traps in the plots from June to August 2011. No other mirid species were caught.

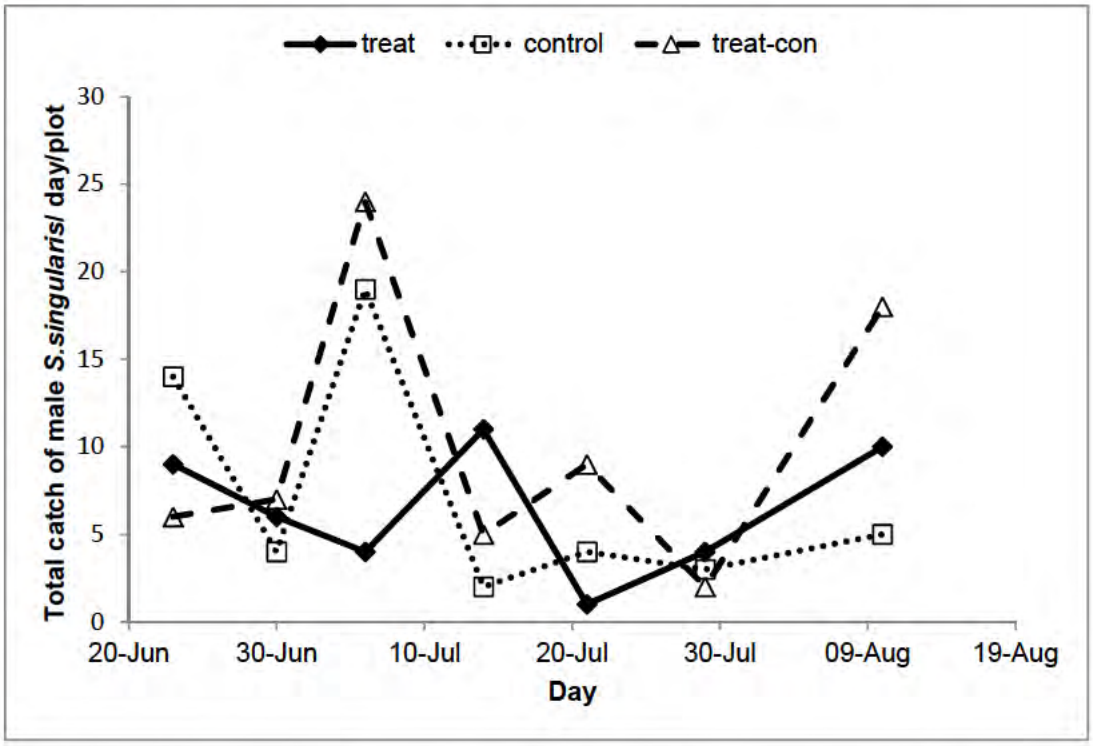

Figure 6.1 Total catches of male *S. singularis* per day per plot by pheromone baited monitoring traps (24) weekly before the deployment of mass trapping traps at Afosu from 20 June to 9 August 2011. Plots designated 'treat', 'con' and 'treat-con' were used for treatment, control and combined chemical and pheromone treated plots respectively (4 plots each).

Figure 6.1 shows a summary of total weekly catches male *S. singularis* per day per plot. Male *S. singularis* were caught in all the plots and at all times throughout the period of monitoring. The highest numbers were caught on 6 July in the plots used for the combined pheromone and chemical treatment. This was followed by

the control also on the same date. The highest catch in the plot for treatment was lower than the controls. After dropping to their lowest points in July, catches in all plots had risen variously at the termination of the experiment in August 2011.

Mean catch per plot from raw data are shown in Figure 6.2. Results of analysis of variance on the raw data are presented here. This is because ANOVA on the data whether raw or transformed to $\sqrt{(x+0.5)}$ gave the same results and also raw data are easy to interpret.

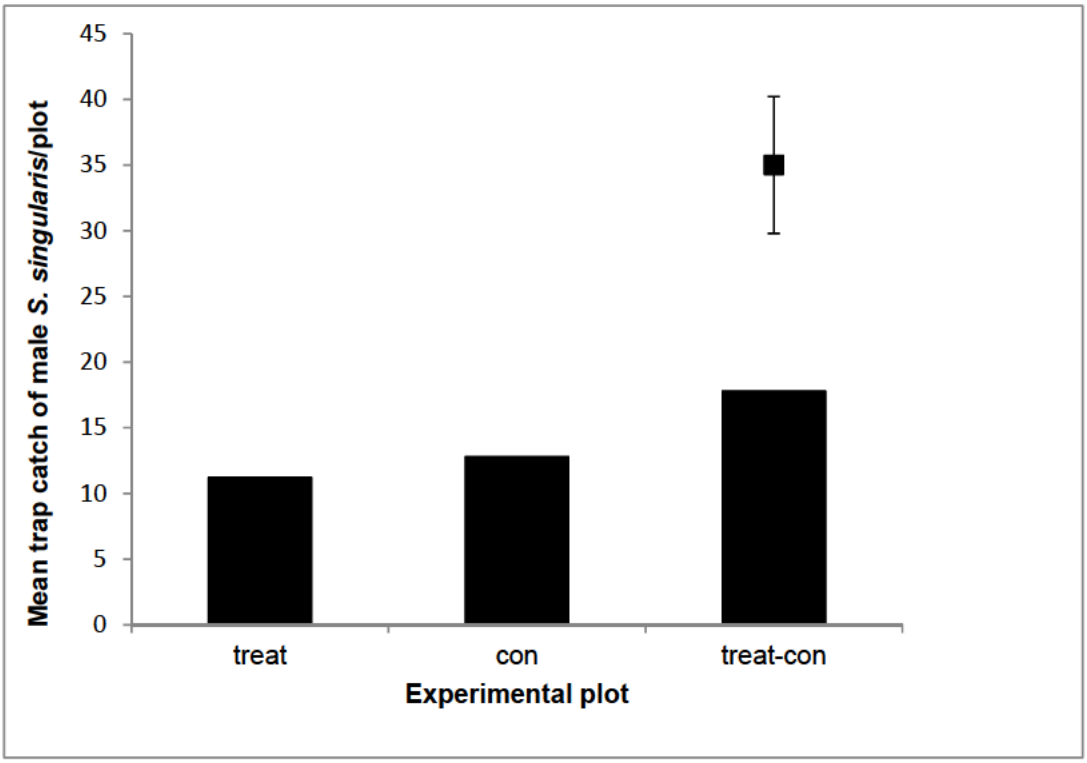

Figure 6.2 Mean catch per plot of male *S. singularis* by pheromone baited monitoring traps (24) on experimental plots before the deployment of mass trapping traps at Afosu from 20 June to 9 August 2011. Error bar shows standard error of difference between means. Bars showing means of plots used for treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

The results indicated parity in *S. singularis* numbers in plots used for mass trapping at Afosu because there were no significant differences between the

means of male *S. singularis* captured on the plots assigned for the respective treatments (F = 0.93, df 2, 6, P = 0.444).

6.3.2 Mirids caught by mass trapping traps at Afosu and Bunso.

A total of 1,230 male mirids consisting of 886 *S. singularis* and 344 *B. laticollis* were recorded in 525 mass trapping traps from 13 September 2011 to 25 January 2012 at Afosu and Bunso. Thirty-six blank traps placed 3 each in replicate plots at Afosu from 20-October to 25- January captured no mirids showing that the *B. laticollis* caught were attracted by the pheromone. Total trap catches per day fortnightly for the period are summarised in Figure 6.3.

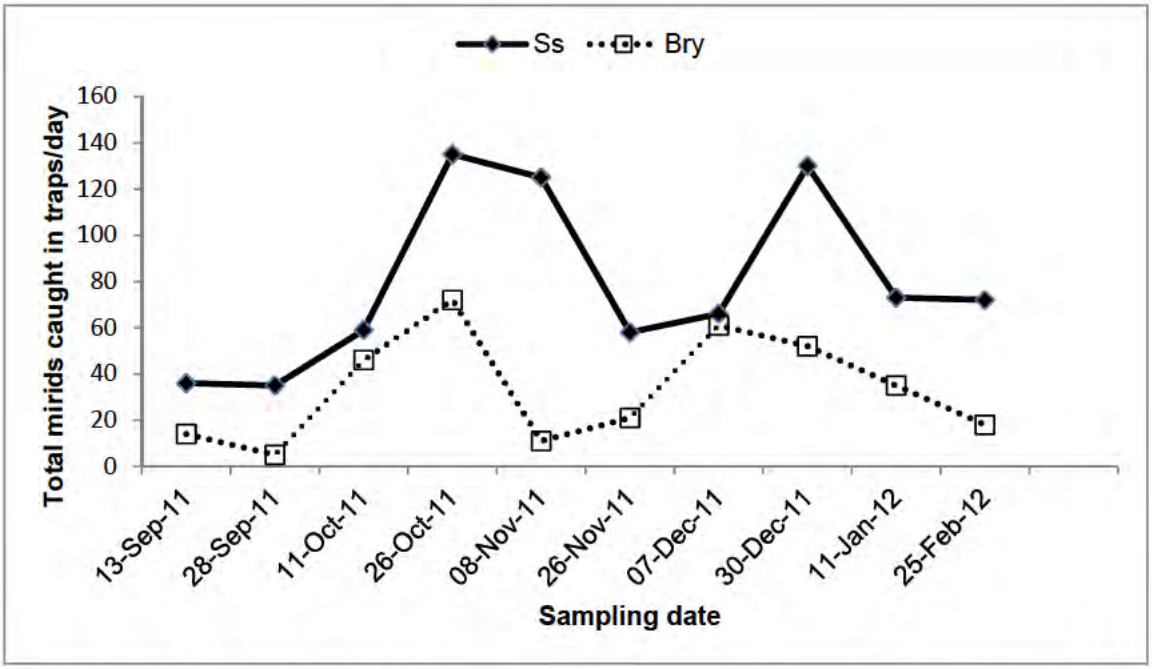

Figure 6.3 Total catches per day of male *S. singularis* (Ss) and *B. laticollis* (Bry) fortnightly by mass trapping pheromone traps (525) at Afosu and Bunso from 13 September 2011 to 25 January 2012.

Male mirids were captured throughout the period of the experiment. More male *S. singularis* were observed to have been captured than *B. laticollis*. Trap catches

of *S. singularis* rose from September to the highest peak in October 2011, declined in November but climbed again in December, before declining gradually through January to February 2012. Generally, trap catches of *B. laticollis* mimicked those of *S. singularis*. Catches of *B. laticollis* also rose from September to the highest peak in October 2011 but not as high as *S. singularis.* Captures then dropped in November 2011 before rising again in December 2011 after which there was a gradual decline to January 2012. Catches of both species reflected the population dynamics of mirids at both Afosu and Bunso.

Analysis of variance carried on the raw and transformed data to $\sqrt{(x+ 0.5)}$ gave the same results and precision. Results from the raw data analysis are therefore presented here because of their ease of interpretation. Mean catches of the male *S. singularis* and *B. laticollis* in the plots are shown in Figures 6.4 and 6.5 respectively. The results for the individual species as well as total mirids showed that isolating trapping plots did not lead to decrease in numbers of mirids trapped.

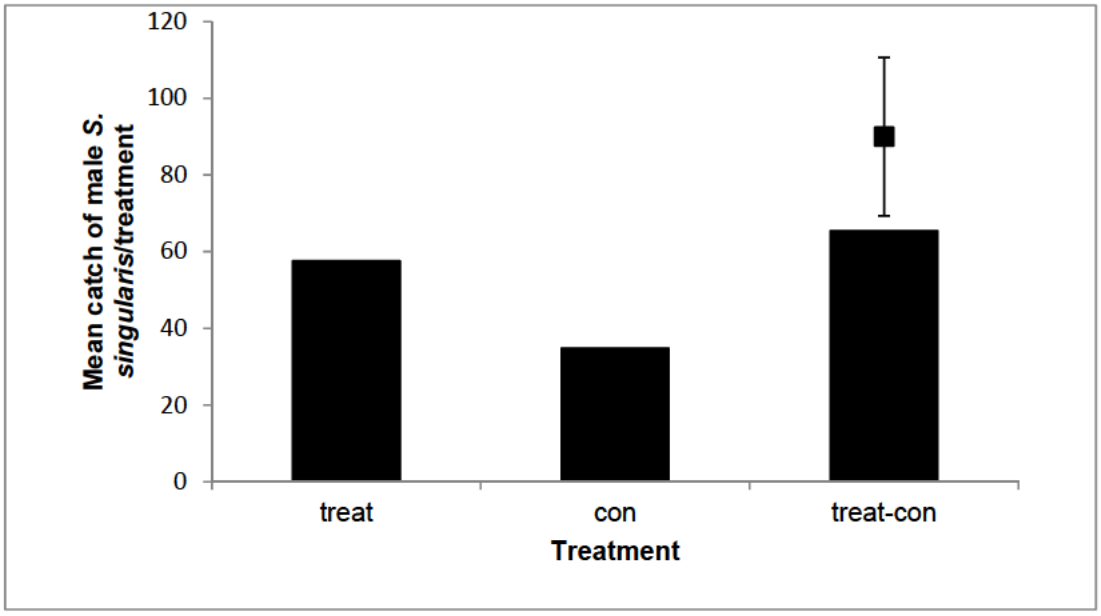

Figure 6.4 Mean catch per treatment of male *S. singularis* in mass trapping traps (175/treatment) baited with synthetic pheromone at Afosu and Bunso (13 September 2011 to 25 January 2012; 5 replicates). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

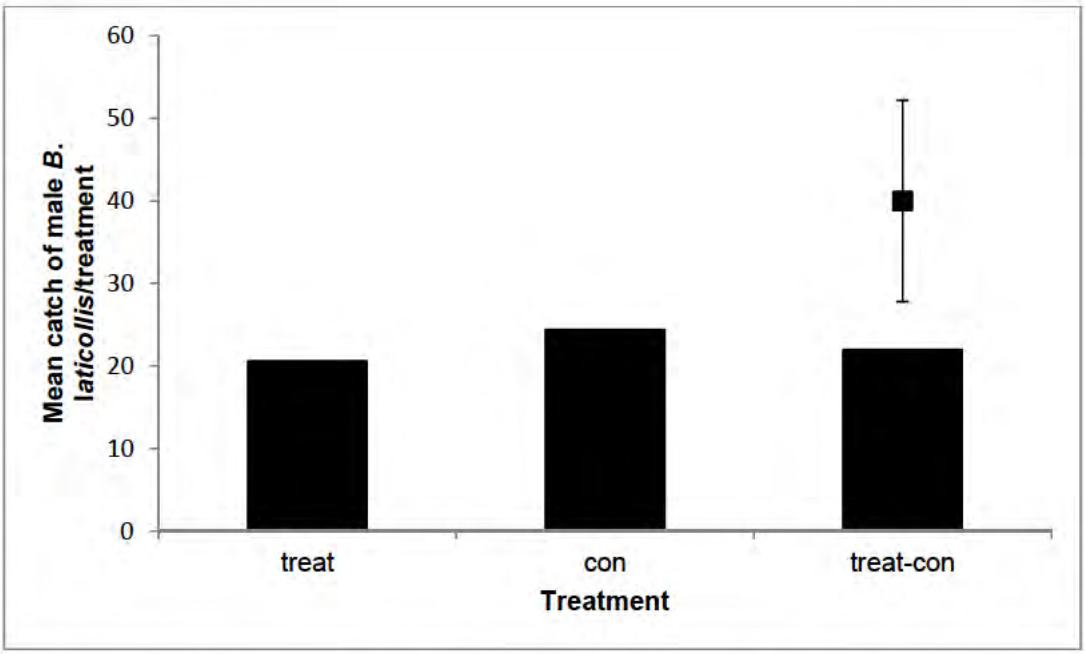

Figure 6.5 Mean catch per treatment of male *B. laticollis* in mass trapping traps (175/treatment) baited with synthetic pheromone at Afosu and Bunso (13 September 2011 to25 January 2012; 5 replicates). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Mean catches of male *S. singularis* among the plots were not significantly different (F = 1.10, *df* 2.8, P = 0.378). Likewise mean catches of *B. laticollis* were not significantly different (F = 0.02, *df* 2, 8, P = 0.977). The results also showed that pheromone trapping combined with one application of imidacloprid did not reduce the mirid numbers.

6.3.3 Catches by monitoring traps during mass trapping

A total of 19 *S. singularis* and eight *B. laticollis* were captured by monitoring traps (30 traps) from 11- October 2011 to 25- January 2012 at Afosu and Bunso. Mean catches of *S. singularis* per trap per day fortnightly are summarised in Figure 6.6.

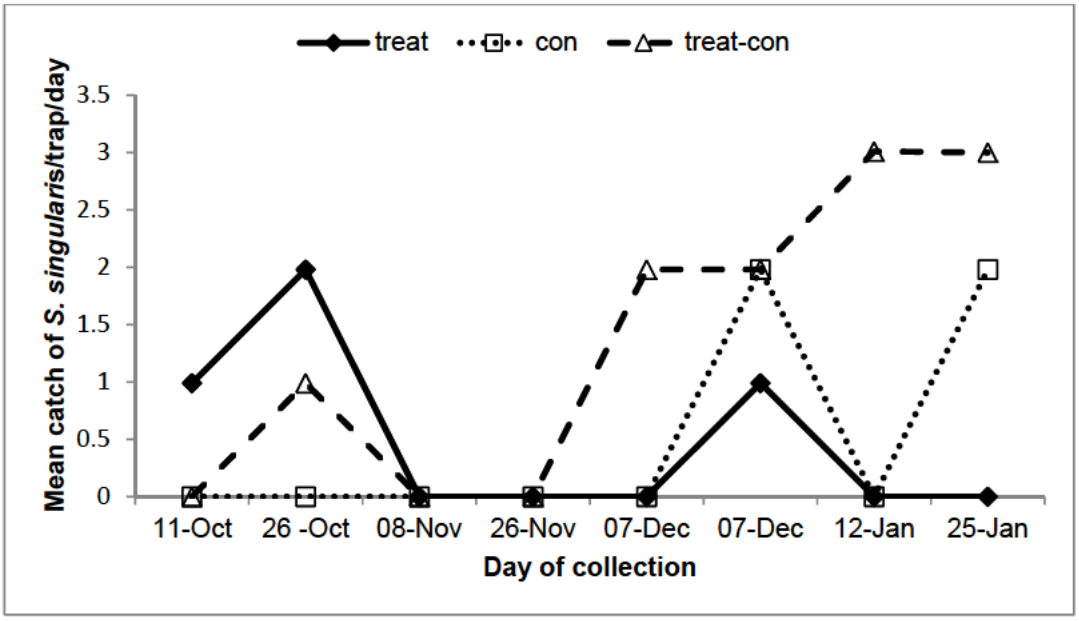

Figure 6.6 Mean catches of male *S. singularis* per trap per day per treatment fortnightly by monitorng traps (10traps/treatment; 5 replicates) baited with synthetic pheromone at Afosu and Bunso (11- October 2010 to 25- January 2012; 5 replicates). Treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Numerically higher catches were recorded in the combined pheromone and chemical treatment than the treated and control plots. Catch of *S. singularis* per trap ranged between zero and 2 in the treated and control plots. In the combined treatment plot it was between zero and 3. Highest catches in treated plot occurred on 26 October 2011 but thereafter dropped to zero. They picked up marginally on 30 December 2011 but again dropped to zero and remained so until the experiment was terminated. There were no catches in the control until 30 December 2011. Catches dropped but had risen at the end of the experiment. After some intial drop catches in the combined treatment plot rose continuously to their highest peak in January when the experiment was terminated.

The results did not alter whether data were analysed by ANOVA raw or transformed to $\sqrt{(x+ 0.5)}$. Therefore, results from the raw data are presented here because they are easy to interpret. Mean catches of male *S. singularis* and *B. laticollis* by monitoring traps are shown in Figures 6.7 and 6.8 respectively. The results showed that pheromone trapping did not reduce male mirid numbers on

isolated plots just as occurred in non-isolated plots in Chapters 4 and 5. Also the imidacloprid combination did not reduce mirid numbers which is consistent with mass trapping above. There were no significant differences in monitoring trap catches of *S. singularis* among the treatments (F = 2.12 df 2, 8, P = 0.183) even though at least twice more were captured in the combined treatment plot than treatment or control. Mean catches of male *B. laticollis* were also not significantly different among the treatments (F = 1.23 df 2, 8, P = 0.342).

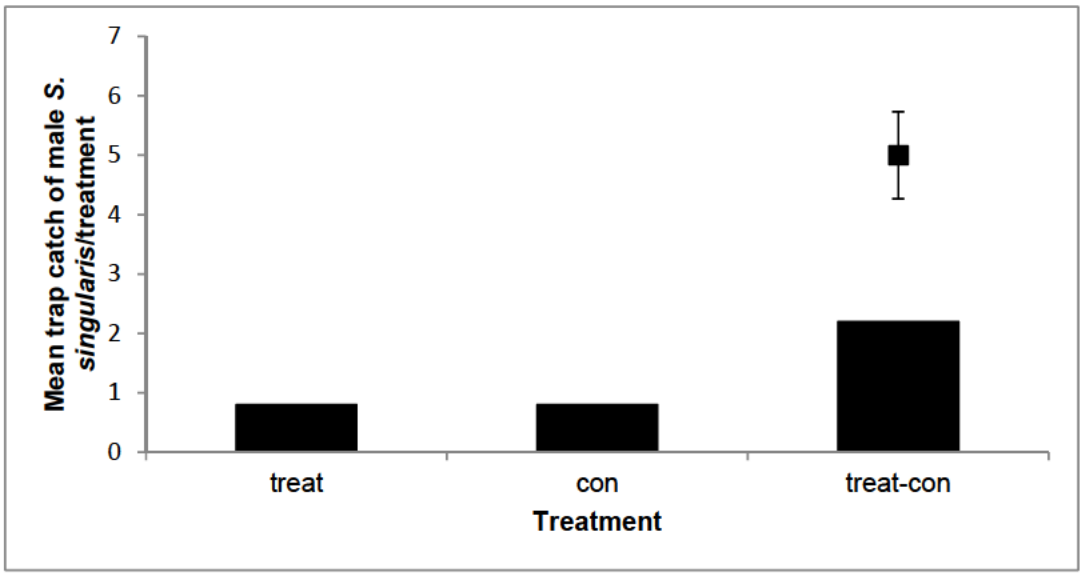

Figure 6.7 Mean catch per treatment of male *Sahlbergella singularis* in monitoring traps (10 traps/treatment) baited with synthetic pheromone at Afosu and Bunso (11- October 2010 to 25- January 2012; 5 replicates). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

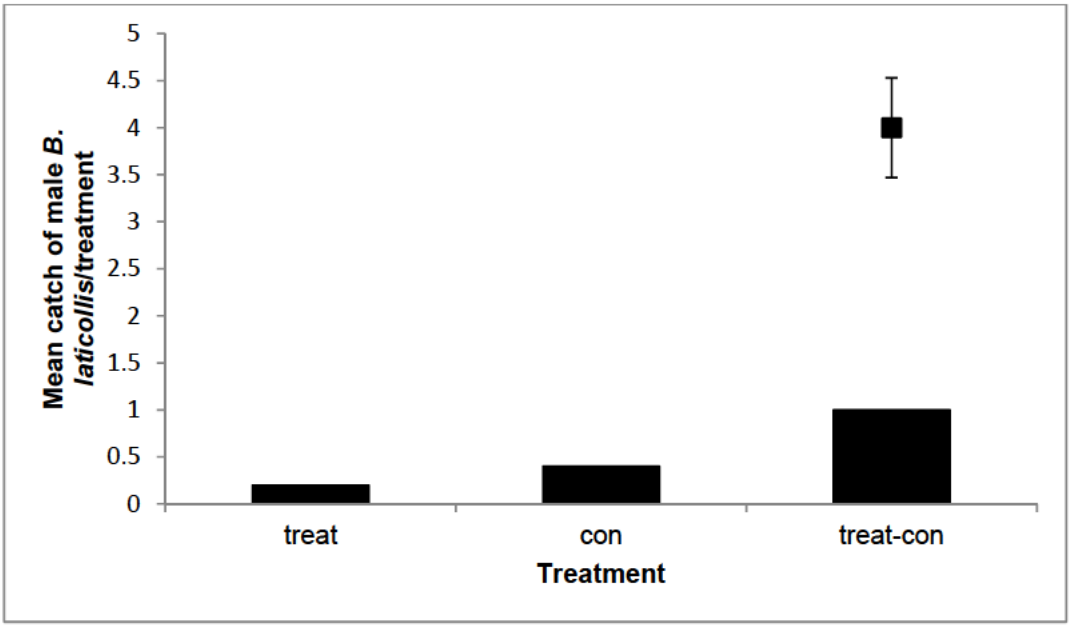

Figure 6.8 Mean catch per treatment of male *B. laticollis* in monitoring traps (10traps/treatment) baited with synthetic pheromone at Afosu and Bunso (11-October 2010 to 25- January 2012; 5 replicates). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

6.3.4 Visual assessment of mirid numbers

Nine *S. singularis* (adults only) and 20 *B. laticollis* (larvae and adults) were manually counted on cocoa trees in the plots from 26-October 2011 to 25-January 2012. Total numbers numbers of *S. singularis* counted per day per treatment every fortnight are summarised in Figure 6.9.

Though *S. singularis* counted was low, numerically more was counted in the treatment than the combined treatment or control. In the treated plot *S. singularis* counted per day per plot ranged from zero to 4. In the control and combined treatment plots the range was from zero to 1. *S. singularis* adult was recorded in the control only on one ocassion in the control. In the treatment and combined treatment plots it was recorded on two and three occasions respectively.

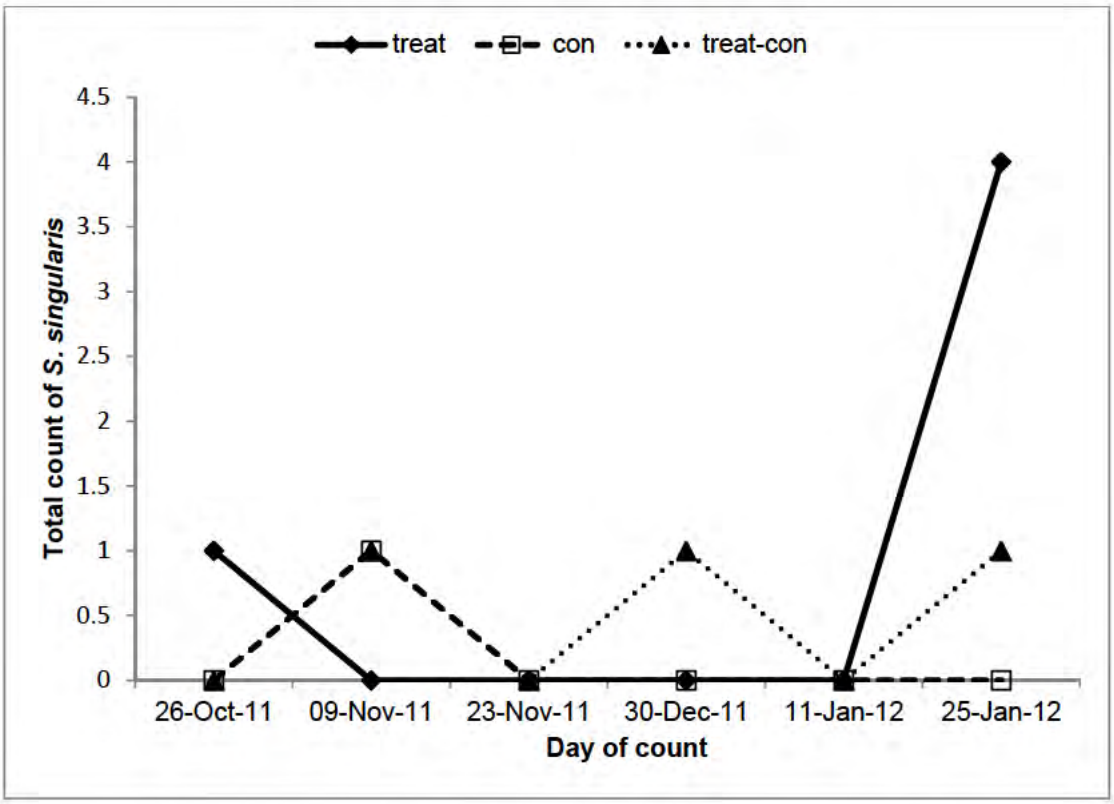

Figure 6.9 Total count per day per treatment of *S. singularis* fortnightly (on 175 trees/treatment; 5 replicates), on mass trapping plots at Afosu and Bunso from 26th October 2011 to 25th January 2012. Treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Neither the results nor the precision changed when analysis of variance was carried on the raw and transformed data to $\sqrt{(x+0.5)}$. The results of the analysis of the raw data are therefore presented here because they are easy to interpret. Mean counts of male *S. singularis* and *B. laticollis* are shown in Figures 6.10 and 6.11 respectively.

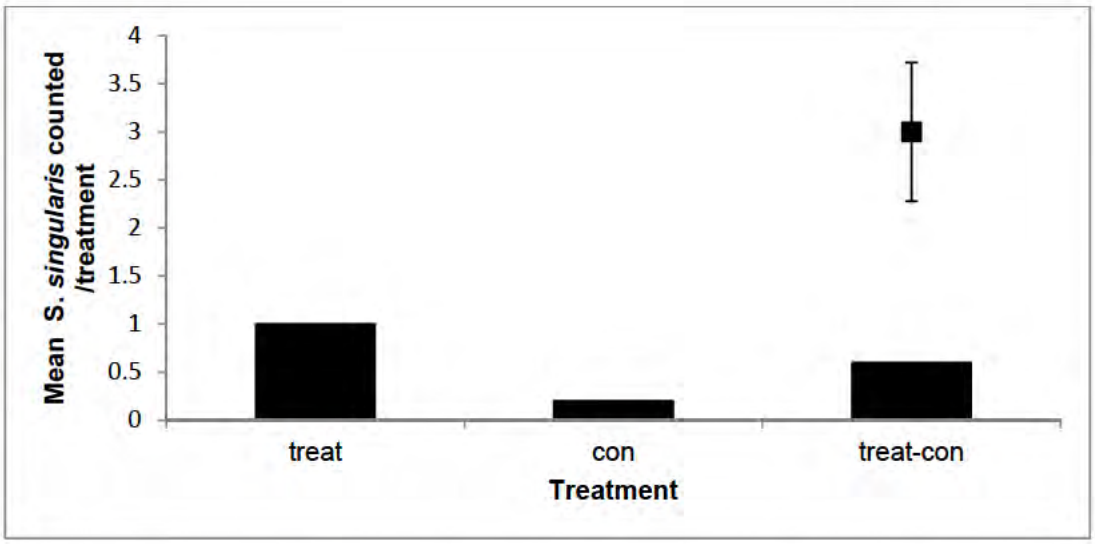

Figure 6.10 Mean *S. singularis* per treatment visually counted on trees (175 trees/treatment; 5 replicates) in pheromone trapped plots at Afosu and Bunso (26-October 2011 to 25- January 2012). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

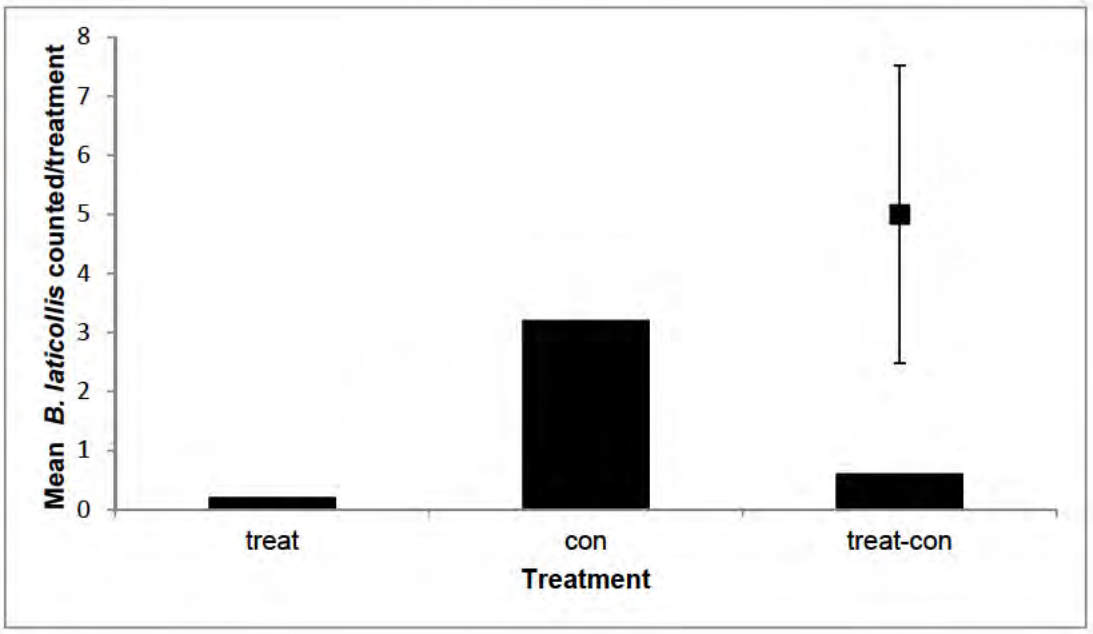

Figure 6.11 Mean *B. laticollis* per treatment visually counted on trees (175 trees/treatment; 5 replicates) in pheromone trapped plots at Afosu and Bunso (26 October 2011 to 25 January 2012). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Mean counts of *S. singularis* in the treatments were not significantly different (F = 0.58, df 2, 8, P = 0.579). In much the same way mean counts of *B. laticollis* also did not differ significantly (F = 0.84, df 2, 8, P = 0.468) despite the numerically high counts recorded in the control. The results confirmed that pheromone trapping of isolated plots and also in combination with one imidacloprid application did not reduce the male mirid numbers.

6.3.5 Visual assessment of mirid damaged shoots

A total of 29 branches were counted in treatment plots from 26 October 2011 to January 25 2012 at Afosu and Bunso. The number of damaged shoots counted fortnightly are summarised in Figure 6.12.

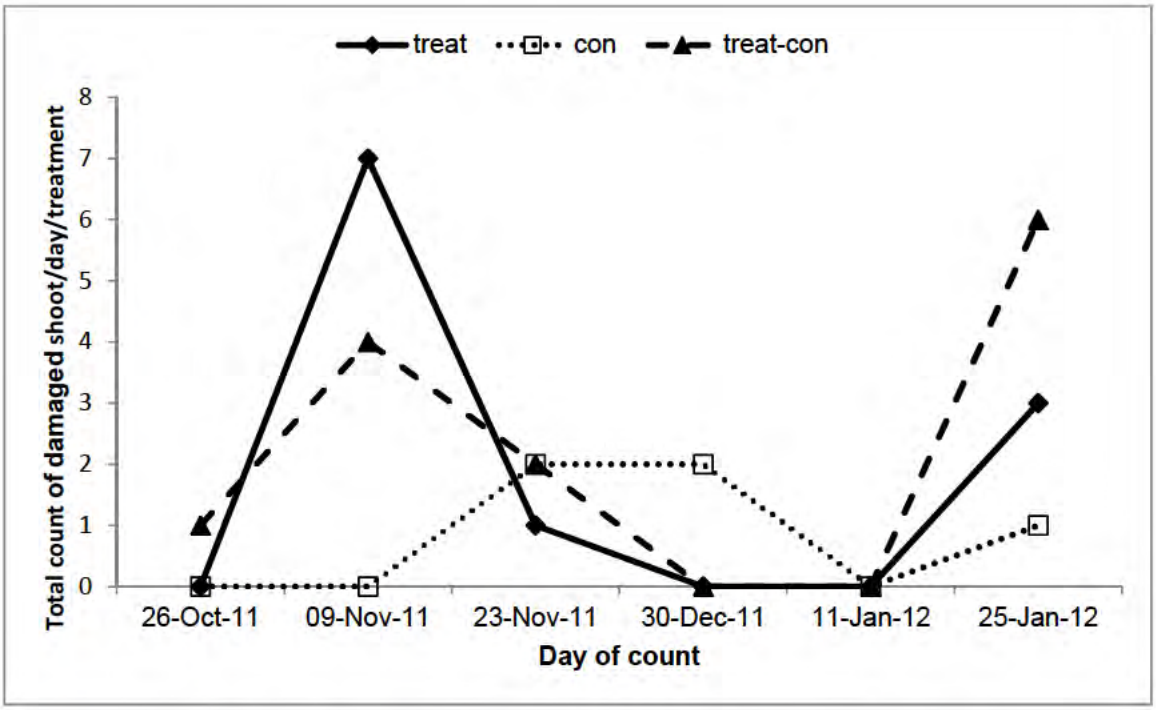

Figure 6.12 Total damaged shoots counted per day per treatment fortnightly, on mass trapping plots at Afosu and Bunso (26 October 2011 to 25 January 2012; 175 trees/treatment; 5 replicates). Treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Highest total count per day was recorded on the treatment plot on 26 October 2011. This was followed by count on combined treatment plot which occurred at the end of the experiment. In treatment and combined chemical and pheromone treated plots high numbers declined together from 26 October and rose together on 25 January2012. Lower numbers of damaged shoot were counted in the control. None was recorded until 23 November 2011 which dropped on 11 January 2012 before rising marginally at the time of termination of the experiment.

Results of analysis of variance on the raw data are presented here because ANOVA on data transformed to $\sqrt{(x+0.5)}$ did not change the results or improve the precision. There were no significant differences between means of damaged shoots in the treatments (F = 0.68, df 2, 8, P = 0.534) (Figure 6.13). However, shoot damage on control was observed to be about half that of either treatment or combined. The results showed that pheromone trapping of isolated plots and also in combination with one imidacloprid application, did not result in decreased mirid damage to shoots. The results are consistent with those of non-isolated plots in Chapters 4 and 5.

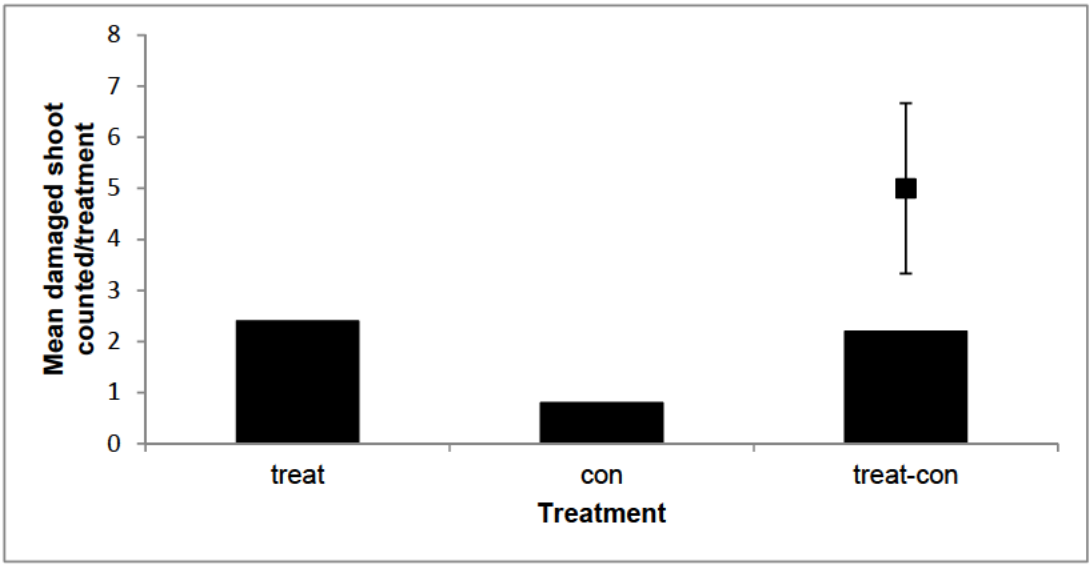

Figure 6.13 Mean numbers of shoots per treatment damaged by mirids in mass trapping plots at Afosu and Bunso from 26 October 2011 to 25 January 2012 (on 175 trees/treatment; 5 replicates). Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

6.3.6 Visual assessment of mirid damaged pods.

A total of 269 pods damaged by mirids were counted in the experimental plots from 26 October 2011 to 25 January 2012 at Afosu and Bunso. A summary of the total counts per treatment per day is shown in figure 6.14

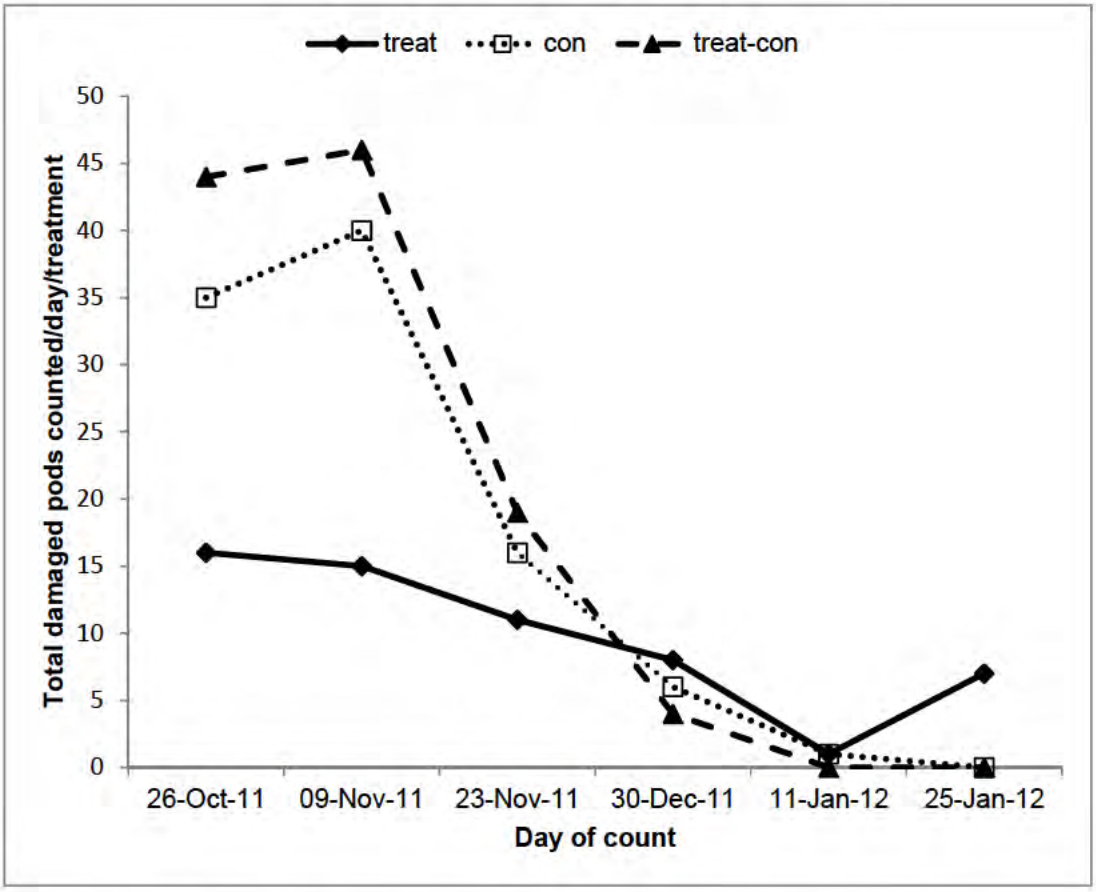

Figure 6.14 Total damaged pods counted per day per treatment fortnightly (on 175 trees/treatment; 5 replicates) in mass trapping plots at Afosu and Bunso from 26 October 2011 to 25 January 2012. Treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

High numbers of damaged pods were counted in October and November 2011 especially in the controls. Highest counts were recorded in the combined plot on 9th November 2011 followed closely by the control also at the same time. In the control and combined, pod damage declined sharply and continuously to the end

of the experiment. Pod damage in treatment was low but its decline was gradual. It had risen marginally at the time the experiment was terminated.

Results of the analysis of variance on the data whether raw or transformed to √(x+0.5) remained the same. Results of ANOVA on raw data are, therefore, presented here. Like the shoots above and also those on non-isolated plots in Chapters 4 and 5, the results showed that pheromone trapping of isolated plots and also in combination with one imidacloprid application, did not result in decreased mirid damage to pods.

There were no significant differences in the mean damaged pods among the treatments (F = 0.55, df 2, 8, P = 0.596) as shown in Figure 6.15.

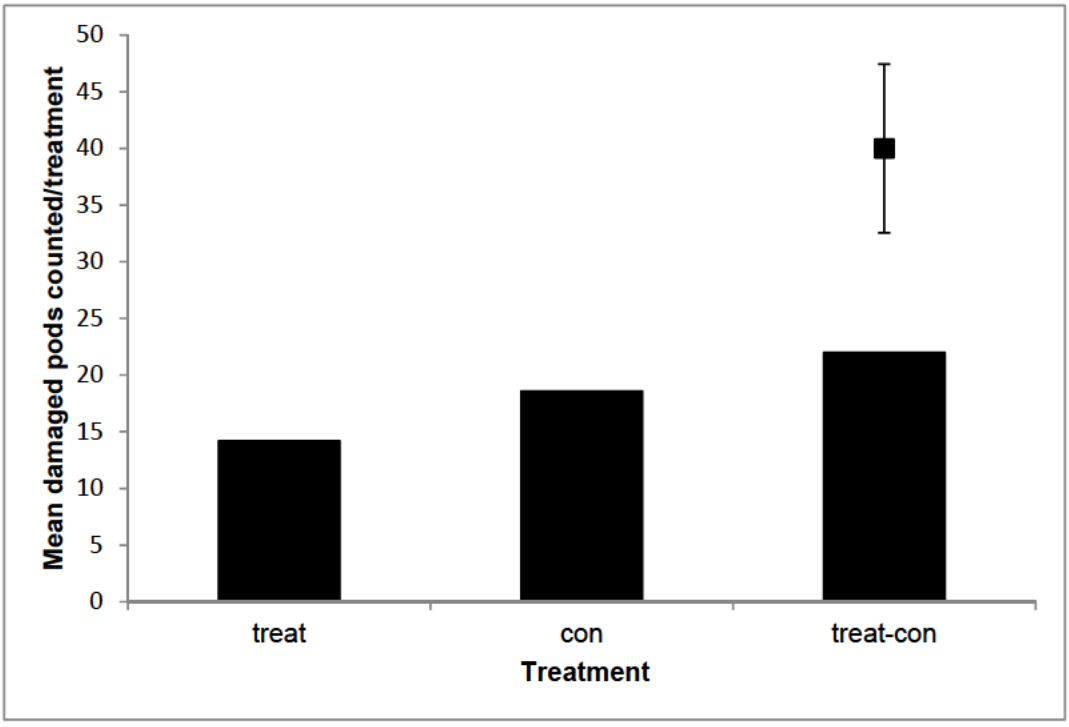

Figure 6.15 Mean counts of pods damaged by mirids per treatment (on 175 trees/treatment; 5 replicates) in mass trapping plots at Afosu and Bunso from 26 October 2011 to 25 January 2012. Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

6.3.7 Assessment of mirid numbers by insecticide knockdown

Twenty-three nymph and adult mirids comprising 19 *S. singularis* and 4 *B. laticollis* were knocked down by the monthly application of imidacloprid in the plots from October 2011 to January 2012 at Afosu and Bunso. Since *B. laticollis* were too few, only numbers of *S. singularis* knocked down per treatment monthly are summarised in Figure 6.16.

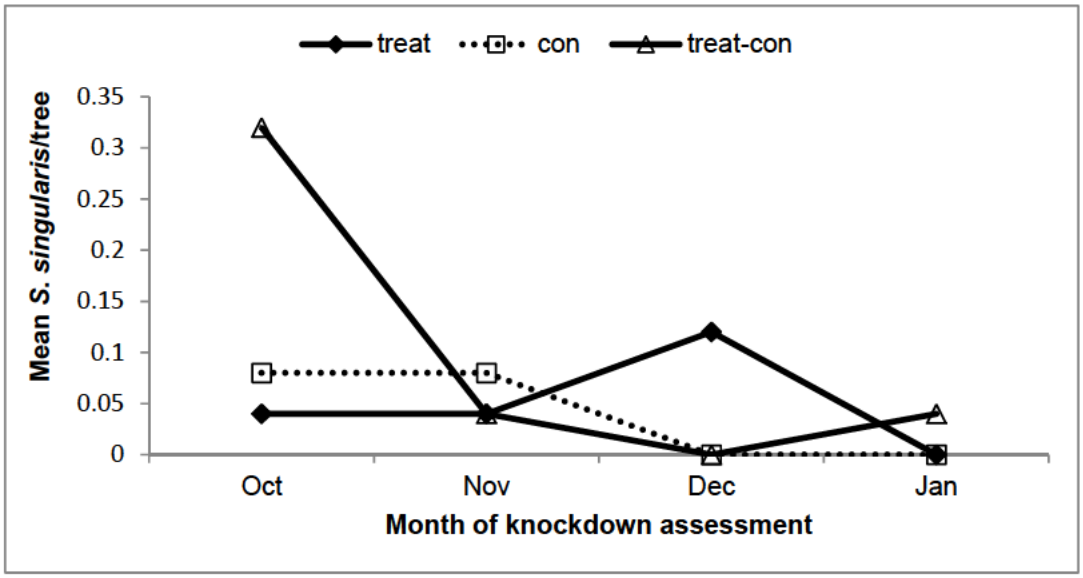

Figure 6.16 Mean *S. singularis* knocked down per tree per treatment monthly (25trees/treatment; 5replicates) in mass trapping plots at Afosu and Bunso from October 2011 to January 2012. Treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

Highest numbers of *S. singularis* were knocked down in the combined treatment plot followed by treatment and control. Mean numbers of *S. singularis* knocked down per tree per month in treatment ranged between zero and 0.14 while that of the control was between zero and 0.06. In the combined treatment plot the range was wider; it was between zero and 0.34. Numbers of *S. singularis* knocked down in treatment increased sharply from November 2011 to a peak in December, before dropping to zero in January 2012. In the combined treatment plot there was a steep decline in November from its peak in October, which continued to

December 2011 before rising marginally in January 2012. Few *S. singularis* were knocked down in the control in October and December 2011 only.

Results of the analysis of variance on the data did not change whether data was transformed to $\sqrt{(x+0.5)}$ or not. Results of ANOVA on raw data are, therefore, presented here. The results were consistent with the visual assessment in showing that neither pheromone trapping of isolated plots nor in combination with one application of imidicloprid insecticide led to reduction in mirid numbers. Mean *S. singularis* or *B. laticollis* knocked down did not differ significantly among the treatments as shown in Figures 6.17 and 6.18 respectively (*S. singularis* $F = 0.58$, df 2, 8, $P = 0.579$: *B. laticollis* $F = 0.58$, df 2, 8, $P = 0.579$). However, at least twice more *S. singularis* were observed in the combined treatment plot than either the treatment or control. This reflected the numerically high numbers of the species on the plot before the experiment and it is further indication of the ineffectiveness of the insecticide-mass trapping combination.

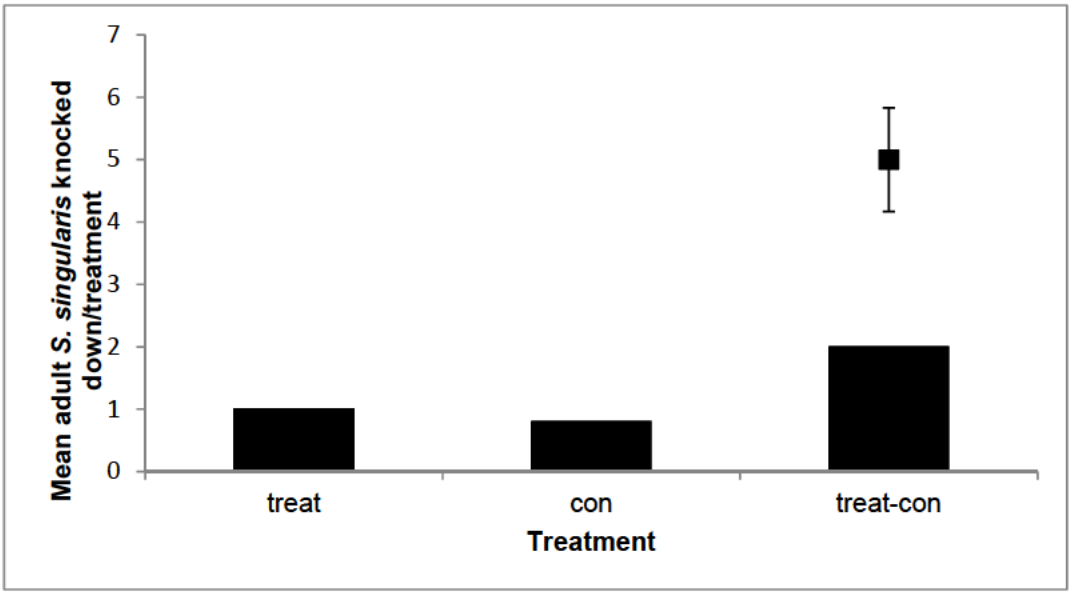

Figure 6.17 Mean number of adult *S. singularis* knocked down per treatment (from 25trees/treatment; 5replicates) in mass trapping plots at Afosu and Bunso from October 2011 to January 2012. Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

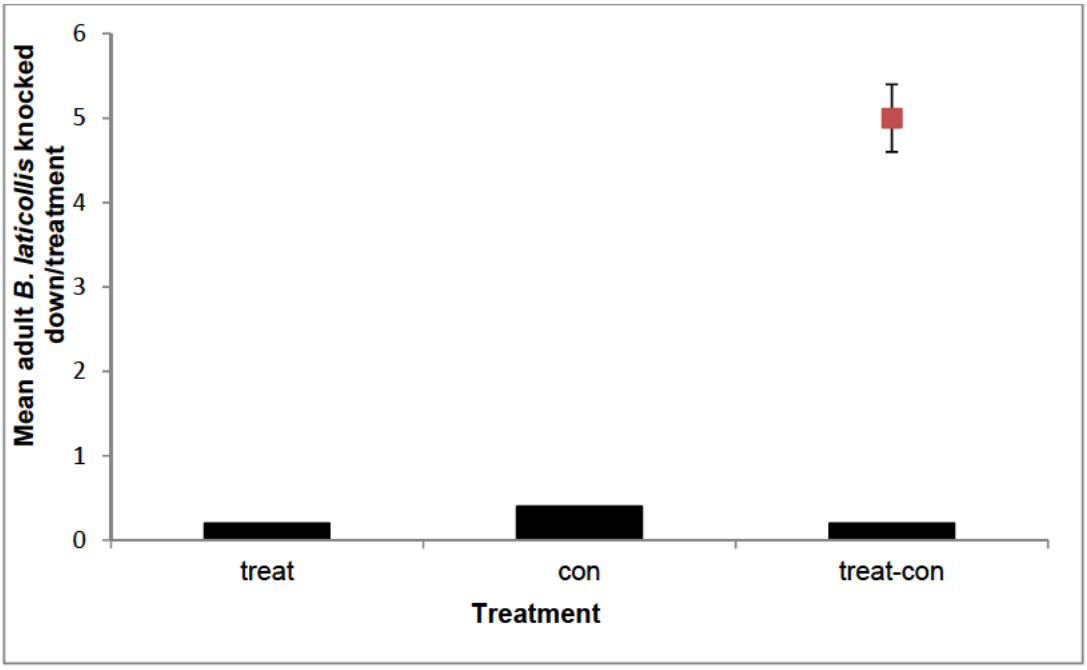

Figure 6.18 Mean number of adult *B. laticollis* knocked down per treatment (from 25trees/treatment; 5replicates) in mass trapping plots at Afosu and Bunso from October 2011 to January 2012. Error bar shows standard error of difference between means. Bars showing means of treatment, control and combined chemical and pheromone treated plots are designated 'treat', 'con' and 'treat-con' respectively.

6.4 DISCUSSION

6.4.1 Mass trapping isolated plots

The present mass trapping which is the third in a series was essentially to find out if isolating trapped plots will result in control because immigration would be prevented. The results of the study, however, did not differ from those in Chapters 4 and 5. Results of mass and monitoring trap catches as well as visual and knockdown assessments all showed that mass trapping isolated plots controlled neither mirid numbers nor their damage just like the results of trapping non-isolated plots. The results therefore, shows that the inability of mass trapping to control the cocoa mirids was not due to the re-infestation from surrounding cocoa or fields.

Since the trap density and positioning were optimised in this study, the inability of mass trapping to control mirids may be due more to their low attraction to pheromone traps, possibly as a result of factors including the inefficiency of traps, eco-biological factors such as density and aggregation (as already discussed in Chapters 4 and 5), missing cues and components in the synthetic pheromone. This is discussed further in Chapter 8. It must however, be stated that the effectiveness or otherwise of the isolating mechanism employed (i.e. trapping around treated plots) in the experiment was not determined.

6.4.2 Mass trapping combined with imidacloprid application

The surprising result of the study was the inability of one imidacloprid insecticide application in combination with pheromone trapping to reduce the mirid numbers. The objective was to find the effect of the combination with the normal application at the station. Spraying was therefore, done by the station staff with insecticide supplied by CRIG. The blanket application of the toxic chemical in combination with pheromone trapping ordinarily should lead to higher mortality of mirids in the plot compared with captures by pheromone traps only.

CRIG recommends four applications of insecticides in a year and one of the aims of this study is to deliver alternatives that would reduce this number of applications because of the adverse effects of synthetic chemicals. However, the apparent ineffectiveness of one chemical application to reduce mirid numbers shown by the results may give justification to the multiple applications recommended by CRIG.

The result may be surprising but it is by no means strange. Combination of mass trapping with chemical application has not always been successful though success in some experiments has always led to recommendations for their use. For example, in Diptera, Boulahia-Kheder *et al.* (2012) reported the reduction of damage at harvest by the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) to only 2% when mass trapping was combined with the insecticide Spinosad and cultural methods. However, in the Lepidoptera, Yamanaka *et al*. (2001) and Hagley (1978) are cited in a review by El-Sayed *et al.* (2006), to have failed to

achieve any improvement in the control of the codling moth and the fall webworm *Hyphantria cunea* (Druny) respectively, with mass trapping combined with chemical control.

6.4.3. Attraction of *Bryocoropsis laticollis*

The significant capture of *B. laticollis* was striking and demonstrates the generic nature of the mirid synthetic pheromone; attracting three of the five genera that attack cocoa in West Africa. As related species (Entwistle, 1972), *B. laticollis* may share common components of the synthetic pheromone with *S. singularis* and *D. theobroma* as occurs in *Phytocoris* spp (Zhang and Aldrich, 2003b). This will make them respond to generic blends of the pheromone but not optimally usually resulting in low trap catches (Gronning *et al*., 2000). This has been discussed further in Chapter 8.

Bryocoropsis laticollis is not considered an important mirid pest of cocoa because, like *Helopeltis* spp., it feeds on pods only and its damage is considered too superficial to cause losses (Raw, 1959). In this study, there were 16x and 5x as many *B. laticollis* counted in control as counted in treatment and combined treatment plots respectively (Figure 6.11). Their pod feeding activity reflected in the pod damage (Figure 6.14) on the plot which was about 23x as high as shoot damage (Figure 6.13). Immature pods massively attacked in the plots where they were dominant presented an unpleasant sight and the pods also appeared smaller in sizes than the un-infested pods. Therefore, it is high time the economic importance of the species was reviewed considering the numbers that were caught in this study.

Trapping of the plots between 20 June and 9 August before the deployment of mass trapping traps did not record the species but thereafter when more traps were deployed they were recorded. The high concentration of the species in the control plot and the great variation in counts shown by high S.E.D. (Figure 6.11) of visual count, suggests aggregated spatial distribution of the species just like *S. singularis* and *D. theobroma*. Their absence in records before the mass

trapping was because their numbers were low and the monitoring traps were too few to capture them. It is welcoming to capture them in traps because it shows their populations could be monitored by the present synthetic pheromone.

Chapter 7
EVALUATION OF PHEROMONE TRAP CATCHES FOR MONITORING MIRID NUMBERS AND DAMAGE

7.1 INTRODUCTION

Monthly catches of male mirids, mainly *S. singularis,* in the optimisation of pheromone blend and trap design experiments in Chapters 2 and 3 respectively at Akwadum, showed that catches were low from March and started rising in November. Catches peaked in January and February and dropped in March. According to Padi and Acheampong (2003), this trend reflected the population dynamics of mirids in the location. This coincidence, therefore, gave an indication of the potential of the cocoa mirids pheromone trap catches for monitoring populations, something already recognised in other mirids such as *C. verbasci* (McBrien *et al.,* 1994; McBrien *et al.,* 1996), *T. caelestialium* (Kakizaki and Sugie, 2001; Yasuda and Higuchi, 2012), *P. difficilis* (Zhang and Aldrich, 2003b), *C. dilutus* (Suzette, 2008) and *S. rubrovittatus* (Yasuda and Higuchi, 2012).

The current schedule for the control of mirids in Ghana is calendar-based, starting in August and ending in December for the whole country. This ignores variations in population dynamics that might exist in places such as Akwadum with possible peak populations in January and February. A schedule of control based on the results of scientific monitoring of mirid populations and damage would hopefully identify variability in the population dynamics and schedule control interventions only where and when necessary.

The objective of this evaluation, therefore, was to establish and quantify the relationships between pheromone trap catches of male *S. singularis* and *D. theobroma* and their field populations, and damage to shoot and pods. The data might be utilised to establish thresholds to synchronise control before damaging pest populations develop. Because of time constraints, a separate experiment was not designed to investigate the potential of cocoa mirid pheromone for

monitoring. Instead, regression analysis was performed on data from monitoring trap catches and visual assessment of numbers and damage in the untreated control plots in the mass trapping experiment at Acherensua (Chapter 4). This was considered a suitable substitute because of the following reasons. Firstly, the consistent trapping and visual assessments done for 11 months provided adequate data for the analysis. Secondly, the population dynamics of the mirids were not believed to have been affected; because plots were neither sprayed nor mass trapped, and captures by the low number of monitoring traps (10 traps per hectare) were too few to affect the population dynamics of the mirids significantly. Thirdly, the simultaneous trapping and visual assessment of the trees gave an instantaneous link between trap catches and the parameters assessed, ensuring accuracy of any relationships that might exist.

7.2 MATERIALS AND METHODS

7.2.1 Data

Material for this chapter was extracted from data from the untreated control plots of the mass trapping trial conducted at Acherensua from 24th September 2008 to 9th July 2009 Each of the five subplots (0.1 ha each) of each whole plot was monitored with a single trap (i.e. density of 10 traps/ha) and visual assessment of mirid populations and mirid damage to shoot and pods was also carried out on 15 trees per subplot. A total of 520 values of each parameter of trap catch and visual assessment of trees recorded from 40 subplots for the period (5 subplots x 8 replicates x 13 recording dates) were analysed. Details of the monitoring are described in section 4.2.

7.2.2 Analysis data

The trap catch data consisted of total male *S. singularis* and *D. theobroma* in 40 traps taken approximately fortnightly. The plant assessment data were total counts of adult *S. singularis, D. theobroma,* nymphs and mirid-damaged shoot

and pods on 600 trees taken on the same occasions as trap catches were counted. Total mirid catches in traps were plotted against total counts of variates in visual assessment to show the patterns of relationships.

These data were square root (x+ 0.5) transformed to normalise the distributions. The potential of using trap catches to predict insect population and damage was assessed in a general linear regression setting. Time (period) was incorporated explicitly in the analysis. Both transformed and untransformed data were analysed. For each analysis block was treated as a factor. The analyses were done using GenStat (Release 9.2).

7.3 RESULTS

7.3.1 Overall descriptive statistics

Wide ranges of numbers of male mirids were recorded in traps in a total of 40 subplots for the period. Similarly, wide ranges of counts were recorded for adult and nymphal mirids and also shoot and pod damage. The mean untransformed pheromone trap catch of *S. singularis* for the period was 0.37 mirid/subplot (range 0–13) and that for *D*. *theobroma* was 0.008 mirid/subplot (range 0–2). From the visual assessments the mean for adult *S. singularis* was 0.03 mirid/subplot (range 0–6), that for adult *D*. *theobroma* was 0.04 mirid/subplot (range 0–8), nymphs 0.29 mirid/subplot (range 0–16), shoot damage 1.30 shoots/subplot (range 0–85), pod damage 1.05 pods/subplot (range 0–83) and total adult and nymphal mirids 7.0 mirids/subplot (range 1–13).

7.3.2 Patterns of relationships between trap catches and visual assessments of adult mirids

Patterns of trap catches of male *D. theobroma* and field populations of mirids and damage were not plotted because of the low numbers of *D. theobroma* caught in traps. The patterns of untransformed catches of *S. singularis* in the pheromone

traps with numbers of adults of *S. singularis* and *D. theobroma* recorded by visual assessment are shown in Figures 7.1 and 7.2 respectively. Numbers of adults of both species recorded were low, but high trap catches of male *S. singularis* were associated with increases in numbers of adults of both species recorded in December 2008.

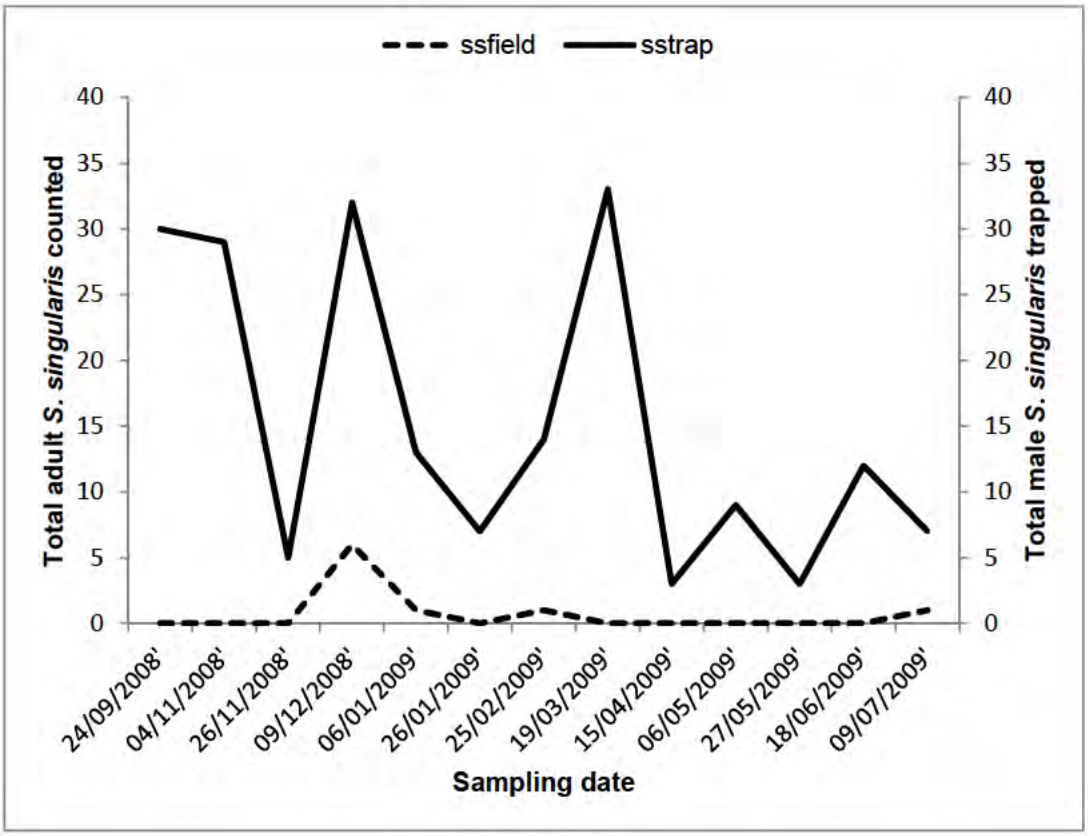

Figure 7.1 Patterns of total untransformed catches of adult male *S. singularis* in pheromone monitoring traps and numbers of adult *S. singularis* recorded by visual assessment in control plots of the mass-trapping experiment at Acherensua (24 September 2008 - 9 July 2009). Trap catches and visual counts of *S. singularis* are represented by 'sstrap' and 'ssfield' respectively.

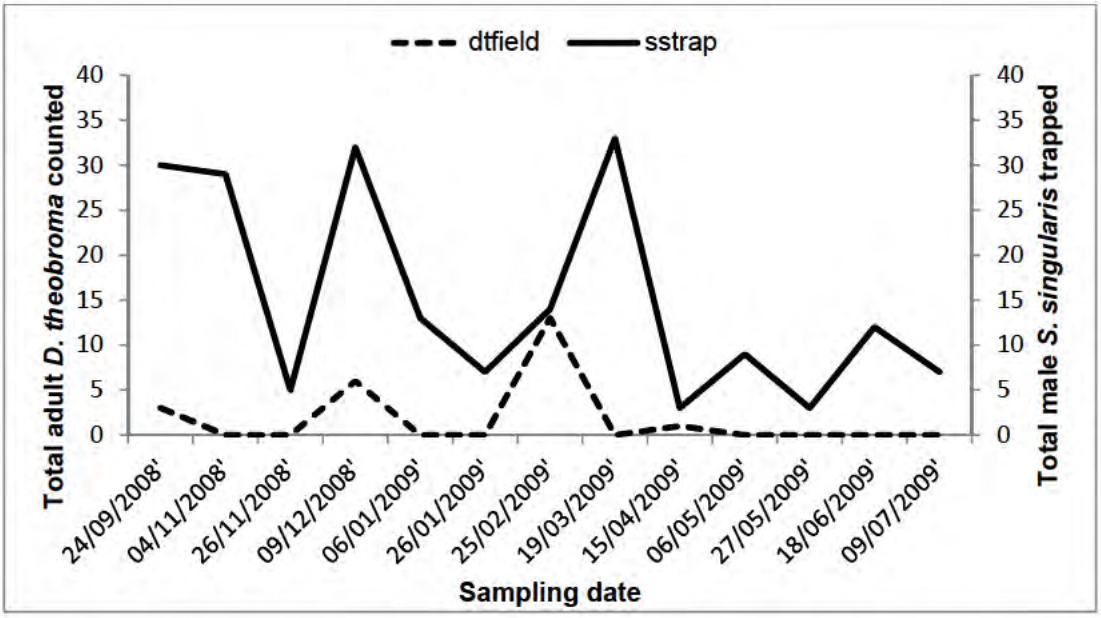

Figure 7.2 Patterns of total untransformed trap catches of adult male *S. singularis* in pheromone monitoring traps and numbers of adult *D. theobroma* recorded by visual assessment in control plots of the mass-trapping experiment at Acherensua (24 September 2008 - 9 July 2009). Trap catches of *S. singularis* and visual counts of *D. theobroma* are represented by 'sstrap' and 'dtfield' respectively.

7.3.3 Patterns of relationship between trap catches and visual assessments of nymphs

The pattern of relationship between untransformed catches of *S. singularis* in the pheromone traps and numbers of mirid nymphs recorded by visual assessment is shown in Figure 7.3. *S. singularis* and *D. theobroma* could not be distinguished as nymphs. Trap catches corresponded with nymph counts on several dates with a peak catch on 09/12/2008 coinciding with peak count. However, trap catches did not correspond with field counts on these dates following dates; 24/09/2008, 04/11/2008, 15/04/2009, 18/06/2009 and 09/07/2009.

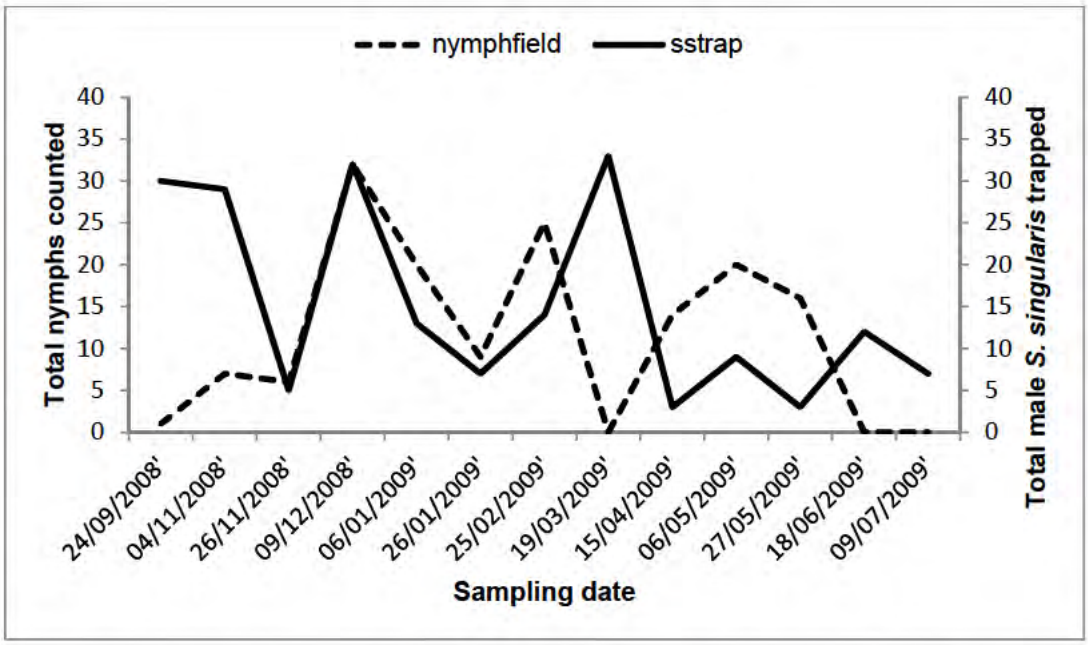

Figure 7.3 Patterns of total untransformed catches of male *S. singularis* in pheromone monitoring traps (sstrap) and numbers of mirid nymphs (nymphfield) recorded by visual assessment in control plots of the mass-trapping experiment at Acherensua. (24 September 2008 - 9 July 2009) Trap catches are shown by 'sstrap' and nymphs are shown by 'nymphfield'

7.3.4 Pattern of relationships between trap catches and visual assessments of total mirid numbers

In Figure 7.4 the total numbers of mirids recorded by visual assessment, both adults and nymphs of the two species, are combined and plotted against catches of *S. singularis* adult males. Trap catches generally corresponded with field populations on all dates except 24/09/2008, 04/11/2008, 19/03/2009 and 18/06/2009 when high catches coincided with low counts; and also on 15/04/2009 when the reverse occurred.

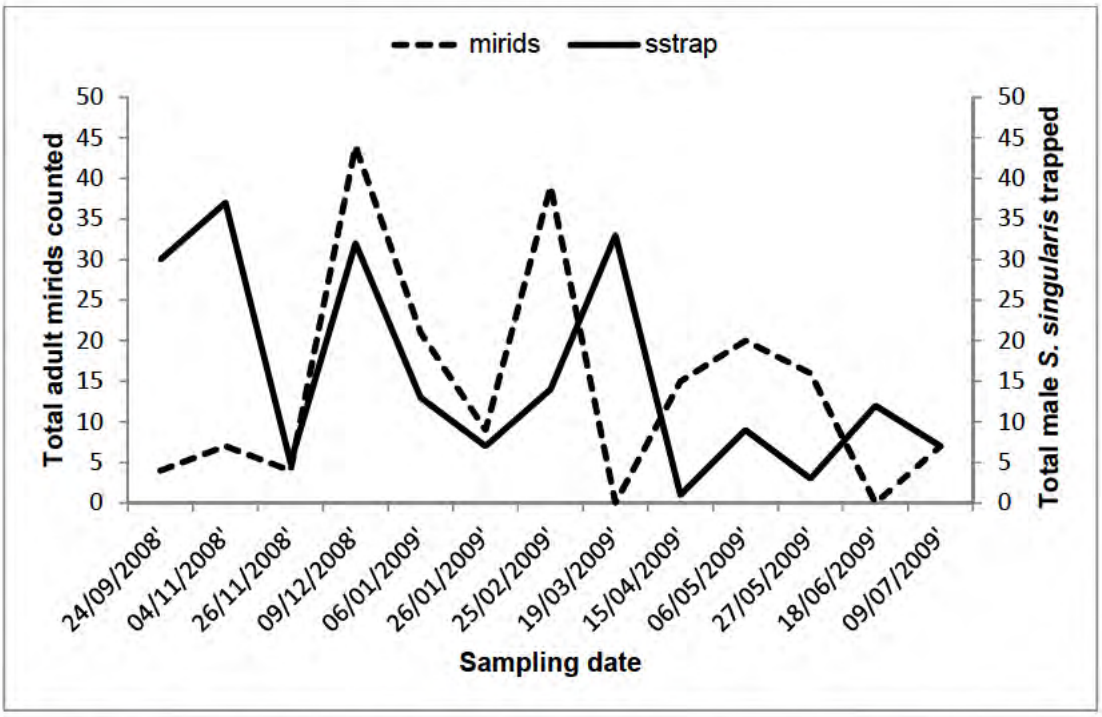

Figure 7.4 Patterns of total untransformed trap catches of male *S. singularis* in pheromone monitoring traps and visually assessed total mirids counted in control plots of the mass-trapping experiment at Acherensua for the whole period (24 September 2008 - 9 July 2009). Trap catches are shown by 'sstrap' and field counts by 'mirids'

7.3.5 Regression relationships between trap catches and mirids

There were no significant regression relationships with associated field populations when transformed or untransformed total counts of adults of *S. singularis* were regressed on male trap catches (N = 520, df 1, 496 , F = 0.03, P = 0.87; N = 520, df 1, 496, F = 0.02, P = 0.888 for *S. singularis* and *D. theobroma* trap catches respectively) or total counts of adult *D. theobroma* regressed on trap catches (N = 520, df 1, 496, F = 2.74, P = 0.098; N = 520, df 1, 496, F = 0.03, P = 0.854 for *S. singularis* and *D. theobroma* trap catches respectively). There was also no significant relationship when total counts of nymphs were regressed on trap catches of male *S. singularis* (N = 520, df 1, 496, F = 2.31, P = 0.129) or trap catches of *D. theobroma* (N = 520, df 1, 496, F = 0.12, P = 0.734). The regressions of total mirid numbers on trap catches of

S. singularis (*N* = 520, *df* 1, 496, *F* = 3.02, *P* = 0.083) and *D. theobroma* (*N* = 520, *df* 1, 496, *F* = 0.80, *P* = 0. 371) also showed no significant relationships.

Examination of the plots (Figures 7.3 and 7.4) showed that for the first two sampling dates, trap catches recorded were low whereas numbers of nymphs (Figure 7.3) and total mirids (Figure 7.4) recorded by visual assessment were high. Thereafter, there seemed to be some general relationship between trap catches and nymphs as well as total mirid numbers (Figures 7.3 and 7.4 respectively). Re-analysis omitting the first two sampling dates using transformed data showed no significant relationship with regression of total mirid counts on trap catches of *D. theobroma* (*N* = 440, *df* 1, 432, *F* = 0.14, *P* = 0.711). However, there was a significant regression relationship when total mirid counts were regressed on trap catches of male *S. singularis* (*N*= 440, *df* 1, 432, *F* = 4.23, *P* = 0.040) among blocks (*N* = 440, *df* 7, 432, *F* = 159.68, *P* = 0.001).

The analysis of the mirid data showed that both trap catches and blocks were significant explanatory variates in a model $y = a + bx + \varepsilon$, where; y= total mirid population, a = constant, b = regression coefficient, x = trap catches and ε = residual. This model accounted for 3.2% of the variation in total mirid counts. Values for the constants and regression coefficients of the equations of the relationship, derived for each block from the parameter estimates of the analysis, are shown in Table 7.1. From the coefficient values, monitoring trap catches of male *S. singularis* predicted total mirid populations consistently in the blocks; thus a unit increase in trap catch predicted an increase of 0.15 in mirid population by visual inspection in the blocks.

Table 7.1 Constants and coefficients of the regression equations of the relationships between trap catches of male *S. singularis* and field populations of total mirids in blocks excluding the first two dates (y= a + bx + ε, where; y= total mirid population, a = constant, b = regression coefficient, x = trap catches and ε = residual).

Block	a	b
1	0.72 (± 0.10	0.15 (± 0.07)
2	0.71 (± 0.09)	0.15 (± 0.07)
3	0.81 (± 0.09)	0.15 (± 0.07)
4	0.86 (± 0.09)	0.15 (± 0.07)
5	0.59 (± 0.09)	0.15 (± 0.07)
6	0.64 (± 0.09)	0.15 (± 0.07)
7	0.59 (± 0.09)	0.15 (± 0.07)
8	0.60 (± 0.09)	0.15 (± 0.07)

7.3.6 Patterns of relationships between trap catches and mirid damage

Patterns of untransformed total trap catches of male *S. singularis* and visually assessed shoot and pod damage are shown in Figures 7.5 and 7.6 respectively. The trend was similar in both figures with reduction in trap catches and damage from high values at 04/11/08 and 06/01/09 to low values between 25/02/09 and 09/07/09. Highest total male *S. singularis* catch coincided with the highest shoots and pods damage on 04/11/2008. A significant exception to the general declining trend, however, was an increase in trap catches on 19/03/09.

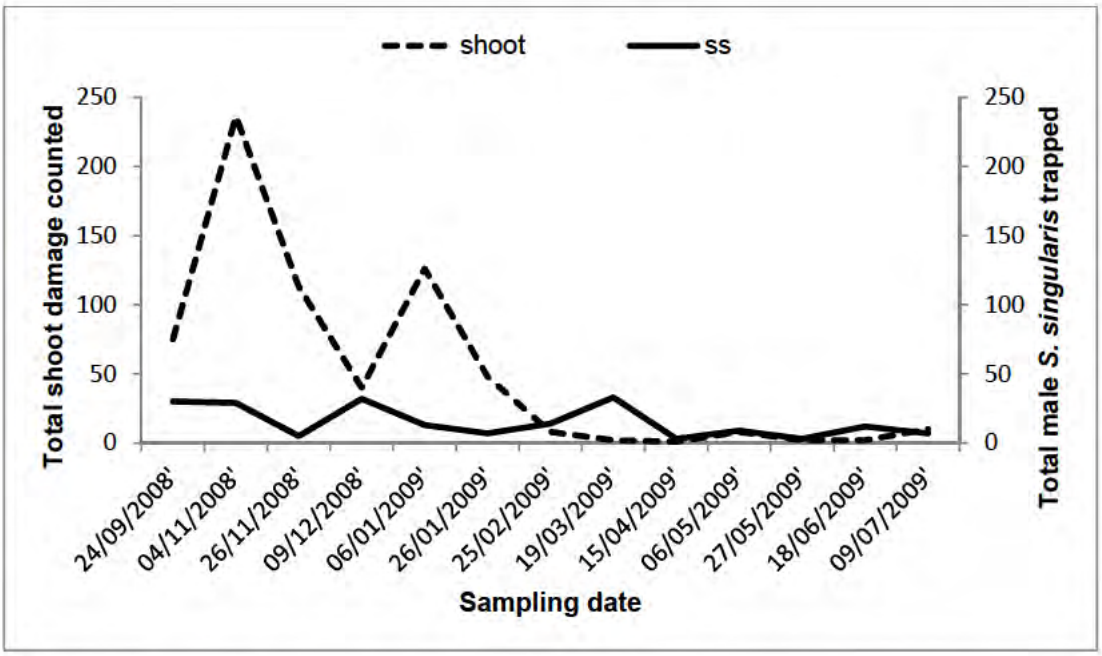

Figure 7.5 Total untransformed trap catches of male *S. singularis* in pheromone monitoring traps and visually assessed damaged shoots counted in control plots of the mass-trapping experiment at Acherensua (24 September 2008 - 9 July 2009). Trap catches are represented by 'ss' and shoot counts by 'shoot'

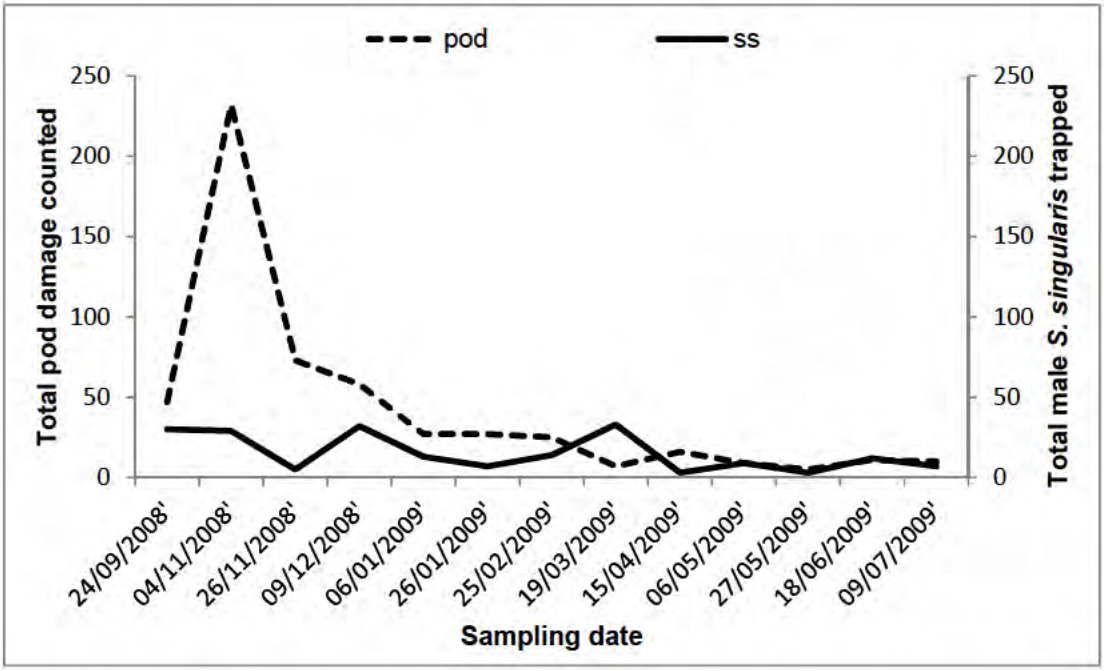

Figure 7.6 Total untransformed trap catches of male *S. singularis* in pheromone monitoring traps and visually assessed damaged pods counted in control plots of the mass-trapping experiment at Acherensua (24 September 2008 - 9 July 2009). Trap catches are represented by 'ss' and pod counts by 'pod'

7.3.7 Regression relationships between trap catches of mirids and field damage

There were no significant regression relationships with field damage when transformed and untransformed total counts of either shoots damage or pods damage were regressed on male trap catches of *D. theobroma* (N = 520, df 7, 499, F = 0.51, P = 0.477; N = 520, df 7, 499, F = 0.74, P = 0.391 for shoots and pods damage respectively).

However, both transformed and untransformed data showed that there were significant (P<0.05) relationships when both visual counts of shoots and pods damage in the associated field were regressed separately on trap catches of male *S. singularis* (Table 7.3). The significant regression of visually counted shoots (N = 520, df 7, 499, F = 4.48, P < 0.001) and pods (N = 520, df 7, 499, F = 4.83, P < 0.001) on monitoring trap catches of male *S. singularis* were different among the blocks. Thus the blocks term was significant in shoots (N= 520, df 7, 499, F = 118.96, P < 0.001) and pods (N = 520, df 7, 499, F = 186.08, P < 0.001). Sampling date also significantly affected shoot damage (N = 520, df 7, 499, F = 9.46, P < 0.001) and pod damage (N = 520, df 7, 499, F = 12.35, P < 0.001) among the blocks. Thus the regression had trap catches, blocks and sampling date as explanatory variables and had the model $y = a + bx + ct + \varepsilon$, where; y= damage, a = constant, b = regression co-efficient, x = trap catches, c = regression co-efficient of period, t = period (sampling date) and ε = residual. This model accounted for 17.3% and 24.7% of the variation in damaged shoot and pod count records respectively. Values for the constants and regression coefficients of the equations of the relationship for shoots and pods damage, derived for each block from the parameter estimates of the analysis, are shown in Table 7.4 and 7.5 respectively.

From the coefficients, monitoring trap catches of male *S. singularis* predicted shoots and pods damaged by mirids inconsistently in the blocks. In the shoot (Table7.4), if the date (period) is fixed, a unit increase in trap catch predicted increases of 0.95, 0.57, 0.11 and 0.36 in shoot damage on trees in blocks 2, 3, 5 and 8 but a decrease of 0.75, 0.06, 0.51 and 0.003 in blocks 1, 4, 6 and 7

respectively. Similarly in the pod (Table7.5), if the date is fixed, a unit increase in trap catch predicted increases of 0.05, 0.78, 0.06 and 0.05 in pod damage on trees in blocks 1, 2, 6, and 7 but a decrease of 0.006, 0.03, 0.85 and 0.06 in blocks 3, 4, 5 and 8 respectively. Overall, fresh damage to both shoots and pods tended to reduce in subsequent sampling dates, therefore, showing a reducing effect on damage in blocks.

Table 7.2 Constants and coefficients of the regression equations of the relationships between trap catches of male *S. singularis* and visual counts of shoot damage on trees in blocks [y= a + bx + ct + ε, where; y= damage, a = constant, b = regression co-efficient, x = trap catches, c = regression co-efficient of period, t = period (sampling date) and ε = residual].

Block	a	b	c
1	1.33 (± 0.30)	– 0.08 (± 0.16)	– 0.05 (± 0.02)
2	1.70 (± 0.42)	+ 0.94 (± 0.34)	– 0.14 (± 0.02)
3	1.50 (± 0.43)	+ 0.57 (± 0.45)	– 0.10 (± 0.02)
4	1.21 (± 0.37)	– 0.06 (± 0.31)	– 0.03 (± 0.02)
5	1.01 (± 0.45)	+ 0.11 (± 0.47)	– 0.02 (± 0.02)
6	1.95 (± 0.45)	– 0.51 (± 0.50)	– 0.06 (± 0.02)
7	1.27 (± 0.30)	– 0.00 (± 0.24)	– 0.04 (± 0.02)
8	1.35 (± 0.36)	+ 0.36 (± 0.26)	– 0.06 (± 0.02)

Table 7.3 Constants and coefficients of the regression equations of the relationships between trap catches of male *S. singularis* and visual counts of pod damage on trees in blocks[y= a + bx + ct + ε, where; y= damage, a = constant, b = regression co-efficient, x = trap catches, c = regression co-efficient of period, t = period (sampling date) and ε = residual].

Block	a	b	c
1	1.63 (± 0.24)	+ 0.05 (± 0.13)	– 0.06 (± 0.02)
2	2.09 (± 0.33)	+ 0.78 (± 0.95)	– 0.15 (± 0.02)
3	1.03 (± 0.35)	– 0.01 (± 0.02)	– 0.01 (± 0.02)
4	1.65 (± 0.28)	– 0.03 (± 0.25)	– 0.06 (± 0.02)
5	1.13 (± 0.36)	– 0.09 (± 0.37)	– 0.02 (± 0.02)
6	1.07 (± 0.36)	+ 0.06 (± 0.40)	– 0.03 (± 0.02)
7	0.94 (± 0.24)	+ 0.05 (± 0.19)	– 0.02 (± 0.02)
8	1.34 (± 0.28)	– 0.06 (± 0.21)	– 0.04 (± 0.02)

7.4 DISCUSSION

7.4.1 Relationships between catches in pheromone traps and visual assessment of mirid numbers

No significant regression relationships were found during the experimental period between catches of male *S. singularis* in pheromone traps and visual assessments of numbers of adults of *S. singularis*, adults of *D. theobroma,* nymphs of both species or total numbers of adults and nymphs. However, examination of the data indicated that increases in trap catches were associated with increases in mirid populations on some occasions, and exclusion of results

from the initial two recording occasions when trap catches were high but numbers of mirids recorded by visual assessment were low, gave a significant regression relationship between trap catches and total mirid numbers.

Similar difficulties have been found in use of pheromone traps for monitoring other species of mirids. For example, Suzette (2008) evaluated the potential of pheromone traps as monitoring tools for the green mirid, *C. dilutes,* at three sites in Australia. Comparison of male trap catches and results from four field assessment methods - visual counts, suction, beat sheets and sweep-nets showed a significant association between pheromone trap catch numbers and the absolute number of adult and nymphs in the field in two locations but not at the third (Suzette, 2008). Smith and Borden (1990) compared field populations of the mullein bug, *C. verbasci,* with male captures in traps baited with live females in 10 apple orchards in the Okanagan Valley, British Columbia, in Canada over five different short periods from 14 September to 13 October, 1987. They found similar positive relationships in the first four intervals when trap catches were high but not for the last one when trap catches were low (Smith and Borden, 1990). Yasuda and Higuchi (2012) also compared pheromone trap catch of males and sweep net assessment of field populations of the rice leaf bugs *T. caelestialium* and *S. rubrovittatus* in paddy fields in Japan, and reported a synchrony between the numbers caught by the two methods. However, no quantitative analysis was done and the graphs shown are similar to those obtained here (Yasuda and Higuchi, 2012).

Low numbers of adults of both *S. singularis* and *D. theobroma* were recorded in this study, suggesting the hand height sampling method used is not very effective. Comparison of mirid assessment by hand height visual counts and whole tree pyrethrum knockdown counts on mature Amelonado cocoa by Collingwood (1971a) showed that the former underestimated the populations by a factor of 12.6 despite the high correlation between the two methods. According to Gibbs *et al.* (1968), there is migration of mirids to the canopy layers from the trunks during the period of dryness and out of range of visual counts because of pod removal among other reasons. Mirid counts for the analysis in this chapter were mainly from a dry period and could have been affected by migration into the canopy

which was not covered by the sampling method though there were pods on the trunks. Therefore, the numerically more adult *D. theobroma* (23) than *S. singularis* (9) counted might be a reflection of the higher preference by the latter for the canopy than the former though both are normally distributed in all parts of the tree (Collingwood, 1971a).

The low numbers of adults counted could also mean that the number of trees sampled was insufficient to have quantified the populations accurately because of the clumped distribution of mirids (Bisseleua *et al.,* 2011; Babin *et al*., 2010; Youdeowei, 1965; Williams, 1953b; Squire, 1947). Increasing the number of trees sampled to ensure adequate coverage of the plot might improve the link between trap capture and associated field populations.

High numbers of nymphs on the other hand could be counted because they were less mobile, particularly on pods (Marchart, 1968; 1969a), and these factors must all be considered in further work on assessing the use of pheromone traps to monitor mirid populations.

7.4.2 Relationships between catches in pheromone traps and assessment of mirid damage

Both transformed and untransformed data showed that there were significant relationships between trap catches of male *S. singularis* and overall visual counts of damage to shoots and pods. However, predictions of future damage by trap catches were inconsistent as a result of different block models. This makes it impossible for individual predictions to be applied across the whole field. The different regression coefficients representing the separate regression relationships suggested the influence of the seasonal incidence and the aggregated spatial distribution of mirid population (Bisseleua *et al.,* 2011; Babin *et al*., 2010; Youdeowei, 1965; Williams, 1953b; Squire, 1947).

Reports of direct significant linkage between pheromone trap catches of mirids and field damage appear lacking but it has been reported with other sampling methods. From several sampling methods including visual counts on whole trees

regenerated after coppicing, visual counts on normal trees to hand height of all trees on 0.5ha plots, knockdown spraying into standard cotton sheets and light trap catches of adult *S. singularis,* Gibbs *et al.* (1968) showed that mirid numbers corresponded with damage. They also showed that population and damage were at peak between August and January and start declining in February to July with some variation between plots. Collingwood *et al.* (1971) showed from their seven-year study of 25 untreated 0.5 ha untreated plots in the Eastern Region of Ghana that high mirid population between 1967-1969 corresponded with high damage within that period.

In other mirids too, Thistlewood *et al.* (1989b) reported a significant relationship between numbers of *C. verbasci* sampled by limb tapping of apples and damage, and this was used to establish thresholds. The authors compared numbers of nymphs and damaged apples at harvest in orchards in Okanagan Valley, Canada, and suggested the establishment of two economic injury levels of one nymph per tap and four nymphs per tap for a susceptible cultivar called the 'Golden Delicious' and a less susceptible one, 'Red Delicious' respectively (Thistlewood *et al.,* 1989b). A link between pheromone trap catches and field damage has been reported in other insect groups such as the Coleoptera. Mathieu *et al*. (1999) estimated damage in the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) by trap catches. By trapping adult females in kairomone baited traps over a 10 month period in a coffee plantation in New Caledonia, Mathieu *et al*., (1999) found a highly significant relationship between the infestation level and log mean trap catch from initial berry colonisation to harvesting.

Pheromone trap catches of *S. singularis* were generally lower than damage counted (Figures 7.5 and 7.6) for the period and this agrees with results from hand-height assessment method reported by Owusu-Manu (1971). In a study on spatial distribution of mirids in Ghana, he assessed mirid numbers by counting both adult and nymphs within hand height along the trunk of mature cocoa trees on farmers' farms. From that method he found that mirid populations were comparatively lower from March to June than the damage counts. Like this study, he also reported a general reduction in the mirid numbers and damage within that

period and attributed this to either the reduced pods on trees or the inability of inexperienced numerators to identify fresh shoot damage. Babin *et al.* (2011) also attributed reduction in mirid populations to reduced numbers of pods. However, mirids are known to feed equally on both pods and shoots (Squire, 1947; Williams, 1953a) and the lack of one need not necessarily reduce fecundity. Nonetheless, reduction in mirid numbers is generally believed to be due to water stress as a result of reduced moisture in the dry season (Collingwood, *et al*., 1971).

7.4.3 Conclusions

In mirids, several synthesised sex pheromones have been recommended for monitoring after encouraging captures by traps baited with the pheromones. For example, Kakizaki and Sugie (2001), after identifying the sex pheromones of *T. caelestialium*, recommended its use in monitoring based on its attraction to males. Zhang and Aldrich (2003b) suggested the use of synthetic sex pheromones of *P. difficilis* to sample and monitor the populations based on trap catches, instead of the tedious and time consuming methods of beating tray and sweep-net sampling. After the identification and synthesis of the female sex pheromones of *C. verbasci* by Smith *et al.* (1991), McBrien *et al.* (1994) followed up to develop traps to monitor the same insect in Canada. However, a serious attempt at developing threshold levels from monitoring mirid populations has only been reported in *C. verbasci* (McBrien *et al.,* 1994; McBrien *et al.,* 1996). The evaluation done in this chapter is exploratory; nonetheless, the results add the sex pheromones of the cocoa mirids to the list of mirid pheromones with the potential for monitoring. The relationships, however, need some improvements which are discussed further in Chapter 8.

Chapter 8
GENERAL DISCUSSION

8.1 AIMS AND OBJECTIVES

Mirids are the most important insect pests of cocoa in West Africa (Dungeon, 1910; Owusu-Manu, 1985, 1996; N'Guessan and Coulibaly, 2000; Sounigo *et al*., 2003; Babin *et al*., 2008) The most important species in Ghana are *Sahlbergella singularis* Hagl and *Distantiella theobroma* (Dist)(Heteroptera : Miridae) (Padi and Owusu, 2001; Owusu-Manu, 1985) with the former being the most widespread attacking cocoa from Sierra Leone in West Africa to Zaire and Central Africa Republic in the East (Padi and Owusu, 2001). Despite the patchiness of their distribution and relative low numbers, they wreak a lot of havoc on cocoa by the feeding activities of both nymph and adult (Williams, 1953a; Entwistle, 1972; Collingwood, 1977). They puncture the branches and pods producing lesions which may be infected by pathogenic fungi *Albonectria* (*Calonectra*) *rigidiuscula* (Brek. & Boome) and *Lasiodiplodia theobromae* (Owen) (Cotterell, 1926; Crowdy, 1947). This causes the wilting of young cherelles and degradation of the canopy (Wright, 1938). Estimates of losses caused by their damage in Ghana range between 25 – 30% per annum and as high as 75% in poorly-managed farms (Anon. 1951; Wills, 1962; Stapley and Hammond, 1959).

The pests are mainly controlled by monthly foliar application of synthetic chemical insecticides (Collingwood and Marchart, 1971; Padi and Owusu, 2001; Owusu-Manu, 2002). It is done four times in the year starting from August to December and omitting November. This blanket application of broad-spectrum insecticides has been problematic since it has resulted in the development of resistance in mirids (Dunn, 1963) and destruction of natural enemies to increase pest incidence (Entwistle *et al.,* 1959; Lodos, 1967). It has also led to the shifting of pest status (Owusu-Manu, 1974). Recommended spray regimes are rarely followed by farmers because of expense and difficulty of application (Davis 2001) resulting in

indiscriminate use of cheap and highly toxic insecticides (Ackonor, *et al*., 2006) with inherent dangers of environmental unfriendliness and human health risks (Murray and Lopez, 1996; Bouwman *et al.*, 2006). In addition, increasingly stringent requirements to reduce insecticide residue levels in cocoa and the prospect that many of the insecticides in use will be banned under new EU legislation, show the unsustainability of this approach. In West Africa, the problems associated with insecticide use have generated great interest in the use of safe non-chemical methods of disinfestation of cocoa such as the use of mirid sex pheromones. Trapping with sex pheromone traps is environmentally friendly because the pheromone is non-toxic and the removal of the pest may only be accompanied by insignificant number of beneficial insects (Witzgall *et al*., 2010)

Studies of a control strategy using the female sex pheromones of the cocoa mirids was initiated by CRIG in collaboration with the NRI and CABI Bioscience, UK and its African Research centre in Nairobi, Kenya in 1998. The work which was funded by DFID, led to the identification and synthesis of the female sex pheromones of *D. theobroma* at NRI. The synthetic sex pheromones consist of a blend of a diester, hexyl (*R*)-3-(*E*)-2-butenoyl butyrate, and a monoester, hexyl (*R*)-3-hydroxy butyrate, in approximately 2:1 ratio (Downham *et al.,* 2002). Field bioassays at CRIG showed attraction of males of both *S. singularis* and *D. theobroma* to the synthetic pheromone dispensed in polyethylene vials at the optimal load of 1.0 mg (Padi *et al.*, 2002). However, other parameters for trapping such as optimal lure dosage, effective age of lure, as well as optimal traps, density and height of placement were not determined conclusively.

This study, which was funded by Cocoa Research UK, followed on from that funded by DFID. The research aimed at refining and building on the pheromone technology developed in previous studies by CRIG and NRI and investigating the use of pheromone trapping in the control of the cocoa mirids. The specific objectives were to optimise lure blends and evaluate effective age of the lures; optimise trap designs, height of placement and density for trapping; determine the suitability of mass trapping for the control of cocoa mirids and finally determine the potential of pheromone trapping for monitoring of mirid populations and their damage. The experiments were designed to provide information and contribute

towards the standardisation of pheromone trapping methods for both control and monitoring of the cocoa mirids in West Africa.

Results from the study satisfy the objectives by providing parameters necessary for standardising pheromone trapping and showing the capacity of pheromone trapping in mirid control. Specifically, optimal lure and its longevity in the field as well as suitable traps, their density and optimal height of placement have been identified and recommended for trapping. The results have shown the failure of mass trapping as a method of control of cocoa mirids and damage. However, positively they have also shown the potential of pheromone trapping in monitoring mirid populations and the damage they cause.

8.2 STANDARDISATION OF TRAPPING PARAMETERS

The study helped to standardise lure blends, lure age, trap designs and placement as well as density of traps for pheromone trapping of mirids.

8.2.1 Lure blends

Preliminary studies conducted on pheromone blends and their ageing during and after the CRIG/CABI/NRI project on mirid pheromone were largely small and un-replicated calling for caution in the application of the results. The present studies, however, were not only large and replicated severally (8-fold), but they also included both the low and peak mirid infestations as well as the wet and dry climates of the year in Ghana during the fifteen calendar months of the experiment. The results are, therefore, believed to be reflective of the holistic cocoa farm environment.

From results of the lure optimization experiment in chapter 2, the blend of diester, hexyl (*R*)-3-(*E*)-2-butenoyl butyrate, and monoester, hexyl (*R*)-3-hydroxy butyrate, impregnated in polyethylene vials in the ratio 1000 µg:500 µg diester to monoester attracted highest numbers of mirids though not significantly more than the 1000 µg:1000 µg blend which attracted lower numbers. These results are in agreement with those of Sarfo and Ackonor (2007a) in which the authors reported that the

same two blends caught the highest number of male mirids. They are, however, in contrast to the predominance of 1000 µg:50 µg as the highest attracting blend reported in the previous DFID-funded pheromone work (Padi *et al.,* 2002). Perhaps the numerically highest attraction by 1000 µg:500 µg should be expected as it is in the same ratio as the female sex pheromones (Downham *et al*., 2002).

8.2.2 Cross-attraction by lure blend

Results of catches by traps using the synthetic pheromones as lure in experiments, particularly in Chapters 2, 4, 5 and 6, demonstrated cross- attraction by the synthetic pheromone. Males of *S. singularis, D. theobroma* and *B. laticollis* of three genera *Sahlbergella, Distantiella* and *Bryocoropsis* respectively, were attracted by the lure. As discussed in chapter 6, this suggests that the major component, hexyl (*R*)-3-(*E*)-2-butenoyl butyrate (Downham *et al.*, 2002; Padi *et al.*, 2002) which is the main attractive component as shown in Chapter 2, may be generic possibly because it is produced by females of both *D. theobroma* and *S. singularis* albeit lower in the latter (Padi *et al*., 2002). This would suggest that this major component may also be produced by *B. laticollis*.

Attributing cross attraction to the sharing of common pheromone components is precedented in mirids and other families of Heteroptera. Zhang and Aldrich (2003b) reported attraction of males of *P. breviusculus* to traps baited with two components of synthetic pheromones of *P. difficilis*, hexyl and (*E*)-2-octenyl acetates, and suggested the cross attraction to be due to the common sharing of these components by the two species in their pheromones. Innocenzi *et al*. (2005) showed that traps baited with synthetic pheromone of *L. rugulipennis* attracted significant numbers of *L. pratensis* in the field and suggested that (*E*)-2–hexenyl butyrate produced by females of both *L. rugulipennis* (Innocenzi *et al.,* 2005) and *L. pratensis* (Fountain *et al*., unpublished data) might be responsible. In related families, Zhang and Aldrich (2003b) observed that a blend of a component of synthetic aggregation pheromone of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae), (*E*)-2-octenyl acetate, and, a non-pheromone compound, (*E,E*)-2,4-hexadienyl acetate, attracted males of *P. difficilis* Knight

(Heteroptera: Miridae). Investigations showed this was due to synergism between the two components. Subsequent analyses of methathoracic scent gland secretions of female *P. difficilis* showed significant amounts of (*E*)-2-octenyl acetate in the sex pheromone (Zhang and Aldrich, 2003b).

It may be an advantage to have a multi-species pheromone lure for trapping, particularly in monitoring because of reduction in cost of logistics. However, the attraction of both species of mirids by the single pheromone lure which is contrary to the conspecific attraction in field trials with virgin females of *S. singularis* and *D. theobroma* as lures (Padi *et al*., 2002), suggest that the blend may not be optimal. Possibly the species employ additional volatiles not included in the present lure or other cues e.g. auditory and visual, in attracting conspecific mates may be missing (Moraal *et al*., 1993; Jones, 1998; Suckling *et al*., 2005). It could also mean that since the two species seemed to produce the two pheromone components in essentially the same ratio, approximately 2:1 diester:monoester (Padi *et al.*, 2002), they utilize the same pheromones for mating, but the different temporal patterns of activity ensured conspecific attraction. This might be supported by the observation by King (1973) that peak flight activity and attraction of male *D. theobroma* to the virgin females coincided with the release of pheromones by the females in the late afternoon from about 15:30 hr GMT to dusk only and also by the report by Padi *et al.* (2002) of the nocturnality of *S. singularis*. The above phenomenon has precedence among sweet potato weevils, *Cylas puncticollis* (Boheman) and *C. brunneus* (Fabricius) according to Downham *et al.* (1999). From pheromone trapping experiments in Uganda, Downham *et al.* (1999) reported cross attraction of *C. puncticollis* to the synthetic pheromone component of *C. brunneus*, dodecyl (*E*)-2-butenoate, in traps. They also observed temporal separation of activity of males of the two species; male *C. brunneus* were captured between 16:00 and 21:00 hrs. and *C. puncticollis* between midnight and 04:00 hrs. providing a mechanism to ensure species-specificity in mating.

Cross-attraction in *S. singularis*, *D. theobroma* and *B. laticollis* needs to be investigated to confirm or reject the reasons advanced for its occurrence in this study above. This will help to produce blends that would be closer to the natural pheromones of the individual species and specific to be more competitive (El-

Sayed and Trimble, 2002; El-Sayed, 2006). Also cross attraction in the mirids with specific pheromones would be of interest because that would be counter to conspecific attraction by sex pheromones and might challenge the classical attribute of sex pheromones as volatiles for attraction of conspecific mates (Karlson and Butenandt, 1959; Karlson and Luscher, 1959).

8.2.3 Relative attraction of *S. singularis* and *D. theobroma* by lures

Although the pheromone lures attracted males of both *S. singularis* and *D. theobroma*, trap captures from all the experiments in which both species were captured showed that more of the former were caught in traps than the latter. However, this difference in numbers trapped was not reflected in the relative numbers of each species recorded by visual inspection. Proportions of the respective species captured in those experiments are shown in Table 8.1. An average of 93.4% and 5.9% of male *S. singularis* and *D. theobroma* were trapped respectively.

Table 8.1 Numbers of male mirid *S. singularis* and *D. theobroma* caught in traps in optimisation of lure blend and trap design experiment at Akwadum, and combined mass trapping experiments at Acherensua(i) Mfranor and Atiebu (ii) relative to numbers observed by visual inspection.

	% Caught in traps relative to visual inspection	
Experiment	***S. singularis***	***D. theobroma***
Optimisation of lure blend	99.7	0.3
Optimisation of trap design	97.3	0.7
Mass trapping (i) and (ii)	83.4	16.6

Chi-square analysis of trap captures of the two species in the mass trapping trial at Achrerensua (Chapter 4) showed clearly that significantly more *S. singularis* were trapped than *D. theobroma*. This is in spite of the fact that the main attractant is produced by both species (Padi *et al*., 2002).

Also Chi-square analysis of trap catch data in mass trapping at Mfranor and Ateibu (Chapter 5) in the two consecutive years of the experiment, showed highly significantly more *D. theobroma* on trees in the visual assessment than were caught in pheromone traps. However, the reverse was the case for *S. singularis* indicating that the pheromone traps were not attracting *D. theobroma* as effectively as they were attracting *S. singularis*.

Collingwood (1977) reported that *D. theobroma* was a more serious pest than *S. singularis* in Ghana, whereas elsewhere in West Africa the latter was dominant. However, Collingwood (1977) also noted that proportions of the two species fluctuate widely, a point supported by distribution studies in Ghana (Gibbs *et al.*, 1968) and in Cote d'Ivoire (Lavabre *et al.*, 1963). Results from all the experiments of this study showed clearly that *S. singularis* was the dominant species. As already discussed in Chapter 3, Owusu-Manu (1996) also made a similar observation but did not assign any reason for it probably because that was not the object of his study. This present study did not provide any answer either but as speculated in Chapter 3, perhaps chupon removal and regular harvesting of pods (Baah *et al.,* 2009; Baah, 2010, 2011) might be reducing the feeding and breeding sites of *D. theobroma* (Entwistle, 1957; King, 1971) to result in their dwindling populations.

According to King (1973), the low attraction of male *D. theobroma* to pheromones might have something to do with better competition by virgin female *D. theobroma* because of closeness and stronger attraction between sexes than traps, due to their aggregated distribution, as discussed in Chapter 4. This present study did not investigate reasons behind the lower catches of *D. theobroma* than *S. singularis* by the traps and it would be worthwhile to evaluate attraction of mirids to pheromone trap in areas with high *D. theobroma* to determine whether

the low catches are an expression of weak attraction to lures or other factors such as declining numbers in Ghana.

8.2.4 Lure age

Results from the ageing experiment in Chapter 2 also showed that the 1000 µg : 500 µg blend attracted male mirids optimally for 4 weeks with slight decline in 8 weeks, and was still attractive at 12 weeks when the experiment was terminated. The loss of attractiveness with age is in conformity with previous results by Sarfo and Ackonor, (2007b), though different methods were used as have already been discussed in Section 2.4. The results indicated however, that the optimal age of attraction could be higher than the 4 weeks and might lie between 4 and 8 weeks but could not be determined from the intervals tested in the experiment. The lure would not be fully effective after ageing but it would be useful for monitoring purposes and the less frequently they are changed the less it would cost both in materials and in labour.

Padi *et al.* (2002) showed that release of the pheromone components from the polyethylene vials was first order, i.e. proportional to the amount remaining, and that release of the monoester component was faster than that of the diester. Thus the reduction in attractiveness of the lures observed here over time may be due to the declining release rate and/or to the declining proportion of the monoester in the blend released.

The results provide researchers with optimal lures and their age for effective trapping as contribution to the standardisation of pheromone trapping of the cocoa mirids.

8.2.5 Trap designs

Standard rectangular and delta traps were tested for mirid pheromone trapping during the initial CRIG/CABI/NRI work on mirid pheromones. However, the

present study is the first time traps made from locally available materials were used for pheromone trapping. Results of the optimisation of trap designs in Chapter 3 showed that both sticky and water traps designed from locally available plastic water cans and plates were as good as the imported standard traps for the capture of mirids. The materials for the traps are available on the local market, cheap and easily accessible. They are also simple, durable, easy to fabricate and easy to handle. This removed the challenge posed by the unavailability and inaccessibility of exotic standard traps.

The study identified designs suitable for both annihilation methods such as mass trapping and lure and kill or monitoring. As already discussed in section 3.4, identifying and counting of catches in water traps three or more weeks after capture, was difficult even in alcohol, because of decomposition of the dead insects. This coupled with the need for frequent refilling with water due to fast evaporation and/or drinking by birds and other animals, make the water traps suitable for use more in methods such as mass trapping and lure and kill than monitoring. Monitoring would require shorter intervals, preferably weekly or shorter for recording of catches. On the other hand catches by the sticky traps remained recognisable even after three weeks and hence suitable for the annihilation methods and monitoring as well. However, not only could their efficiencies be reduced by the accumulation of debris, dust and dead insects, necessitating frequent maintenance, but also the sustainability of their use is not in the least helped by the unavailability of the polybutene sticker on the local market in Ghana.

The results also showed that the addition of the external surface for trapping maximised trap catches by significantly increasing trap catches more than four-fold. Thus the mean catch of the normal bottle increased from 2.12 to 9.24 male *S. singularis* per trap per day when the external surface was included in the trapping with the application of sticky glue on the outside. In percentage terms the normal bottle trapped about 23% of the total catch when the external surface was included. Also, the application of glue on the outside of the trap appeared to have boosted catches inside significantly by 70.7% (mean catch per trap per day increased from 2.12 to 3.62). As already discussed in Chapter 3, this might

possibly be due to deflection of attracted mirids from the surface by residual warning signals released by dying mirids caught on the outer surface as reported in the Coleopteran *Tribolium castaneum* by Trematerra *et al*. (1996). Though Yasuda and Higuchi (2012) also found parity in mirid catches by water and sticky traps, this is the first time mirids have been trapped in both surfaces of the trap.

8.2.6 Trap placement

Results of the trap height experiments in Chapter 3 showed captures of highest numbers of male mirids inside the canopy or around the canopy, i.e. just below the canopy at 2.7 m and 0.3 m above the canopy. This indicated the importance of canopy in influencing trap catches and suggested the placement of traps in the canopy to maximise catches in pheromone trapping for monitoring or attraction and annihilation purposes.

Significantly different captures at the various heights suggest very little overlapping of plumes vertically. This is because with long periods of calmness under the canopy (Murlis *et al.,* 2000), the turbulence necessary to mix the pheromone in the vertical plane is reduced and mirids might have been attracted to traps in their path only. It would be good to test this hypothesis in areas of high mirid populations in both closed and open cocoa canopies.

Having several traps on a single pole or cocoa tree and having them on different cocoa trees produced similar distribution patterns. However, the former method is novel for pheromone trapping of mirids. It was more efficient because all the two experiments where the method was used at Acherensua and Suhyen had lower standard errors of difference between means (S.E.D. of 0.241 and 0.238 respectively) than the latter method at Akwadum (S.E.D. = 0.510). Thus putting several traps on a single pole or cocoa tree appears to have minimised the trap to trap variation so prevalent in pheromone trap catches of mirids, because of the clumped spatial distribution of cocoa mirids (Bisseleua, *et al.,* 2011; Babin *et al*., 2010; Gibbs *et. al.,* 1968). This suggests that perhaps the setting up of

pheromone trapping of mirids in smaller plots such as pockets where possible, would help produce reliable results.

The results in Chapter 3 provide a procedure for the development of traps from local materials, and also provide researchers with optimal height for the placement of traps. These are a contribution to the development of a standard pheromone trapping method for the cocoa mirids.

8.3 MASS TRAPPING AS A METHOD OF CONTROL OF COCOA MIRIDS

The effectiveness of mass trapping as a direct annihilation method of controlling cocoa mirids was tested in research and smallholders' farms. The experimental design used in research farm was adapted from Stelinski *et al*. (2005) who originally used it for mating disruption studies. It is therefore, novel to mass trapping and different from the routine method of trapping whole plot as a block against whole un-trapped plot. The design was a split plot involving the use of different densities of traps for monitoring which also helped provide the relationship required for the determination of optimal densities for trapping each of the two species of mirids. A third mass trapping experiment was conducted on a research farm at optimal trap densities and height to investigate a possible reason for the results of the preceding experiments. This experiment also investigated the effect of a combination of insecticide and pheromone trapping on mirid control.

From the results of the study, mass trapping on research and smallholders' farms did not demonstrate the ability to control mirid numbers and their damage. This is in spite of the fact that assessments of the effectiveness or otherwise of mass trapping by monitoring traps consistently showed significant reduction in numbers of male *S. singularis.* Insecticide knockdown and visual assessments, however, showed definitively that not enough reduction had occurred in population numbers of the mirids to halt their damage to pods and shoots.

Possible reasons for the failure, including re-infestation by immigrant mirids, wrong placement of traps and high density of traps, have been discussed in

Chapter 4. The possible effect of re-infestation by immigrant mirids tested in a mass trapping experiment of isolated plots with traps at optimal height and density at Afosu and Bunso (Chapter 6) showed that immigration from un-trapped plots was not the cause of the failure. This is the conclusion from the results of mass and monitoring trap catches as well as visual and knockdown assessments; they clearly showed that, just like the results of trapping non-isolated plots, neither mirid numbers nor their damage was controlled. This is probably because there is less dispersal of mirids from their spatially aggregated locations. Mirid populations in cocoa are normally strongly aggregated (Johnson, 1971; Gibbs *et al.,* 1968; Babin *et al.,* 2010; Bisseleua *et al.,* 2011), more in areas with broken and open canopy with sunlight (Williams 1953a; King, 1971) and fresh growth to feed on than in shaded area of closed canopy (Babin *et al.,* 2010). Oviposition normally occurs on trees previously fed on by mirids and movement of nymphs is usually within trees and sometimes only as far as adjacent ones through interlocking branches (Collingwood, 1971). Mirids, therefore, are confined to localised pockets where successive discrete generations might develop from original colonising individuals (Gibbs, *et al.,* 1968) with little or no deterioration in stock (Piart. 1970). There appears to be little incentive for mirids to disperse to colonise other areas and this might help to explain the absence of re-infestation of trapped areas. Nonetheless, adult mirids may exhibit some dispersal (Collingwood, 1971). According to Gibbs *et al.* (1968), there was extensive dispersal of *S. singularis* within 3.5 km^2 area after harvest, to infest manly vegetative tissues in new areas, in the absence of pods. The mass trapping experiment was started at the beginning of harvest and ended about two months after harvest. However, this did not appear to have affected dispersal into trapped areas probably because the hybrid crop was not completely devoid of pods.

The results of the field bioassay on the behavioural responses of mirids to sticky traps and different heights in Chapter 3 help to provide some reasons for the failure of mass trapping. Based on the results, the failure may be due to the apparent capture of mirids from the horizontal path only and also the inability of the trap to capture all mirids that were attracted to it. The apparent inefficiency in trapping the mirids might also have been aggravated by a combination of eco-

biological factors such as the erratic air flow under the cocoa canopy, the patchy distribution of mirids and the high mirid population in the mirid season observed in the study.

The significant results of the height experiments as discussed in Chapter 3 convincingly show that traps at a single height catch males in only the horizontal path as they do not appear to move upwards or downwards in response to the pheromone. Therefore, males outside the attraction zone of the traps in the mass trapping trials could respond to any female calling to mate which would also lead to increase in population and damage.

The results of the field bioassay clearly show the inability of traps to capture all mirids that were attracted to it. From results of mirid attraction to both inside and outside surfaces of the bottle trap, the normal trap caught about 23% only of mirids that were attracted to it. Compared to other reports, this is on the high side for it has been shown in other pest species that many trapping systems are inherently inefficient, capturing as low as 0.4% to 8.7% of insects attracted to traps, despite the recruitment of about 95% of available insects to within 0.5 m of the source by the odour plumes (Howse *et al.,* 1998). Notwithstanding the relatively high proportion trapped, the mirids and damage were not controlled. Therefore, attracted males that escaped trapping might have augmented mating leading to increase in population. This might also have been cause of the observed increased damage in some treated plots particularly in mass trapped farmers' farms already discussed in Chapter 5.

The contribution of the combination of eco-biological factors are the erratic air flow under the cocoa canopy, the patchy distribution of mirids and the high mirid population to the failure of mass trapping is deduced mainly from reported work. As discussed in Chapter 4, Roelofs *et al*. (1970) explain that at high densities traps are overwhelmed by the sheer numbers of virgin females and therefore lose out in the competition for available males. This might not adequately explain the case in mirids because there would be so much diffused pheromone cloud in their aggregated locations in the field that males would have difficulty locating either females or traps. Witzgall *et al.* (2010) on the other hand suggest that distances

between males and females become shorter and communication becomes stronger, leaving external synthetic pheromones with less influence on the communication between the sexes at high density.

In the cocoa mirids too, it is suggested that communication between the sexes are strong with more attraction towards virgin females than traps (King, 1973). It is believed the communication and hence attraction becomes stronger at high pest density for the combination of factors below. Results in the height experiment in Chapter 3 suggest that the horizontal as well as vertical movement of pheromone under the cocoa canopy may be restrained probably because of the relatively calm air under the canopy. However, the air may not always be calm because work by Murlis *et al.* (2000) showed that wind movement under the closed canopy of forest trees, typified by the cocoa canopy, is characterised by long periods of calm, interspersed with periods of air flow which continually changes direction. Calmness under the canopy will restrict the opportunity to create plumes of pheromone and instead promote the simple diffusion of homogenous cloud of pheromone from the traps which will 'arrest' upward flight of males (Kennedy, *et al.,* 1980, 1981; Wills and Baker, 1984). This is because insects need a plume-like structure of pheromone in order to be able to follow it to its source. They follow a pheromone plume by a behaviour known as zig-zag tracking (e.g. Justus *et al*., 2002), flying upwind towards the source using their antennae to maintain track and tracking back into a plume once they temporarily lose it. They would thus follow a defined edge from a lower to higher concentration. The continually changing direction of wind under the canopy results in carrying bursts of pheromone plumes away from traps without immediate follow up thereby creating long breaks between sequential bursts even if there is a repeat flow of air in that direction. Insects can only follow up plumes if breaks between bursts are very short otherwise they lose track. For example Vickers and Baker (1992) noted that males of the moth species, *Heliothis virescens* (Fabricius), must encounter a pheromone pulse within 250 ms of leaving the last pulse if it is to continue upward flight; for the oriental fruit moth, *Grapholita molesta* (Busck), it is 150 ms (Wills and Baker, 1988), and it is about 300 ms for the large silk moth, *Antheraea polyphemus* (Cramer) (Baker and Vogt, 1988). Therefore the male mirid detecting

occasional intermittent plumes a distance away from the trap cannot routinely navigate to the trap as was observed by Elkinton *et al.* (1987) for the male gipsy moth in the forest.

Mirids are close together because of their clumped distribution but they get closer at higher densities and the number of calling females would also increase with the increased population. Whereas the closeness would help males to respond to calling females quicker, increased female numbers would also make more calling females available to attract 'arrested' males in homogenous cloud of pheromone or those that lose track of plume. Therefore, with increase in numbers at high density, virgin females would out-compete the traps. Also damage under high density is aggravated by the fact that a high proportion of mirids made up of females and nymphs that are not trapped also feed on the pods and shoots.

8.4 POTENTIAL OF PHEROMONE TRAPPING FOR MONITORING

Results from all the trapping experiments showed the capture of mirids by traps in both low and high populations. This demonstrated the capacity of pheromone trapping for the detection of mirids and the potential to serve as a suitable replacement for the difficult, tiring and cumbersome method of scouting for the presence of mirids in the field through visual search as practiced presently. In Chapter 7, results of evaluation established a general significant relationship between pheromone trap catches of male *S. singularis* and total mirid population which include adult *S singularis*, adult *D. theobroma* and nymphs for general prediction. This supports the observation made in Chapters 2 and 3 of the reflection of the total monthly catches in the blend and trap design optimisation trials respectively, with the reported population dynamics of mirids at Akwadum. The relationship demonstrates the link between trap catches and field populations (Suckling, 2000) and shows the potential of pheromone trapping in monitoring seasonal incidence of cocoa mirids. Significant relationships were also established between trap catches of male *S. singularis* and mirid damage to shoots and pods.

It may be an advantage to have trap catches of the more abundant mirid species indexed to monitoring total adult and nymph populations of the two species, as well as damage to both shoots and pods, because of a possible single model being developed for future estimates of all parameters. However, as already discussed in Chapter 7, while the link between trap catches and populations appear weak, the link between trap catches and damage is inconsistent and cannot be applied generally. This provides the basis, in both cases, for further investigation for improvement before they can be applied for general predictive purposes.

Results of the present study highlighted the difficulty in developing a general relationship between pheromone trap catches and field variates such as damage for patchily distributed mirids (Gibbs *et al.,* 1968; Babin *et al.,* 2010; Bisseleua *et al.,* 2011) with definite seasonal incidence, because of variation (King, 1973). It appeared that traps or trees sampled from the mirid pockets (areas of high concentration of mirids) recorded higher catches of *S. singularis* or counts of damage than those outside the pockets, therefore, making the results dependant on the spatial locations of the selected trees for trap placement and counting relative to mirid pocket. This may create the scenarios where the relationship between trap catch inside mirid pocket and damage counted outside the pocket in a block would probably be negative while the reverse in another block would be positive. Estimates were thus affected by the seasonal changes in numbers of mirids which impacted the incidence, and also the distribution of damage in blocks, because the insects occur patchily.

The results of this study established for the first time a link between pheromone trap catches of male *S. singularis* and field populations of mirids and damage. It would provide valuable source of information for subsequent development of threshold levels for controlling cocoa mirids in a bio-rational way, particularly in West Africa. To improve the link between trap catch and associated field populations and damage of mirids for predictive purposes, factors likely to affect the relationship as suggested by the results should be included in the experimental design. Consequently, populations and visible crop damage in three categories of tree quality; almost pristine, mild symptoms and moderately severe

should be considered. The high variation in relationships between monitoring trap catches and damage also emphasises the need to determine the optimal density of trees to be sampled. Field population counts of mirids could be improved with additional methods of assessment such as pyrethrum knockdown.

8.5 SUGGESTIONS FOR FURTHER WORK

The faster release of the monoester might have shifted the ratio of the blend increasing to the diester for most of the trapping period. However, catches were maximised when monoester was at least half of the diester in the mixture of the two components. Therefore, the lures could be further improved by delaying the release of the minor component, possibly by employing a composite dispenser with a slower release rate for the monoester.

Results of the lure age experiment showed the optimal age of attraction at 4 weeks but there was strong indication that it could be optimal between 4 and 8 weeks and could also stay longer than the 12 weeks tested. Therefore, it would be necessary to test the daily attractiveness of the blends aged weekly from 4 - 8 weeks and also 12 - 24 weeks for two months or more in high mirid populations, to determine when attraction begins to reduce and finish.

As already discussed in Chapter 3, the presence of dead mirids might be deflecting incoming mirids into the traps probably because of volatiles from the dead mirids as in other insects (Trematerra *et al.,* 1996). In order to test this hypothesis, it would be necessary to study the effects of dead mirids on trap catches including investigation of the composition of volatiles that might be produced from captured mirids.

It would be necessary to improve the trapping system to maximise captures in view of the apparent inefficiency of the traps. Therefore, in addition to the modification of trap designs already suggested in Chapter 4, further studies on the sex pheromones of the mirids are suggested to look at the identification of possible additional components and/ or refinement of ratios. The study should

also investigate the possibility of the use of cues in addition to pheromones in conspecific attraction in cocoa mirids.

In view of the failure of mass trapping to control mirids it would be necessary to explore other options to control mirids with pheromones. Considering the calmness and apparent reduced turbulence under the canopy it would be appropriate to saturate the field with pheromone from several point sources that would mask any plumes from calling females and disrupt the mating process of the mirids. Also suggested is the attraction of male mirids to lures in areas saturated with pathogens or alternatively to be knocked down with insecticides in a lure and kill tactic.

Results from the regression analysis clearly demonstrate that experiments to improve the regression relationships between pheromone trap catches of mirids and associated populations and damage should be designed to explicitly to reduce to the barest minimum the effect of seasonal incidence and the aggregated spatial distribution of the mirids. These factors were not incorporated in the design in Chapter 7 because the experiment was not originally set up for this evaluation.

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APPENDIX

DATA ANALYSIS

CHAPTER 2 DATA

Pheromone blend

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r	Fpr.
Block stratum	7	606.1	86.6	0.40	
block.*units* stratum					
Treat	5	5791.9	1158.4	5.32	<0.001
Residual	35	7618.0	217.7		
Total	47	14016.0			

Tables of means

Grand mean 14.5

Treat	a	b	c	d	e	f
	13.0	20.6	27.6	25.1	0.5	0.3

Standard errors of differences of means = 7.38

Least significant differences of means = 14.98

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
block stratum	7	5.832	0.833	0.34	
block.*units* stratum					
Treat	5	167.397	33.479	13.86	<0.001
Residual	35	84.520	2.415		
Total	47	257.748			

Tables of means

Grand mean 3.03

Treat	a	b	c	d	e	f
	3.34	4.10	4.91	4.85	0.57	0.42

Standard errors of differences of means = 0.549

Least significant differences of means = 1.577

Age of synthetic pheromone

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r	Fpr.
block stratum	7	152.80	21.83	0.91	
Block.*units* stratum					
Treat	4	402.40	100.60	4.21	<0.009
Residual	28	669.20	23.90		
Total	39	1224.40			

Tables of means

Grand mean 6.80

Treat	fresh	2-week	4-week	8-week	12-week
	10.38	10.38	6.62	3.37	2.87

Standard errors of differences of means = 2.444

Least significant differences of means = 5.00

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r	Fpr.
Block stratum	7	5.4388	0.7770 .	0.88	
Block.*units* stratum					
Treat	4	17.5109	4.3777	4.98	<0.004
Residual	28	24.6125	0.8790		
Total	39	47.5622			

Tables of means

Grand mean 2.38

Treat	fresh	2-week	4-week	8-week	12-week
	3.09	3.13	2.42	1.70	1.56

Standard errors of differences of means = 0.469

Least significant differences of means = 0.96

CHAPTER 3 DATA

Trap design

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r	Fpr.
block stratum	7	812.70	116.10	2.25	
block.*units* stratum					
Treat	4	34.35	8.59	0.17	0.954
Residual	28	1444.05	51.57		
Total	39	2291.10			

Tables of means

Grand mean 7.2

Treat	a	b	c	d	e
	5.6	7.7	6.6	8.3	7.5

Standard errors of differences of means = 3.59

Least significant differences of means = 7.36

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	21.749	3.107	2.12	
Block.*units* stratum					
Treat	4	0.584	0.146	0.10	0.982
Residual	28	41.072	1.467		
Total	39	63.404			

Tables of means

Grand mean 2.37

Treat	a	b	c	d	e
	2.26	2.38	2.53	2.47	2.21

Standard errors of differences of means = 0.428

Least significant differences of means = 1.240

Captures by outside surface of trap

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	7	80.479	11.497	4.14	0.002
Catch	5	105.687	21.137	7.62	<.001
ingluetrap v innonglue	1	15.125	15.125	5.45	0.025
Deviations	4	90.562	22.641	8.16	<.001
Residual	35	97.146	2.776		
Total	47	283.312			

Tables of means

Grand mean 2.81

Block	I	II	III	IV	V	VI	VII	VIII
	3.17	3.83	2.67	1.17	1.00	3.33	5.17	2.17

Catch	A	Bin	Bout	C	Din	Dout

2.12	3.62	5.62	1.50	2.75	1.25

Standard errors of differences of means

Block	Catch
0.962	0.833

LSD5% = 1.70

LSD1% = 2.29

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	7	11.6835	1.6691	5.21	<.001
Catch	5	9.5873	1.9175	5.99	<.001
ingluetrap v innonglue	1	1.4649	1.4649	4.57	0.039
Deviations	4	8.1224	2.0306	6.34	<.001
Residual	35	11.2073	0.3202		
Total	47	32.4781			

Tables of means

Grand mean 1.461

Block	I	II	III	IV	V	VI	VII	VIII
	1.549	1.883	1.496	0.858	0.577	1.740	2.191	1.397

Catch	A	Bin	Bout	C	Din	Dout
	1.332	1.724	2.267	1.018	1.482	0.945

Standard errors of differences of means

Block	Catch
0.3267	0.2829

LSD5% = 0.577

LSD1% = 0.778

Analysis of variance

Variate: male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	7	99.813	14.259	4.31	0.002
Catch	5	122.354	24.471	7.40	<.001
ingluetrap v innonglue	1	15.125	15.125	4.57	0.040
Deviations	4	107.229	26.807	8.10	<.001
Residual	35	115.812	3.309		
Total	47	337.979			

Tables of means

Grand mean 3.15

Block	I	II	III	IV	V	VI	VII	VIII
	3.17	4.33	3.17	1.50	1.00	4.33	5.50	2.17

Catch	A	Bin	Bout	C	Din	Dout
	2.25	3.75	6.38	1.63	2.88	2.00

Standard errors of differences of means

	Block	Catch
	1.050	0.910

LSD5% = 1.86

LSD1% = 2.50

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	7	12.5859	1.7980	5.87	<.001
Catch	5	10.3031	2.0606	6.73	<.001
ingluetrap v innonglue	1	1.7158	1.7158	5.60	0.024
Deviations	4	8.5873	2.1468	7.01	<.001
Residual	35	10.7165	0.3062		
Total	47	33.6055			

Tables of means

Grand mean 1.564

Block	I	II	III	IV	V	VI	VII	VIII
	1.549	2.021	1.646	1.093	0.577	1.946	2.280	1.397

Catch	A	Bin	Bout	C	Din	Dout
	1.384	1.849	2.428	1.048	1.508	1.166

Standard errors of differences of means

Block	Catch
0.3195	0.2767

LSD5% = 0.564

LSD1% = 0.761

Trap placement (Acherensua)

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.

Treat	2	120.844	60.422	8.97	<.001
Residual	42	283.067	6.740		
Total	44	403.911			

Tables of means

Grand mean 2.16

Treat	a	b	c
	0.53	1.53	4.40

Standard errors of means = 0.670

Standard errors of differences of means = 0.948

Least significant differences of means (5% level) = 1.913

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Treat	2	9.4990	4.7495	10.93	<.001
Residual	42	18.2533	0.4346		
Total	44	27.7523			

Tables of means

Grand mean 1.428

Treat	a	b	c
	0.962	1.269	2.053

Standard errors of means = 0.1702

Standard errors of differences of means = 0.2407

Least significant differences of means (5% level) = 0.4858

Trap placement (Suhyen)

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	9	21.400	2.378	0.76	
Block.*Units* stratum					
Height	3	316.200	105.400	33.56	<.001
Residual	27	84.800	3.141		
Total	39	422.400			

Tables of means

Grand mean 3.70

Height	1.8	2.7	incan	abovcan
	0.60	1.50	5.10	7.60

Standard errors of differences of means = 0.793

Least significant difference (5%) = 1.63

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	9	2.5828	0.2870	1.01	
Block.*Units* stratum					
Height	3	28.8611	9.6204	33.98	<.001
Residual	27	7.6432	0.2831		
Total	39	39.0871			

Tables of means

Grand mean 1.650

Height	1.8	2.7	incan	abovcan
	0.600	1.080	2.189	2.731

Standard errors of differences of means = 0.2379

Least significant difference (5%) = 0.488

Analysis of variance

Variate: male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	9	32.025	3.558	0.98	
Block.*Units* stratum					
Height	3	358.875	119.625	33.00	<.001
Residual	27	97.875	3.625		
Total	39	488.775			

Tables of means

Grand mean 3.92

Height	1.8	2.7	incan	abovcan
	0.60	1.50	5.70	7.90

Standard errors of differences of means = 0.851

Least significant difference (5%) = 1.74

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	9	2.9974	0.3330	1.13	
Block.*Units* stratum					

Height	3	31.3545	10.4515	35.48	<.001
Residual	27	7.9539	0.2946		
Total	39	42.3059			

Tables of means

Grand mean 1.693

Height	1.8	2.7	incan	abovcan
	0.600	1.080	2.314	2.779

Standard errors of differences of means = 0.2427

Least significant difference (5%) = 0.498

Trap placement (Akwadum)

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	5	30.800	6.160	0.68	
Block.*Units* stratum					
Height	4	127.533	31.883	3.53	0.025
Residual	20	180.867	9.043		
Total	29	339.200			

Tables of means

Grand mean 3.60

Height	can<1.8	1.8	2.7	incantl	abvcantl
	2.17	0.67	3.83	6.67	4.67

Standard errors of differences of means = 1.736

Least significant difference (5%) = 3.63

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	5	1.4221	0.2844	0.36	
Block.*Units* stratum					
Height	4	10.9691	2.7423	3.52	0.025
Residual	20	15.5931	0.7797		
Total	29	27.9843			

Tables of means

Grand mean 1.63

Height	can<1.8	1.8	2.7	incantl	abvcantl
	1.40	0.57	1.89	2.28	2.03

Standard errors of differences of means = 0.510

Least significant difference (5%) = 1.07

Analysis of variance

Variate: male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	5	43.87	8.77	0.74	
Block.*Units* stratum					
Height	4	163.80	40.95	3.47	0.026
Residual	20	235.80	11.79		
Total	29	443.47			

Tables of means

Grand mean 3.87

Height	can<1.8	1.8	2.7	incantl	abvcantl

	2.17	0.67	3.83	7.33	5.33

Standard errors of differences of means = 1.982

Least significant difference (5%) = 4.14

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	5	1.8663	0.3733	0.45	
Block.*Units* stratum					
Height	4	12.6514	3.1628	3.78	0.019
Residual	20	16.7241	0.8362		
Total	29	31.2418			

Tables of means

Grand mean 1.68

Height	can<1.8	1.8	2.7	incantl	abvcantl
	1.40	0.57	1.89	2.42	2.13

Standard errors of differences of means = 0.528

Least significant difference (5%) = 1.10

CHAPTER 4 DATA

Total catches in treatment plots prior to deploying monitoring traps.

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	175.100	25.014	4.74	
Block.*Units* stratum					
Treatmnt	4	2.900	0.725	0.14	0.967
Residual	28	147.900	5.282		
Total	39	325.900			

Tables of means

Grand mean 3.05

Treatmnt	a	b	c	d	e
	2.87	3.37	2.87	2.75	3.37

Standard errors of differences of means = 1.149

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	15.9554	2.2793	3.54	
Block.*Units* stratum					
Treatmnt	4	0.2246	0.0561	0.09	0.986
Residual	28	18.0073	0.6431		
Total	39	34.1874			

Tables of means

Grand mean 1.48

Treatment	a	b	c	d	e
	1.52	1.48	1.59	1.46	1.36

Standard errors of differences of means = 0.401

Analysis of variance

Variate: male *D. theobroma*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	211.975	30.282	3.43	
Block.*Units* stratum					
Treatmnt	4	27.100	6.775	0.77	0.555
Residual	28	246.900	8.818		
Total	39	485.975			

Tables of means

Grand mean 2.47

Treatmnt	a	b	c	d	e
	0.78	1.08	0.97	1.50	1.30

Standard errors of differences of means = 0.378

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	29.6868	4.2410	7.41	
Block.*Units* stratum					
Treatmnt	4	2.5226	0.6307	1.10	0.375
Residual	28	16.0300	0.5725		
Total	39	48.2394			

Tables of means

Grand mean 1.13

Treatment	a	b	c	d	e
	0.78	1.08	0.97	1.50	1.30

Standard errors of differences of means = 0.378

Analysis of variance

Variate: male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	732.78	104.68	6.27	
Block.*Units* stratum					
Treatmnt	4	35.35	8.84	0.53	0.715
Residual	28	467.85	16.71		
Total	39	1235.98			

Tables of means

Grand mean 5.53

Treatment	a	b	c	d	e
	4.25	5.37	5.12	5.75	7.12

Standard errors of differences of means = 2.044

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.

Block stratum	7	34.8801	4.9829	7.24	
Block.*Units* stratum					
Treatmnt	4	0.6688	0.1672	0.24	0.911
Residual	28	19.2606	0.6879		
Total	39	54.8094			

Tables of means

Grand mean 2.04

Treatment	a	b	c	d	e
	1.91	1.88	2.03	2.17	2.20

Standard errors of differences of means = 0.415

Total catches in the release traps only after deploying monitoring traps

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	33.200	4.743	0.79	
Block.*Units* stratum					
Treatment	4	8.350	2.087	0.35	0.843
Residual	28	168.050	6.002		
Total	39	209.600			

Tables of means

Grand mean 3.60

Treatment	a	b	c	d	e
	2.75	4.00	3.62	4.00	3.62

Standard errors of differences of means = 1.225

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	2.8561	0.4080	0.87	
Block.*Units* stratum					
Treatment	4	0.4867	0.1217	0.26	0.901
Residual	28	13.1160	0.4684		
Total	39	16.4588			

Tables of means

Grand mean 1.79

Treatment	a	b	c	d	e
	1.63	1.90	1.71	1.92	1.77

Standard errors of differences of means = 0.342

Analysis of variance

Variate: male *D. theobroma*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	7	232.575	33.225	6.50	
Block.*Units* stratum					

Treatmnt	4	37.350	9.338	1.83	0.152
Residual	28	143.050	5.109		
Total	39	412.975			

Tables of means

Grand mean 2.22

Treatment	a	b	c	d	e
	2.00	2.37	4.00	1.37	1.37

Standard errors of differences of means =1.130

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	26.7796	3.8257	8.63	
Block.*Units* stratum					
Treatment	4	1.2703	0.3176	0.72	0.588
Residual	28	12.4094	0.4432		
Total	39	40.4594			

Tables of means

Grand mean 1.10

Treatment	a	b	c	d	e
	0.91	1.32	1.31	0.98	0.98

Standard errors of differences of means = 0.333

Variate: male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	386.18	55.17	5.27	
Block.*Units* stratum					
Treatmnt	4	44.65	11.16	1.07	0.391
Residual	28	292.95	10.46		
Total	39	723.78			

Tables of means

Grand mean 5.83

Treatment	a	b	c	d	e
	4.75	6.37	7.62	5.37	5.00

Standard errors of differences of means = 1.617

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	14.8682	2.1240	4.57	
Block.*Units* stratum					
Treatment	4	1.1517	0.2879	0.62	0.653
Residual	28	13.0273	0.4653		
Total	39	29.0473			

Tables of means

Grand mean 2.26

Treatment	a	b	c	d	e
	2.06	2.37	2.53	2.22	2.12

Standard errors of differences of means = 0.341

Total catches in monitoring traps only

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	7	38.000	5.429	0.46	
Block.Wplots stratum					
Treat	1	85.563	85.563	7.22	0.031
Residual	7	82.938	11.848	1.31	
Block.Wplots.Splots stratum					
Montraps	3	212.625	70.875	7.86	<.001
Lin	1	176.446	176.446	19.58	<.001
Quad	1	7.079	7.079	0.79	0.381
Deviations	1	29.100	29.100	3.23	0.080
Montraps.Treat	3	21.312	7.104	0.79	0.507
Lin.Treat	1	2.884	2.884	0.32	0.575
Quad.Treat	1	3.374	3.374	0.37	0.544
Deviations	1	15.055	15.055	1.67	0.203
Residual	42	378.562	9.013		
Total	63	819.000			

Tables of means

Grand mean 3.37

Montraps	2	4	8	15
	1.87	1.31	4.69	5.62

Treat	Treat	Cont
	2.22	4.53
Montraps	Treat	Cont
2	0.75	3.00
4	1.00	1.62
8	2.75	6.62
15	4.37	6.87

Standard errors of differences of means

	Montraps	Treat	Montraps Treat
	1.061	0.861	1.559
LSD =	2.143	2.032	3.149

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	7	4.7976	0.6854	0.71	
Block.Wplots stratum					
Treat	1	8.4892	8.4892	8.82	0.021
Residual	7	6.7359	0.9623	1.45	
block.Wplots.Splots stratum					
Montraps	3	23.3747	7.7916	11.73	<.001
Lin	1	20.1049	20.1049	30.26	<.001
Quad	1	0.9660	0.9660	1.45	0.235
Deviations	1	2.3038	2.3038	3.47	0.070
Montraps.Treat	3	0.5338	0.1779	0.27	0.848
Lin.Treat	1	0.0772	0.0772	0.12	0.735

Quad.Treat	1	0.0832	0.0832	0.13	0.725
Deviations	1	0.3734	0.3734	0.56	0.458
Residual	42	27.9056	0.6644		
Total	63	71.8369			

Tables of means

Grand mean 1.50

Montraps	2	4	8	15
	0.95	0.87	1.93	2.26

Treat	Treat	Cont
	1.14	1.87

Montraps	Treat	Cont
2	0.52	1.38
4	0.59	1.14
8	1.45	2.41
15	1.99	2.53

Standard error of differences of means

	Montraps	Treat	Montraps Treat
	0.288	0.245	0.430
LSD =	0.582	0.578	0.869

Variate: male *D. theobroma*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	7	12.234	1.748	2.45	

Block.Wplots stratum				
Treat	1	0.141	0.141	0.20	0.670
Residual	7	4.984	0.712	0.53	
Block.Wplots.Splots stratum				
Montraps	3	18.547	6.182	4.62	0.007
Lin	1	18.453	18.453	13.80	<.001
Quad	1	0.070	0.070	0.05	0.820
Deviations	1	0.024	0.024	0.02	0.895
Montraps.Treat	3	3.047	1.016	0.76	0.523
Lin.Treat	1	0.005	0.005	0.00	0.953
Quad.Treat	1	2.959	2.959	2.21	0.144
Deviations	1	0.083	0.083	0.06	0.804
Residual	42	56.156	1.337		
Total	63	95.109			

Tables of means

Grand mean 0.83

Montraps	2	4	8	15
	0.31	0.44	0.88	1.69

Treat	Treat	Cont
	0.88	0.78

Montraps	Treat	Treat	Cont
2	0.63	0.00	
4	0.38	0.50	
8	0.63	1.12	
15	1.87	1.50	

Standard error of differences of means

Montraps	Treat	Montraps Treat
0.409	0.211	0.543
LSD = 0.826	0.498	1.097

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	2.7207	0.3887	1.40	
Block.Wplots stratum					
Treat	1	0.0125	0.0125	0.05	0.838
Residual	7	1.9430	0.2776	0.67	
Block.Wplots.Splots stratum					
Montraps	3	7.0025	2.3342	5.66	0.002
Lin	1	6.9676	6.9676	16.89	<.001
Quad	1	0.0236	0.0236	0.06	0.812
Deviations	1	0.0114	0.0114	0.03	0.869
Montraps.Treat	3	1.2568	0.4189	1.02	0.395
Lin.Treat	1	0.1787	0.1787	0.43	0.514
Treat	1	0.9192	0.9192	2.23	0.143
Deviations	1	0.1588	0.1588	0.38	0.538
Residual	42	17.3289	0.4126		
Total	63	30.2644			

Tables of means

Grand mean 0.596

Montraps	2	4	8	15
	0.239	0.364	0.686	1.095

Treat	Treat	Cont
	0.610	0.582

Montraps	Treat	Treat	Cont
2		0.479	0.000
4		0.302	0.427
8		0.552	0.820
15		1.108	1.081

Standard errors of differences of means

	Montraps	Treat	Montraps Treat
	0.2271	0.1317	0.3077
LSD =	0.4587	0.3108	0.6216

Variate: total male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	38.98	5.57	0.43	
Block.Wplots stratum					
Treat	1	78.77	78.77	6.07	0.043
Residual	7	90.86	12.98	1.07	
Block.Wplots.Splots stratum					
Montraps	3	345.55	115.18	9.49	<.001
Lin	1	309.02	309.02	25.47	<.001
Quad	1	5.74	5.74	0.47	0.495
Deviations	1	30.79	30.79	2.54	0.119
Montraps.Treat	3	28.67	9.56	0.79	0.508

Lin.Treat	1	3.12	3.12	0.26	0.615
Quad.Treat	1	12.65	2.65	1.04	0.313
Deviations	1	12.90	2.90	1.06	0.308
Residual	42	509.53	12.13		
Total	63	1092.36			

Tables of means

Grand mean 4.20

Montraps	2	4	8	15
	2.19	1.75	5.56	7.31

Treat	Treat	Cont
	3.09	5.31

Montraps	Treat	Treat	Cont
2		1.37	3.00
4		1.37	2.12
8		3.37	7.75
15		6.25	8.38

Standard errors of differences of means

	Montraps	Treat	Montraps Treat
	1.231	0.901	1.757
LSD =	2.487	2.126	3.549

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	5.2515	0.7502	0.74	
Block.Wplots stratum					
Treat	1	6.9319	6.9319	6.84	0.035

Residual	7	7.0927	1.0132	1.34	
Block.Wplots.Splots stratum					
Montraps	3	30.2815	10.0938	13.37	<.001
Lin	1	26.4217	26.4217	35.00	<.001
Quad	1	1.0883	1.0883	1.44	0.237
Deviations	1	2.7715	2.7715	3.67	0.062
Montraps.Treat	3	0.6713	0.2238	0.30	0.828
Lin.Treat	1	0.0603	0.0603	0.08	0.779
Quad.Treat	1	0.5213	0.5213	0.69	0.411
Deviations	1	0.0897	0.0897	0.12	0.732
Residual	42	31.7074	0.7549		
Total	63	81.9361			

Tables of means

Grand mean 1.71

Montraps	2	4	8	15
	1.08	1.00	2.18	2.59

Treat	Treat	Cont
	1.38	2.04

Montraps	Treat	Treat	Cont
2		0.78	1.38
4		0.68	1.31
8		1.68	2.67
15		2.38	2.80

Standard errors of differences of means

	Montraps	Treat	Montraps Treat

	0.307	0.252	0.453
LSD =	0.620	0.595	0.915

Total catches per plot in all traps after deploying monitoring traps

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	46.67	6.67	0.50	
Block.*Units* stratum					
Traps	7	242.94	34.71	2.62	0.021
Lin	1	146.11	146.11	11.01	0.002
Quad	1	0.82	0.82	0.06	0.805
Deviations	5	96.01	19.20	1.45	0.222
Residual	57	756.40	13.27		
Total	71	1046.00			

Tables of means

Grand mean 5.00

Traps	2	4	8	15	17	19	23	30
	3.00	1.62	6.62	4.81	4.75	4.62	6.75	8.00

	s.e.d.		LSD
	1.821	min.rep	3.642
	1.577	max-min	3.154
	1.288X	max.rep	2.576

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	3.9443	0.5635	0.88	
Block.*Units* stratum					
Traps	7	16.7334	2.3905	3.72	0.002
Lin	1	11.1961	11.1961	17.40	<.001
Quad	1	0.1917	0.1917	0.30	0.587
Deviations	5	5.3456	1.0691	1.66	0.159
Residual	57	36.6702	0.6433		
Total	71	57.3479			

Tables of means

Grand mean 2.050

Traps	2	4	8	15	17	19	23	30
	1.381	1.143	2.405	2.080	2.049	2.044	2.563	2.706

s.e.d.		LSD	
0.4010	min.rep	0.802	
0.3473	max-min	0.6946	
0.2836X	max.rep	0.5672	

Variate: male *D. theobroma*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
block stratum	7	199.278	28.468	4.83	
block.*Units* stratum					

Traps	7	122.694	17.528	2.97	0.010
Lin	1	76.750	76.750	13.02	<.001
Quad	1	9.237	9.237	1.57	0.216
Deviations	5	36.708	7.342	1.25	0.300
Residual	57	335.972	5.894		
Total	71	657.944			

Tables of means

Grand mean 1.97

Traps	2	4	8	15	17	19	23	30
	0.00	0.50	1.12	1.75	3.00	4.37	2.00	3.25

s.e.d.	LSD
1.214 min.rep	2.428
1.051 max-min	2.102
0.858X max.rep	1.716

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	17.1268	2.4467	3.99	
Block.*Units* stratum					
Traps	7	17.5002	2.5000	4.08	0.001
Lin	1	14.6853	14.6853	23.98	<.001
Quad	1	1.5695	1.5695	2.56	0.115

Deviations	5	1.2454	0.2491	0.41	0.842
Residual	57	34.9116	0.6125		
Total	71	69.5387			

Tables of means

Grand mean 1.003

Traps	2	4	8	15	17	19	23	30
	0.000	0.427	0.820	0.996	1.4	1.39	1.360	1.567

s.e.d		LSD
0.3913	min.rep	0.7826
0.3389	max-min	0.6778
0.2767X	max.rep	0.5534

Variate: total male mirids

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	7	329.28	47.04	2.13	
Block.*Units* stratum					
Traps	7	531.13	75.88	3.43	0.004
Lin	1	434.65	434.65	19.67	<.001
Quad	1	4.55	4.55	0.21	0.652
Deviations	5	91.93	18.39	0.83	0.532
Residual	57	1259.53	22.10		
Total	71	2119.94			

Tables of means

Grand mean 6.97

Traps	2	4	8	15	17	19	23	30
	3.00	2.17	7.75	6.56	7.75	9.00	8.75	11.25

s.e.d		LSD
2.350	min.rep	4.700
2.035	max-min	4.070
1.662X	max.rep	3.324

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	7	11.0731	1.5819	2.02	
Block.*Units* stratum					
Traps	7	27.3314	3.9045	4.99	<.001
Lin	1	21.4924	21.4924	27.44	<.001
Quad	1	1.1774	1.1774	1.50	0.225
Deviations	5	4.6616	0.9323	1.19	0.325
Residual	57	44.6415	0.7832		
Total	71	83.0460			

Tables of means

Grand mean 2.41

Traps	2	4	8	15	17	19	23	30
	1.38	1.31	2.67	2.43	2.57	2.78	2.91	3.22

s.e.d		LSD
0.442	min.rep	0.884
0.383	max-min	0.766

0.313X max.rep 0.626

Knockdown counts

Two-sample t-test

Variates: adult *D. theobroma* in treatment and control.

Test for equality of sample variances

Test statistic F = 1.14 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.68

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
kddtT	40	0.2250	0.2814	0.5305	0.08388
kddtC	40	0.1000	0.2462	0.4961	0.07845

Difference of means = 0.125

Standard error of difference = 0.115

95% confidence interval for difference in means: (-0.1036 0.3536,)

Test of null hypothesis that mean of kddtT is equal to mean of kddtC

Test statistic t =1.09 on 78 d.f.

Probability = 0.280

Variates: adult S. singularis in treatment and control.

Test for equality of sample variances

Test statistic F = 1.85 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.06

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
kdssT	40	0.1750	0.1994	0.4465	0.07060
kdssC	40	0.3000	0.3692	0.6076	0.09608

Difference of means: -0.125

Standard error of difference: 0.119

95% confidence interval for difference in means: (-0.3624, 0.1124,)

Test of null hypothesis that mean of kdssC is equal to mean of kdssT

Test statistict=1.05on78d.f.

Probability= 0.298

Variates: nymph *S.singularis* in treatment and control

Test for equality of sample variances

Test statistic F = 30.51 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) < 0.001
Note: strong evidence of unequal sample variances -

variances estimated separately for each group.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
kdssnT	40	0.0500	0.0487	0.2207	0.0349
kdssnC	40	0.2750	1.4865	1.2192	0.1928

Difference of means: -0.225

Standard error of difference: 0.196

95% confidence interval for difference in means: (-0.6205, 0.1705)

Test of null hypothesis that mean of kdssnT is equal to mean of kdssnC

Test statistic t = -1.15 on approximately 41.55 d.f.

Probability = 0.257

Variates: nymph D. theobroma in treatment and control.

Test for equality of sample variances

Test statistic F = 3.06 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) < 0.001
Note: strong evidence of unequal sample variances -

variances estimated separately for each group.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
kdtnT	40	1.1250	7.753	2.784	0.4403
kdtnC	40	0.6750	2.533	1.591	0.2516

Difference of means = 0.450

Standard error of difference = 0.507

95% confidence interval for difference in means: (-0.5637, 1.464)

Test of null hypothesis that mean of kdtnT is equal to mean of kdtnC

Test statistic t = 0.89 on 78 d.f.

Probability = 0.378

Single trap monitoring

Two-sample t-test

Variates: male *S. singularis* in treatment and control

Test for equality of sample variances

Test statistic F = 58.15 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) < 0.001

Note: strong evidence of unequal sample variances -

variances estimated separately for each group.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
ssT	40	0.725	0.77	0.877	0.1386
ssC	40	6.025	44.69	6.685	1.0570

Difference of means = -5.300

Standard error of difference =1.066

95% confidence interval for difference in means: (-7.454, 3.146)

Test of null hypothesis that mean of ssT is equal to mean of ssC

Test statistic t = 4.97 on approximately 40.34 d.f

Probability < 0.001

Variates: male *D. theobroma* in treatment and control.

Test for equality of sample variances

Test statistic F = * on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 1.00

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
DtT	40	0.00000	0.00000	0.00000	0.00000
DtC	40	0.17500	0.19936	0.4465	0.07060

Difference of means = -0.1750

Standard error of difference = 0.0706

95% confidence interval for difference in means: (0.3155, 0.03445)

Test of null hypothesis that mean of dtC is equal to mean of dtT

Test statistic t = 2.48 on 78 d.f.

Probability = 0.015

Visual assessment

Two-sample t-test

Variates: adult *S. singularis* in treatment and control.

Test for equality of sample variances

Test statistic F = 1.96 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.04
Note: evidence of unequal sample variances -

variances estimated separately for each group.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
SsT	40	0.6500	4.079	2.020	0.3194
ssC	40	0.3750	2.087	1.444	0.2284

Difference of means = 0.275

Standard error of difference = 0.393

95% confidence interval for difference in means: (-0.5079, 1.058)

Test of null hypothesis that mean of SsT is equal to mean of ssC

Test statistic t = 0.70 on 78 d.f.

Probability = 0.486

Variates: adult *D. theobroma* in treatment and control

Test for equality of sample variances

Test statistic F = 1.32 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.39
Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
DtT	40	0.9750	3.717	1.928	0.3048
DtC	40	0.5750	2.815	1.678	0.2653

Difference of means = 0.400

Standard error of difference = 0.404

95% confidence interval for difference in means: (-0.4045, 1.205)

Test of null hypothesis that mean of DtT is equal to mean of dtC

Test statistic t = 0.99 on 78 d.f.

Probability = 0.325

Variates: nymphs *S. singularis* and *D. theobroma* combined in treatment and control.

Test for equality of sample variances

Test statistic F = 1.84 on 39 and 38 d.f.

Probability (under null hypothesis of equal variances) = 0.06

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
NymphT	40	5.275	96.67	9.832	1.555
nymphC	39	3.846	52.45	7.242	1.160

Difference of means: 1.429

Standard error of difference: 1.947

95% confidence interval for difference in means: (-2.448, 5.306)

Test of null hypothesis that mean of NymphT is equal to mean of nymphC

Test statistic t = 0.73 on 77 d.f

Variates: mirid damaged shoot in treatment and control.

Test for equality of sample variances

Test statistic F = 2.87 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.00
Note: strong evidence of unequal sample variances -

variances estimated separately for each group.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
ShootT	40	15.63	150.4	12.26	1.939
ShootC	40	16.95	431.7	20.78	3.285

Difference of means = -1.325

Standard error of difference = 3.815

95% confidence interval for difference in means: (-8.948, 6.298)

Test of null hypothesis that mean of ShootT is equal to mean of shootC

Test statistic t = -0.35 on 78 d.f.

Probability = 0.729

Variates: mirid damaged pods in treatment and control

Test for equality of sample variances

Test statistic F = 1.09 on 39 and 39 d.f.

Probability (under null hypothesis of equal variances) = 0.80
Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
PodsT	40	14.98	349.7	18.70	2.957

PodC	40	13.67	321.8	17.94	2.836

Difference of means = 1.300

Standard error of difference = 4.097

95% confidence interval for difference in means: (-6.857, 9.457)

Test of null hypothesis that mean of PodsT is equal to mean of podC

Test static t = 0.32 on 78 d.f.

Probability = 0.752

CHAPTER 5 DATA

Visual assessment of *S. singularis*

Year 2009

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	233.000	58.250	17.92	0.008
Treat	1	10.000	10.000	3.08	0.154
Residual	4	13.000	3.250		
Total	9	256.000			

Tables of means

Grand mean 4.00

Block	Akote	Alhassan	Osafo	Appiah	Ayittey

	3.00	0.50	13.50	1.00	2.00

Treat	Phero	Control
	5.00	3.00

	Block	Treat
Standard errors of means	= 1.275	0.806
Standard errors of differences of means	= 1.803	1.140

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	13.1779	3.2945	4.75	0.080
Treat	1	0.9595	0.9595	1.38	0.305
Residual	4	2.7740	0.6935		
Total	9	16.9114			

Tables of means

Grand mean 1.52

Block	Akote	Alhassan	Osafo	Appiah	Ayittey
	1.73	0.50	3.66	0.71	1.00

Treat	Phero	Control
	1.83	1.21

	Block	Treat
Standard errors of means	= 0.589	0.372
Standard errors of differences of means	= 0.833	0.527

Year 2010

Analysis of variance

Untransfrmed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	769.7	153.9	0.86	0.565
Treat	1	456.3	456.3	2.54	0.172
Residual	5	898.7	179.7		
Total	11	2124.7			

Tables of means

Grand mean 8.7

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	1.0	0.0	6.5	24.0	8.5	12.0

Treat	Phero	Control
	14.8	2.5

Standard errors of differences of means

Block	Treat
13.41	7.74

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	20.534	4.107	0.91	0.539
Treat	1	12.192	12.192	2.71	0.161
Residual	5	22.498	4.500		
Total	11	55.224			

Tables of means

Grand mean 2.02

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	1.0	0.00	2.37	4.22	2.06	2.45

Treat	Phero	Control
	3.02	1.01

Standard errors of differences of means

	Block	Treat
	2.121	1.225

Visual assessment of male mirids (*S. singularis* and *D. theobroma*)

Year 2009

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	1053.60	263.40	7.89	0.035
Treat	1	102.40	102.40	3.07	0.155
Residual	4	133.60	33.40		
Total	9	1289.60			

Tables of means

Grand mean 7.8

Block	Akote	Alhassan	Osafo	Appiah	Ayittey
	3.0	1.0	28.0	1.0	6.0

Treat	Phero	Control
	11.0	4.6

Standard errors of differences of means

Block	Treat
5.78	3.66

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	26.316	6.579	6.38	0.050
Treat	1	4.359	4.359	4.22	0.109
Residual	4	4.127	1.032		
Total	9	34.802			

Tables of means

Grand mean 2.08

Block	Akote	Alhassan	Osafo	Appiah	Ayittey
	1.73	1.00	5.22	0.71	1.73

Treat	Phero	Control
	2.74	1.42

Standard errors of differences of means

Block	Treat
1.016	0.642

Year 2010

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	1867.8	373.6	0.95	0.520
Treat	1	1026.8	1026.8	2.62	0.166
Residual	5	1959.8	391.9		
Total	11	4854.2			

Tables of means

Grand mean 11.8

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	1.0	0.0	8.0	37.0	8.5	16.0

Treat	Phero	Control
	21.0	2.5

Standard errors of differences of means

Block	Treat
19.80	11.43

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	30.532	6.106	1.10	0.459
Treat	1	19.747	19.747	3.56	0.118
Residual	5	27.743	5.549		
Total	11	78.021			

Tables of means

Grand mean 2.29

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	1.00	0.00	2.78	5.08	2.06	2.83

Treat	Phero	Control

3.57 1.01

Standard errors of differences of means

Block	Treat
2.356	1.360

2x2 chi- square test of independence to compare the numbers of male mirid species in pheromone trap and total number of mirid species (both male and female) counted in trees.

2009

	Observed		
	S. singularis	*D. theobroma*	Total
Pheromone	398	9	407
Visual assessment	47	48	95
Total	445	57	502

	Expected		
	S. singularis	*D. theobroma*	Total
Pheromone	360.78	46.22	407
Visual assessment	84.22	10.78	95
Total	445	57	502

	Chi-square		
	S. singularis	*D. theobroma*	Total
Pheromone	3.84	29.97	
Visual assessment	16.44	128.5	
Total	20.28	158.47	178.75

2010

	Observed		
	S. singularis	*D. theobroma*	Total
Pheromone	194	17	211
Visual assessment	104	37	141
Total	298	54	352

	Expected		
	S. singularis	*D. theobroma*	Total
Pheromone	179	32	211
Visual assessment	119	22	141
Total	298	54	352

	Chi-square		
	S. singularis	*D. theobroma*	Total
Pheromone	1.26	7.03	
Visual assessment	1.89	10.23	
Total	3.15	17.26	20.41

Visual assessment of pod damage

Year 2009

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	231.40	57.85	1.68	0.313
Treat	1	8.10	8.10	0.24	0.653
Residual	4	137.40	34.35		
Total	9	376.90			

Tables of means

Grand mean 6.9

Block	Akote	Alhassan	Osafo	Appiah	Ayittey
	3.5	4.5	16.0	3.0	7.5

Treat	Phero	Control
	7.8	6.0

Standard errors of differences of means

Block	Treat
5.86	3.71

Year 2010

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	955.8	191.2	0.80	0.595
Treat	1	1064.1	1064.1	4.44	0.089
Residual	5	1198.4	239.7		
Total	11	3218.2			

Tables of means

Grand mean 17.2

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	12.0	5.0	11.0	32.0	23.0	20.5

Treat	Phero	Control
	26.7	7.8

Standard errors of differences of means

Block	Treat

15.48 8.94

Visual assessment of shoot damage

Year 2009

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	55.00	13.75	0.27	0.887
Treat	1	96.10	96.10	1.85	0.245
Residual	4	207.40	51.85		
Total	9	358.50			

Tables of means

Grand mean 7.5

Block	Akote	Alhassan	Osafo	Appiah	Ayittey
	8.5	5.0	10.5	4.5	9.0

Treat	Phero	Control
	10.6	4.4

Standard errors of differences of means

Block	Treat
7.20	4.55

Year 2010

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	5	1218.0	243.6	0.78	0.604
Treat	1	456.3	456.3	1.46	0.281
Residual	5	1561.7	312.3		
Total	11	3236.0			

Tables of means

Grand mean 10.0

Block	Alhassan	Akote	Margaret	Ayittey	Appiah	Osarfo
	2.0	1.5	9.5	31.5	7.5	8.0

Treat Phero Control

16.2	3.8

Standard errors of differences of means

Block	Treat
17.67	10.20

CHAPTER 6 DATA

Monitoring before deployment of traps

Analysis of variance

Variate: male *S. singularis*

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	3	200.92	66.97	1.24	
Block.*Units* stratum					
Treat	2	92.67	46.33	0.85	0.471
Residual	6	325.33	54.22		
Total	11	618.92			

Tables of means

Grand mean 13.9

Treat	a	b	c
	12.8	11.2	17.8

Standard errors of differences of means = 5.21

Least significant differences of means (5% level) = 12.74

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	3	3.2534	1.0845	1.55	
Block.*Units* stratum					
Treat	2	1.3049	0.6525	0.93	0.444

Residual	6	4.1969	0.6995
Total	11	8.7553	

Tables of means

Grand mean 3.70

Treat	a	b	c
	3.64	3.33	4.13

Standard errors of differences of means = 0.591

Least significant differences of means (5% level) = 1.447

Mass trap catches of male *S. singularis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	6916.00	1729.00	1.62	0.259
Treat	2	2528.00	1264.00	1.19	0.354
Residual	8	8517.00	1065.00		
Total	14	17962.00			

Tables of means

Grand mean 52.6

Block	I	II	III	IV	V
	54.7	56.0	78.0	61.3	13.0

Treat	a	b	c
	34.8	57.6	65.4

Standard errors of differences of means

Block	Treat
26.64	20.64

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	45.048	11.262	1.99	0.189
Treat	2	12.454	6.227	1.10	0.378
Residual	8	45.204	5.650		
Total	14	102.705			

Tables of means

Grand mean 6.80

Block	I	II	III	IV	V
	7.38	7.47	8.38	7.35	3.42

Treat	a	b	c
	5.51	7.46	7.43

Standard errors of differences of means

Block	Treat
1.941	1.503

Mass trap catches of *B. laticollis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	3168.7	792.2	2.13	0.168

Treat	2	36.9	18.5	0.05	0.952
Residual	8	2975.7	372.0		
Total	14	6181.3			

Tables of means

Grand mean 22.3

Block	I	II	III	IV	V
	13.3	43.7	23.3	30.3	1.0

Treat	a	b	c
	24.4	20.6	22.0

Standard errors of differences of means

	Block	Treat
	15.75	12.20

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	48.170	12.043	3.83	0.050
Treat	2	0.146	0.073	0.02	0.977
Residual	8	25.131	3.141		
Total	14	73.447			

Tables of means

Grand mean 4.24

Block	I	II	III	IV	V
	3.54	6.42	4.74	5.32	1.17

Treat	a	b	c
	4.31	4.10	4.30

Standard errors of differences of means

Block	Treat
1.447	1.121

Monitoring trap catches of *S. singularis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	4	11.600	2.900	2.15	
Block.*Units* stratum					
Treat	2	6.533	3.267	2.42	0.151
Residual	8	10.800	1.350		
Total	14	28.933			

Tables of means

Grand mean 1.27

Treat	a	b	c
	0.80	0.80	2.20

Standard errors of differences of means = 0.735

Least significant differences of means (5% level) = 1.695

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	4	1.6261	0.4065	2.30	
Block.*Units* stratum					
Treat	2	0.7482	0.3741	2.12	0.183

Residual	8	1.4138	0.1767	
Total	14	3.7881		

Tables of means

Grand mean 1.23

Treat	a	b	c
	1.06	1.09	1.55

Standard errors of differences of means = 0.266

Least significant differences of means (5% level) = 0.613

Monitoring trap catches of *B. laticollis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	4	2.4000	0.6000	0.86	
Block.*Units* stratum					
Treat	2	1.7333	0.8667	1.24	0.340
Residual	8	5.6000	0.7000		
Total	14	9.7333			

Tables of means

Grand mean 0.53

Treat	a	b	c
	0.40	0.20	1.00

Standard errors of differences of means = 0.529

Least significant differences of means (5% level) = 1.220

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block stratum	4	0.4954	0.1239	1.03	
Block.*Units* stratum					
Treat	2	0.2966	0.1483	1.23	0.342
Residual	8	0.9635	0.1204		
Total	14	1.7555			

Tables of means

Grand mean 0.957

Treat	a	b	c
	0.914	0.811	1.147

Standard errors of differences of means = 0.2195

Least significant differences of means (5% level) = 0.5061

Visual counts of adult *S. singularis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	5.600	1.400	1.08	0.428
Treat	2	1.600	0.800	0.62	0.564
Residual	8	10.400	1.300		
Total	14	17.600			

Tables of means

Grand mean 0.60

Block	I	II	III	IV	V

0.00	1.33	0.33	0.00	1.33

Treat	a	b	c
	0.20	1.00	0.60

Standard errors of differences of means

Block	Treat
0.931	0.721

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	0.9875	0.2469	1.42	0.312
Treat	2	0.2037	0.1019	0.58	0.579
Residual	8	1.3929	0.1741		
Total	14	2.5841			

Tables of means

Grand mean 0.96

Block	I	II	III	IV	V
	0.71	1.34	0.88	0.71	1.18

Treat	a	b	c
	0.81	1.09	0.99

Standard errors of differences of means

Block	Treat
0.341	0.264

Visual counts of adult *B. laticollis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	82.00	20.50	1.29	0.350
Treat	2	26.53	13.27	0.84	0.468
Residual	8	126.80	15.85		
Total	14	235.33			

Tables of means

Grand mean 1.3

Block	I	II	III	IV	V
	0.3	6.0	0.3	0.0	0.0

Treat	a	b	c
	3.2	0.2	0.6

Standard errors of differences of means

Block	Treat
3.25	2.52

Visual counts of shoot damage

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	5.067	1.267	0.18	0.941
Treat	2	7.600	3.800	0.55	0.600
Residual	8	55.733	6.967		

Total	14	68.400

Tables of means

Grand mean 1.80

Block	I	II	III	IV	V
	1.33	2.00	2.67	1.00	2.00

Treat	a	b	c
	0.80	2.40	2.20

Standard errors of differences of means

Block	Treat
2.155	1.669

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Block	4	0.2697	0.0674	0.11	0.976
Treat	2	0.8350	0.4175	0.68	0.534
Residual	8	4.9235	0.6154		
Total	14	6.0282			

Tables of means

Grand mean 1.38

Block	I	II	III	IV	V
	1.29	1.56	1.44	1.17	1.43

Treat	a	b	c
	1.06	1.62	1.46

Standard errors of differences of means

Block	Treat
0.641	0.496

Visual counts of pod damage

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	2569.6	642.4	4.64	0.031
Treat	2	152.9	76.5	0.55	0.596
Residual	8	1106.4	138.3		
Total	14	3828.9			

Tables of means

Grand mean 18.3

Block	I	II	III	IV	V
	14.7	42.7	9.3	19.3	5.3

Treat	a	b	c
	18.6	14.2	22.0

Standard errors of differences of means

Block	Treat
9.60	7.44

Insecticide knockdown of adult *S. singularis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	38.933	9.733	5.62	0.019

Treat	2	4.133	2.067	1.19	0.352
Residual	8	13.867	1.733		
Total	14	56.933			

Tables of means

Grand mean 1.27

Block	I	II	III	IV	V
	0.00	4.33	0.67	1.33	0.00

Treat	a	b	c
	0.80	1.00	2.00

Standard errors of differences of means

Block	Treat
1.075	0.833

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	4.2190	1.0547	6.43	0.013
Treat	2	0.2167	0.1083	0.66	0.543
Residual	8	1.3127	0.1641		
Total	14	5.7483			

Tables of means

Grand mean 1.18

Block	I	II	III	IV	V
	0.71	2.15	1.05	1.27	0.71

Treat	a	b	c
	1.09	1.09	1.35

Standard errors of differences of means

Block	Treat
0.331	0.256

Insecticide knockdown of adult *B. laticollis*

Analysis of variance

Untransformed data

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.6000	0.4000	1.00	0.461
Treat	2	0.1333	0.0667	0.17	0.849
Residual	8	3.2000	0.4000		
Total	14	4.9333			

Tables of means

Grand mean 0.27

Block	I	II	III	IV	V
	0.67	0.00	0.67	0.00	0.00

Treat	a	b	c
	0.40	0.20	0.20

Standard errors of differences of means

Block	Treat
0.516	0.400

Data transformed to Square roots (+0.5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.36888	0.09222	1.10	0.419
Treat	2	0.01694	0.00847	0.10	0.905

Residual	8	0.67099	0.08387	
Total	14	1.05680		

Tables of means

Grand mean 0.834

Block	I	II	III	IV	V
	0.998	0.707	1.052	0.707	0.707

Treat	a	b	c
	0.882	0.811	0.811

Standard errors of differences of means

Block	Treat
0.2365	0.1832

CHAPTER 7 DATA

Regression relationship between monitoring trap catches of male *S. singularis* and visual count of total mirids after omitting first two sampling occasions

Data transformed to Square roots (+0.5)

Regression analysis

Response variate: visual count of total mirids

Fitted terms: blocks and trap catches of male *S. singularis*

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	Fpr.
Regression	8	5.18	0.6471	2.81	0.005

Residual	431	99.40	0.2306
Total	439	104.58	0.2382

Percentage variance accounted for 3.2

Standard error of observations is estimated to be 0.480.

Estimates of parameters

Parameter	estimate	s.e.	t(431)	t pr.
blk 1	0.7170	0.1020	7.05	<.001
blk 2	0.7073	0.0866	8.16	<.001
blk 3	0.8120	0.0853	9.52	<.001
blk 4	0.8603	0.0852	10.10	<.001
blk 5	0.5937	0.0851	6.98	<.001
blk 6	0.6421	0.0842	7.63	<.001
blk 7	0.5859	0.0876	6.69	<.001
blk 8	0.6017	0.0894	6.73	<.001
css	0.1472	0.0716	2.06	0.040

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	Fpr.
- Constant	-1	-290.4200	290.4200	1259.23	<.001
+ blk	8	294.6226	36.8278	159.68	<.001
+ css	1	0.9746	0.9746	4.23	0.040
Residual	431	99.4029	0.2306		
Total	439	104.5800	0.2382		

Regression relationship between monitoring trap catches of male *S. singularis* and visual count of shoot damage

Untransformed data

Regression analysis

Response variate: visual count of shoot damage

Fitted terms: blocks, interaction between trap catches of *S. singularis* and block (css.blk) and interaction between period and block (period.blk).

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r	Fpr.
Regression	23	2657.00	115.54	4.17	<.001
Residual	496	13733.00	27.69		
Total	519	16390.00	31.58		

Percentage variance accounted for 12.3

Standard error of observations is estimated to be 5.26.

Estimates of parameters

Parameter	estimate	s.e.	t(496)	t pr.
Blk 1	1.740	1.530	1.14	0.254
Blk 2	10.570	1.640	6.45	<.001
Blk 3	4.190	1.490	2.82	0.005
Blk 4	1.310	1.500	0.88	0.382
Blk 5	1.050	1.500	0.70	0.486
Blk 6	2.960	1.460	2.03	0.043
Blk 7	2.120	1.470	1.44	0.149

Blk 8	3.020	1.560	1.94	0.053
css.Blk 1	-0.056	0.308	-0.18	0.856
css.Blk 2	2.999	0.951	3.15	0.002
css.Blk 3	1.080	1.360	0.79	0.429
css.Blk 4	-0.084	0.708	-0.12	0.905
css.Blk 5	0.070	1.510	0.04	0.965
css.Blk 6	-0.580	1.270	-0.46	0.645
css.Blk 7	-0.065	0.501	-0.13	0.897
css.Blk 8	0.648	0.623	1.04	0.299
period.Blk 1	-0.117	0.153	-0.77	0.445
period.Blk 2	-0.902	0.161	-5.60	<.001
period.Blk 3	-0.364	0.152	-2.39	0.017
period.Blk 4	-0.089	0.154	-0.58	0.564
period.Blk 5	-0.067	0.153	-0.44	0.663
period.Blk 6	-0.207	0.152	-1.36	0.175
period.Blk 7	-0.151	0.152	-0.99	0.320
period.Blk 8	-0.213	0.156	-1.36	0.173

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	Fpr.
- Constant	-1	-884.01	884.01	31.93	<.001
+ Blk	8	1464.95	183.12	6.61	<.001
+ css.Blk	8	888.36	111.05	4.01	<.001
+ period.Blk	8	1188.04	148.50	5.36	<.001
Residual	496	13732.64	27.69		
Total	519	16389.99	31.58		

Data transformed to Square roots (+0.5)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	Fpr.
Regression	23	79.0	3.4347	5.71	<.001
Residual	496	298.4	0.6016		
Total	519	377.4	0.7271		

Percentage variance accounted for 17.3

Standard error of observations is estimated to be 0.776.

Estimates of parameters

Parameter	estimate	s.e.	t(496)	t pr.
Blk 1	1.332	0.297	4.48	<.001
Blk 2	1.702	0.421	4.05	<.001
Blk 3	1.496	0.434	3.44	<.001
Blk 4	1.206	0.357	3.38	<.001
Blk 5	1.005	0.448	2.25	0.025
Blk 6	1.951	0.446	4.37	<.001
Blk 7	1.265	0.302	4.19	<.001
Blk 8	1.345	0.357	3.77	<.001
css.Blk 1	-0.075	0.161	-0.46	0.643
css.Blk 2	0.948	0.338	2.80	0.005
css.Blk 3	0.572	0.447	1.28	0.202
css.Blk 4	-0.060	0.310	-0.19	0.846
css.Blk 5	0.107	0.470	0.23	0.821
css.Blk 6	-0.506	0.499	-1.01	0.311

css.Blk 7	-0.003	0.243	-0.01	0.989
css.Blk 8	0.357	0.262	1.36	0.174
period.Blk 1	-0.036	0.023	-1.62	0.107
period.Blk 2	-0.137	0.024	-5.75	<.001
period.Blk 3	-0.099	0.023	-4.40	<.001
period.Blk 4	-0.030	0.023	-1.34	0.181
period.Blk 5	-0.024	0.023	-1.05	0.294
period.Blk 6	-0.059	0.023	-2.66	0.008
period.Blk 7	-0.041	0.022	-1.81	0.070
period.Blk 8	-0.063	0.023	-2.73	0.007

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	Fpr.
- Constant	-1	-560.6177	560.6177	931.91	<.001
+ Blk	8	572.5163	71.5645	118.96	<.001
+ css.Blk	8	21.5542	2.6943	4.48	<.001
+ period.Blk	8	45.5456	5.6932	9.46	<.001
Residual	496	298.3839	0.6016		
Total	519	377.3823	0.7271		

Regression relationship between monitoring trap catches of male *S. singularis* and visual count of pod damage

Untransformed data

Regression analysis

Response variate: visual count of pod damage

Fitted terms: blocks, interaction between trap catches of *S. singularis* and block (css.blk) and interaction between period and block (period.blk).

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	Fpr.
Regression	23	2164.00	94.07	5.30	<.001
Residual	496	8802.00	17.75		
Total	519	10966.00	21.13		

Percentage variance accounted for 16.0

Standard error of observations is estimated to be 4.21.

Estimates of parameters

Parameter	estimate	s.e.	t(496)	t pr.
Blk 1	2.910	1.220	2.38	0.018
Blk 2	10.300	1.310	7.85	<.001
Blk 3	0.740	1.190	0.63	0.532
Blk 4	2.670	1.200	2.22	0.027
Blk 5	0.950	1.200	0.79	0.431
Blk 6	0.940	1.170	0.81	0.421
Blk 7	0.610	1.170	0.52	0.603
Blk 8	1.460	1.250	1.17	0.243
css.Blk 1	0.010	0.247	0.04	0.968
css.Blk 2	2.509	0.762	3.29	0.001
css.Blk 3	-0.070	1.090	-0.07	0.946
css.Blk 4	-0.127	0.567	-0.22	0.823
css.Blk 5	-0.160	1.210	-0.13	0.893
css.Blk 6	0.040	1.01	0.04	0.971
css.Blk 7	0.026	0.401	0.06	0.949

css.Blk 8	-0.062	0.499	-0.12	0.902
period.Blk 1	-0.196	0.122	-1.60	0.110
period.Blk 2	-0.863	0.129	-6.69	<.001
period.Blk 3	-0.025	0.122	-0.20	0.840
period.Blk 4	-0.171	0.123	-1.39	0.166
period.Blk 5	-0.063	0.123	-0.52	0.605
period.Blk 6	-0.066	0.122	-0.54	0.587
period.Blk 7	-0.037	0.122	-0.30	0.762
period.Blk 8	-0.107	0.125	-0.86	0.392

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	Fpr.
- Constant	-1	-575.40	575.40	32.42	<.001
+ Blk	8	1217.15	152.14	8.57	<.001
+ css.Blk	8	621.72	77.72	4.38	<.001
+ period.Blk	8	900.24	112.53	6.34	<.001
Residual	496	8801.88	17.75		
Total	519	10965.60	21.13		

Data transformed to Square roots (+0.5)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	Fpr.
Regression	23	73.5	3.1964	8.41	<.001
Residual	496	188.6	0.3803		
Total	519	262.1	0.5051		

Percentage variance accounted for 24.7

Standard error of observations is estimated to be 0.617.

Estimates of parameters

Parameter	estimate	s.e.	t(496)	t pr.
Blk 1	1.6330	0.2360	6.91	<.001
Blk 2	2.0930	0.3340	6.26	<.001
Blk 3	1.0270	0.3450	2.97	0.003
Blk 4	1.6530	0.2840	5.83	<.001
Blk 5	1.1280	0.3560	3.17	0.002
Blk 6	1.0700	0.3550	3.02	0.003
Blk 7	0.9400	0.2400	3.92	<.001
Blk 8	1.3370	0.2840	4.71	<.001
css.Blk 1	0.0500	0.1280	0.39	0.695
css.Blk 2	0.7750	0.2690	2.88	0.004
css.Blk 3	-0.0060	0.3560	-0.02	0.986
css.Blk 4	-0.0260	0.2470	-0.11	0.915
css.Blk 5	-0.0850	0.3740	-0.23	0.821
css.Blk 6	0.0603	0.3970	0.16	0.873
css.Blk 7	0.0450	0.1940	0.23	0.816
css.Blk 8	-0.0640	0.2090	-0.31	0.760
period.Blk 1	-0.0626	0.0179	-3.49	<.001
period.Blk 2	-0.1529	0.0190	-8.06	<.001
period.Blk 3	-0.0107	0.0179	-0.60	0.550
period.Blk 4	-0.0595	0.0180	-3.31	0.001
period.Blk 5	-0.0231	0.0179	-1.29	0.198
period.Blk 6	-0.0289	0.0179	-1.62	0.106
period.Blk 7	-0.0162	0.0178	-0.91	0.363
period.Blk 8	-0.0424	0.0185	-2.29	0.022

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
- Constant	-1	-544.8580	544.8580	1432.74	<.001
+ Blk	8	566.1234	70.7654	186.08	<.001
+ css.Blk	8	14.6833	1.8354	4.83	<.001
+ period.Blk	8	37.5695	4.6962	12.35	<.001
Residual	496	188.6238	0.3803		
Total	519	262.1420	0.5051		