Host plant finding by *Acraea acerata* Hew. (Lepidoptera: Nymphalidae), the sweet potato butterfly: Implications for pest management

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Abstract

Phytophagous insects such as Lepidopteran species utilise both olfactory and visual cues to locate their host-plants used as mating or oviposition sites, shelter or food. Larvae of *Acraea acerata* feed on sweet potato plant leaves causing more that 50 % loss of sweet potato tuber yield in some East African countries. Attempting to elaborate a management strategy to control *A. acerata* suitable to a tropical resource-poor farming system, it was essential to investigate how the butterfly finds its host-plants. The results of a wind tunnel bioassay using glass-screened, muslin-screened and non-screened sweet potato plants suggested that sweet potato plant volatiles play an important role in attracting *A. acerata* to its host-plant. This was supported by both the distance moved by female *A. acerata* towards muslin-screened plants (olfactory cues) and the percentage of butterflies which landed on the screen. Visual stimuli seemed to have a negative effect. The attractiveness of sweet potato plant volatiles to *A. acerata* was later confirmed by the use of volatiles collected by headspace entrainment from sweet potato plants.

The main components of sweet potato plant volatiles were tentatively identified by GC-MS (Gas Chromatography coupled with Mass Spectrometry) analysis and electrophysiological responses were recorded for some of them. Compared to ethylbenzene, 3-carene and (-) trans-caryophyllene, 3-hexen-1-ol,(Z), a general green leaf alcohol, elicited far more substantial EAG (electroantennogram) responses in *A. acerata*. This result suggested that *A. acerata* might well respond to a specific blend of volatiles made up of the different chemical components of sweet potato plant volatiles instead of one or two specific chemical components.

Considering the important role of sweet potato plant volatiles in attracting *A. acerata*, a number of plants reported to be repellent to herbivorous insects were mixed with sweet potato plants and screened for repelling/disorienting of female *A. acerata* in olfactometer and wind tunnel bioassays. Two plant mixtures with opposite effects on the response of *A. acerata* to their volatiles were identified: sweet potato + *Desmodium* plant volatiles were found to be more attractive to the butterfly than sweet potato plant volatiles alone, and sweet potato + onion plant volatiles which reduced considerably the attractiveness of sweet potato plant volatiles to *A. acerata*. As the trichomes of *Desmodium* plants were reported to trap insects, a 'push-pull' management strategy for *A. acerata* involving the two intercrops was suggested: the intercrop sweet potato + onion plants would 'push' away ovipositing *A. acerata* whereas the intercrop sweet potato + *Desmodium* plants would attract the butterflies which would be trapped by *Desmodium* trichomes. The results of a preliminary field experiment carried out in Uganda suggested that the intercrop sweet potato + onion plants had a negative effect on the number of egg batches laid by *A. acerata* on sweet potato plants. There is, therefore, a need for comprehensive field experimentation of the whole strategy to validate these laboratory and field experimental findings.

Dedication

To all the people who really care, the sustainers of our daily living.

Declaration

I hereby declare that this thesis is my own composition and all the work reported was carried out by myself except where clearly and specifically acknowledged.

Nicolas Hitimana

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Table of contents

List of Figures

List of Tables

List of Plates

List of Diagrams

Chapter 1

General introduction

Chapter 1 General introduction

1.1 Sweet potato production in Africa

The sweet potato (*Ipomoea batatas* L. (Lam.)) is a perennial creeping plant of the Convolvulaceae family but cultivated as an annual crop. Its origins are traced in north west of South America (Villareal, 1982, Thurston, 1984, Jansson and Raman, 1991) from where, towards the end of the fifteenth century, European explorers spread it throughout the world (Thurston, 1984). It was taken to Africa by European colonialists as early as the beginning of the 16th century and it reached China, the world's largest producer, at the end of the same century. There is however a question mark over how it reached Polynesia and New Zealand, where it was known before the first Europeans arrived in South America (Purseglove, 1968, Villareal, 1982).

Sweet potato is grown from 32° South to 40° North in the tropics and in warm temperate latitudes, on a variety of soils and in altitudes ranging from sea level to 3000 metres (Kay, 1973, Varma and Naskar, 1986, Woolfe, 1992). It is a hardy crop, well adapted to marginal lands but is more productive on sandy-loam soils with a pH of 5.6-6.6 (Kay, 1973). It is propagated predominantly from vine cuttings or from tuber sprouts, planted on ridges, mounds, furrows, raised and flat beds in monocropping as well as mixed cropping (Kay, 1973, Ndamage, 1987, Nayar and Rajendran, 1989, Qiwei, Rilian, Liyu, Pinlian, Changping, Yisi, and Peiliang, 1990, IITA, 1992, Kapinga, Ewell, Jeremiah and Kileo, 1995). In Africa, the second largest producer of sweet potato (Figure 1.1, Figure 1.2), farmers grow sweet potato mostly in mixed cropping systems (Kay, 1973, Ndamage, 1987, Nayar and Rajendran, 1989, Qiwei *et al.*, 1990, IITA, 1992, Kapinga *et al.*, 1995). Sweet potato, cultivated mainly for its tubers, is the eighth most important food crop in the world (after maize, wheat, rice, potato, cassava, soybeans and barley) and ranks fourth in roots and tuber production in Africa (after cassava, *Manihot esculentum* Crantz, yams, *Dioscorea* spp and Irish potato, *Solanum tuberosum* L. (FAO, 1999). World-wide, sweet potato tubers are used for direct human consumption, processing and animal feed (Woolfe, 1992) whereas in Africa, almost all sweet potato tuber production is

production is used for human consumption (Ewell, 1993). Young leaves and tips of sweet potato are used as a nutritious vegetable in some countries of Asia, and South America as well as Southern, Eastern and Western Africa (Woolfe, 1992, Kapinga *et al.*, 1995).

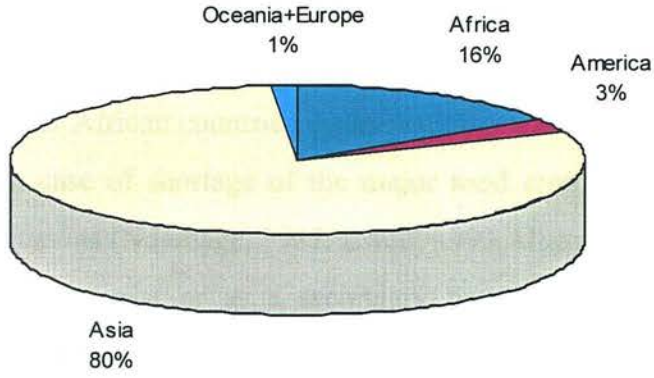


Figure 1.1 Partition of the sweet potato area (%) in the world by continent: average area harvested in 10 years (1989-1998): total = 9,117,000 hectares (ha). The figure shows 10 year average hectarage figures from FAO production yearbooks 1996-1998.

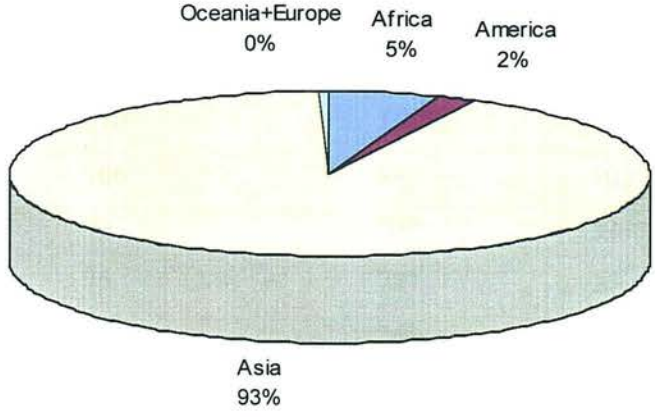


Figure 1.2 Partition of sweet potato production (%) in the world by continent: average production in 10 years (1989-1998): total = 1,388,683,000 tonnes (t). The

According to FAO production statistics (FAO, 1999), the top ten African countries producing sweet potato are: Uganda, Rwanda, Kenya, Burundi, Madagascar, Democratic Republic of Congo (DRC), Tanzania, Cameroon, Angola and Ethiopia (Table 1.1). Uganda, Tanzania and Rwanda are the major sweet potato producers in terms of acreage, with Rwanda and Burundi the most efficient per capita producers with 165 kg and 102 kg per head of population respectively. The per capita production of sweet potato in Africa reveals the two different statuses of the crop. It is high in Rwanda, Burundi and Uganda where sweet potato is a major staple food crop, whereas in other African countries sweet potato is a secondary food crop, used as food security in case of shortage of the major food crops or as a food reserve between growing seasons (Ndamage, 1987, Ewell, 1993, Hitimana, 1996). Both roles of sweet potato, as a major or as a secondary food crop, are vital in African agricultural farming systems where people rely on low input subsistence farming to feed large families.

Table 1.1 Major producers of sweet potato in Africa for the period 1996-1998

Country	Area harvested (1000 hectares)	Production (1000 tonnes)	Production per capita (kilograms)
Uganda	525	1,777	86
Tanzania	245	385	12
Rwanda	150	982	165
DRC	110	413	9
Burundi	106	647	102
Madagascar	90	507	33
Kenya	75	727	25
Cameroon	45	240	17
Angola	23	198	17
Ethiopia	20	159	3
All other countries	160	902	2
Africa	1,549	6,938	9

Source: FAO 1996-1998

The figures in Table 1.1 were calculated by averaging three years data on area harvested, production and population from FAO production yearbooks 1996, 1997, 1998 (Vol. 50, 51 and 52). As emphasised by Ewell (1993), FAO data are not accurate, but rather, they constitute the only source of sweet potato production data available for generalised comparisons between countries and continents. With 70,000 Kcal/ha/day of edible energy and 22 kg/ha/day of dry matter, sweet potato is one of the most productive of the major food crops (Horton, Prain and Gregory, 1989 quoted by Ewell, 1993). Its tubers contain appreciable quantities of vitamins A, B, and C and minerals and cultivars with dark yellow or orange flesh are rich in carotenoids, precursors of vitamin A. The top greens contain about 3 to 4% protein, a higher value than those of conventional temperate vegetables with the exception of spinach (Woolfe, 1992).

With its adaptability to different growing conditions, high dry matter content, high edible energy productivity and its high esteem in some rich countries (it was listed among eight crops to test in future American space missions and it is believed by the American Cancer Society to reduce the risk of cancer (Jones and Bouwkamp, 1992)), sweet potato could potentially lead an agricultural revolution if there were advances in post-harvest technologies and utilisation, two major constraints. Other constraints on the production and the yield of sweet potato, especially in developing countries are: non application of good agricultural techniques, lack of good quality planting material, and pests and diseases (Tardif-Douglin and Rwalinda, 1993, Ewell, 1993, Hitimana, 1996).

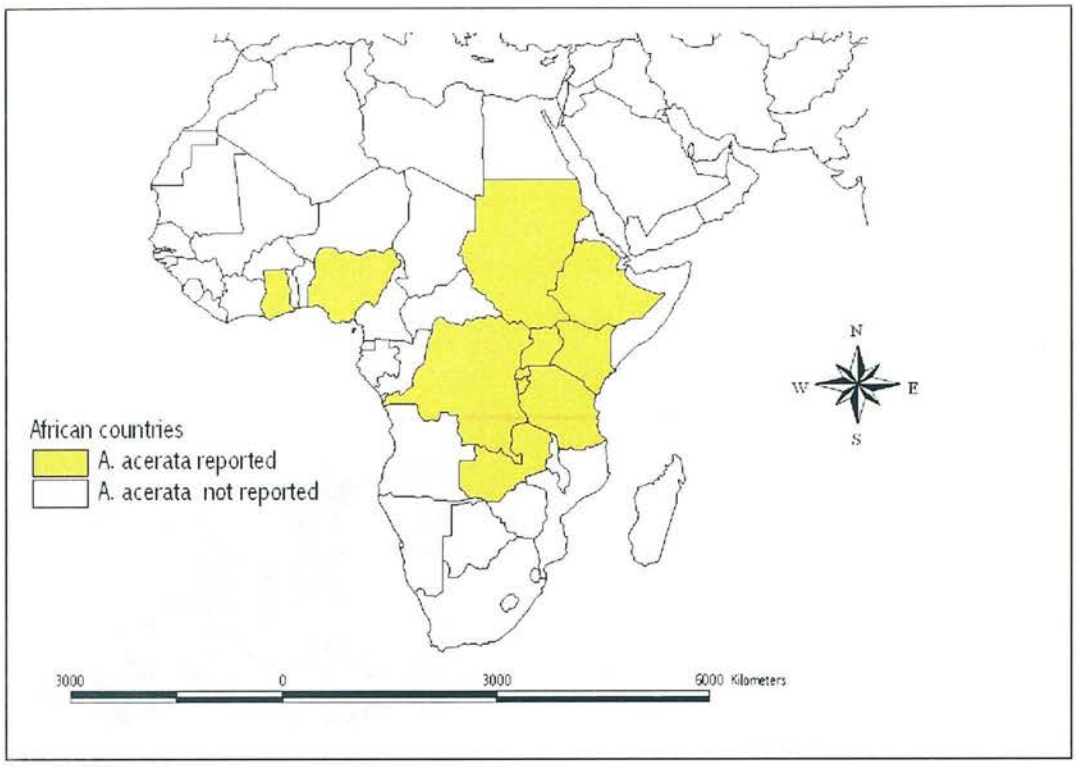
Pests and diseases constrain production in all sweet potato growing countries. Virus and fungal diseases are very common and cause significant damage to the sweet potato crop (Terry, 1982, Moyer, 1982, Ndamage, 1987, Skoglund and Smit, 1994). Jansson and Raman (1991) reported that a list of 280 insect species and 18 mite species which attack sweet potato world-wide has been established. The sweet potato weevils (*Cylas formicarius* complex.: Coleoptera; Curculionidae) have a world-wide distribution and are known to be the most damaging pests of sweet potato (Talekar,

1982, Hill, 1983, Chalfant, Jansson, Seal and Schalk, 1990). Eggs are laid in sweet potato stems and roots where larvae feed and develop with adults feeding on leaves, stems and roots causing significant losses (Starr, Severson and Kays, 1991). Other sweet potato pests of regional importance have also been described (Franssen, 1986, Chalfant *et al.*, 1990, Skoglund and Smit, 1994). They include: sweet potato vine borer (*Megastes grandalis*), white fringed beetles (*Graphognathus* spp) and cucumber beetles (*Diabrotica* spp) in South America and USA, the sweet potato stem borer, *Ophisa anastonosalis* Guen (Lepidoptera: Pyralidae), and the green tortoise beetle, *Metriona circumdata* (Herbst.) (Chrysomelidae) in Asia; and *C. puncticollis*, *C. brunneus* and *Acraea acerata*, the sweet potato butterfly in Africa (Schmutterer, 1969, Hill, 1983, Chalfant *et al.*, 1990, Skoglund and Smit, 1994).

1.2 The sweet potato butterfly (SPB)

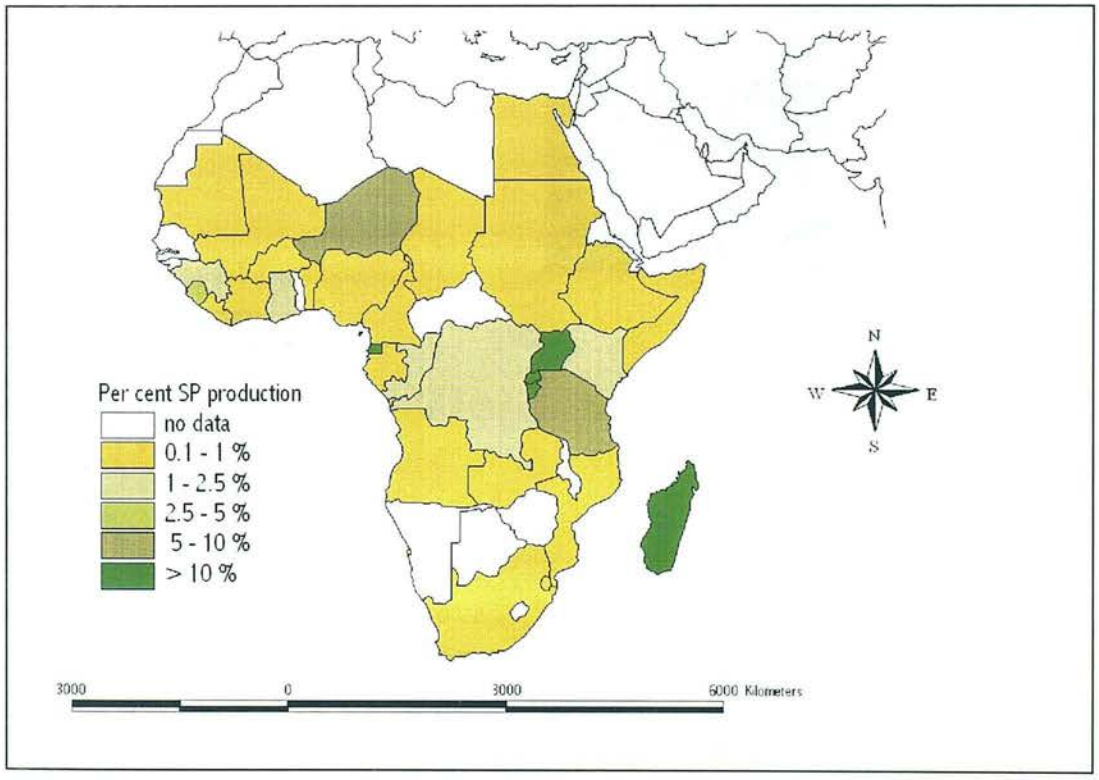
Acraea acerata (Lepidoptera: Nymphalidae), the SPB is an aposematic insect, coloured in orange and black patterns (Plate 1.3), reported only in Africa. It is widespread in East Africa (Uganda, Tanzania, Kenya, Rwanda, Burundi), East of the Democratic Republic of Congo (Kivu and Ituri regions) (Lefèvre, 1948; Hill, 1983, Subukino, 1987) and Ethiopia (Azerefegne, 1999). It has been reported in Nigeria and described as a serious pest of sweet potato in southern Sudan (Schmutterer, 1969, Matanmi and Hassan, 1987). Hewiston, the first person to describe the species in 1874, used specimens from Kumasi in Ghana (Matanmi and Hassan, 1987 citing Van Son (1963)). In their reviews on SPB, Leblanc (1993) and Smit, Luggoja and Ogenga-Latigo (1997) reported its records as a pest of sweet potato in southern Ethiopia and northern Zambia. Larsen (1991) affirmed that the SPB was found throughout the wetter parts of the Afrotropical region. A comparison of the maps showing countries where the SPB has been reported (Plate 1.1), and the sweet potato production area as per cent of the arable land (Plate 1.2), lends supports to Larsen's affirmation but more field reports are needed to substantiate it.

The life cycle of the SPB is in four stages: egg, larva (with five instars), pupa and adult (Plate1.3). These stages vary in length from 27 to 52 days under laboratory conditions depending on temperature and relative humidity (Lefèvre, 1948, Subukino, 1987, Smit *et al.*, 1997). This life cycle variability suggests that it may be theoretically possible for the butterfly to produce between 7 to 13 generations per year. The larval stage (it is only during this stage that the SPB feeds on the sweet potato) is the longest growth stage, representing on average more that 50 % of the life cycle of the butterfly. According to data presented by Lefèvre (1948), Subukino (1987) and Smit *et al.*(1997) when the life cycle of the SPB (from one generation to another: egg to egg) becomes shorter, the larval stage becomes relatively longer, and vice-versa. As eggs are laid in clusters of some hundreds, with such a relatively long larval stage, one may expect a very high level of damage to be sustained by host plants.



Source: FAO, 2000

Plate 1.1 Distribution of sweet potato butterfly (*Acraea acerata*) in Africa



Source: FAO, 2000

Plate 1.2 Sweet potato production area as per cent of the arable land in Africa

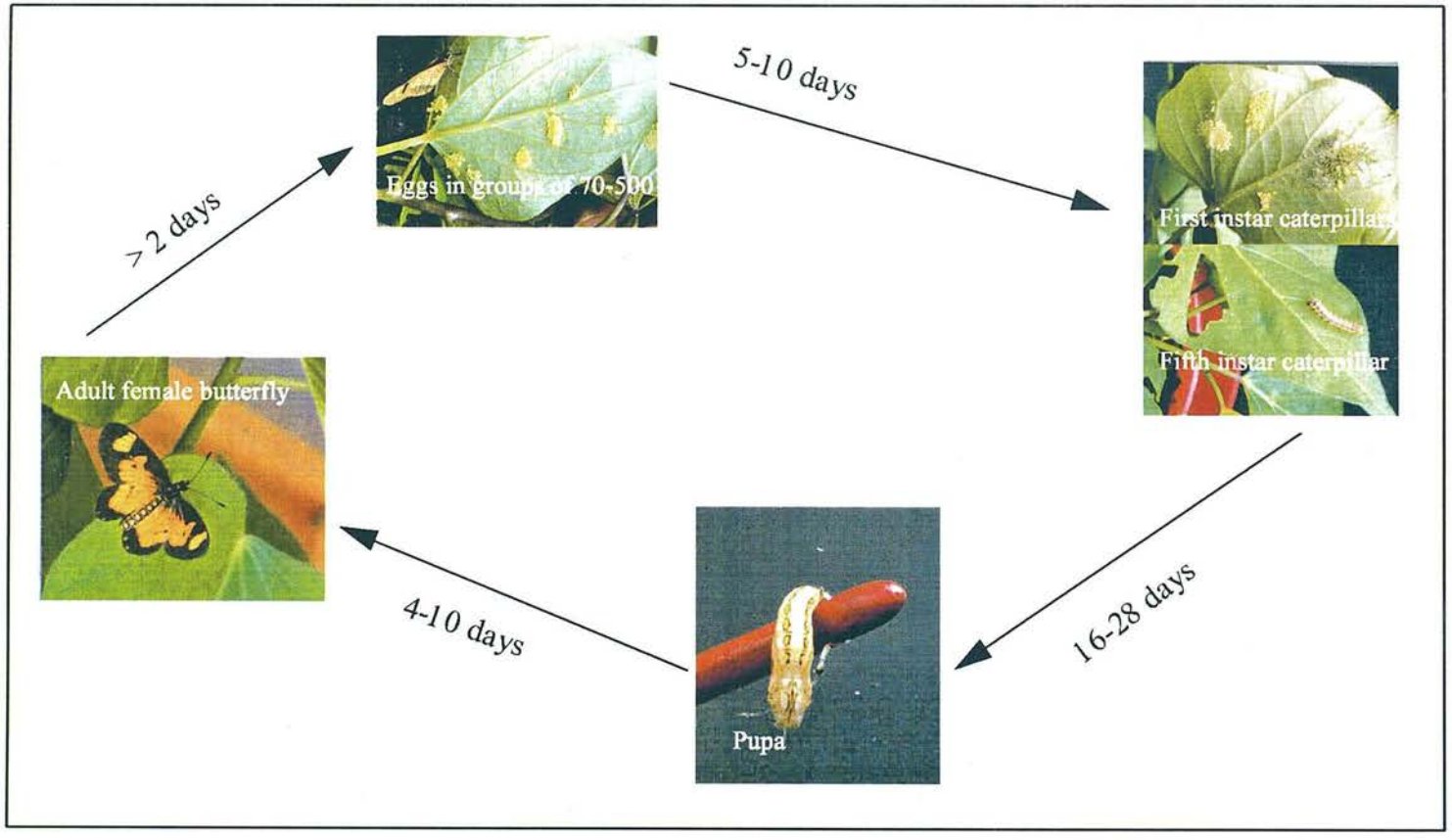


Plate 1.3 Life cycle of *Acraea acerata*

The SPB is a specialist feeder on a few plant species of the Convolvulaceae family, of which the sweet potato appears to be the most preferred food plant (Lefèvre, 1948, Subukino, 1987, Azerefegne, 1999). This prompted some researchers to look for African indigenous host plants of the SPB. In DRC, Lefèvre (1948) tested the acceptability of indigenous Convolvulaceae and some weed plants of Gramineae, Cyperaceae, Commelinaceae, Amaranthaceae, Capparidaceae, Malvaceae and Compositae families for the SPB caterpillars. Only *Ipomoea kentrocarpa*, *I. tenuirostris* and *I. lilacina* were found to be host plants whereas *I. cairica* had a low toxicity to the SPB larvae. In Uganda, another indigenous Convolvulaceae, *Lepistemon africanum* has been reported by Hargreaves (quoted by Lefèvre (1948)) as a host plant of the butterfly. In Nigeria, Matanmi and Hassan (1987) found the SPB larvae feeding on another species of the same genus, *L. ovariense* in a region which does not produce sweet potato. In Rwanda, Subukino (1987) tested the acceptability of indigenous Convolvulaceae to the SPB. Only *I. wightii* was found to be a host plant. In Ethiopia, Azerefegne (1999) reported that the SPB larvae were observed feeding on two indigeneous species, *I. obscura* (in fields) and *I. cairica* (in laboratory). The caterpillars of the SPB would starve to death instead of feeding on *Corchorus olitorius*, a Leguminosae host plant of *A. eponina* (Matanmi and Hassan, 1987) which had been mis-identified as the SPB in Burundi while feeding on another Leguminosae, *Smithia bequaertii* (Lefèvre, 1948).

Available information suggests that the SPB can feed on species of only two genera of the Convolvulaceae family: *Ipomoea* and *Lepistemon* (Table 1.2). As stated by Jaenike (1990), the colonisation of an introduced plant by an insect may be explained not only by the known hosts belonging to the same taxonomical family but also by plant chemistry similarities existing between the indigenous host plants and the introduced plant. Host plant chemistry is even more critical for the SPB, an unpalatable species (for avian predators) which might use toxic sequestered host plant compounds for defence (Raubenheimer, 1989, Azerefegne, 1999). Furthermore, it is normal for herbivorous insects to appear to prefer the more nutritious

domesticated crop plants with their luxuriant foliage covering a large area (large quantity of food supply) than their related wild species (Hill, 1983).

Table 1.2 Indigenous host plant species of *A. acerata*

Plant species	First reported	References
Ipomoea kentrocarpa	DRC	Lefèvre,1948
I. tenuirostris, I. lilacina	DRC	Lefèvre,1948
I. cairica	DRC	Lefèvre,1948
Lepistemon africanum	Uganda	Lefèvre,1948
L. ovariense	Nigeria	Matanmi *et al.*,1987
I. wightii	Rwanda	Subukino,1987
I. obscura,	Ethiopia	Azerefegne, 1999

1.3 Damage of *Acraea acerata* and economic importance

The caterpillars of the SPB feed on sweet potato leaves eating the whole leaf lamina except the primary midribs (Lefèvre, 1948, Skoglund and Smit, 1994, Smit *et al.*, 1997). Early instars are gregarious whereas late instars are solitary. The SPB has been reported to have outbreaks which cause very important yield losses or even complete crop losses associated sometimes with serious food shortages (Lefèvre, 1948, Ndamage, 1987, Azefegene, 1999, Odongo, unpublished report). Such crop losses normally extend to the following growing seasons as sweet potato planting material becomes very scarce.

Reports on sweet potato crop losses due to the SPB have been mostly qualitative with limited quantitative data. In Rwanda, in a nationwide survey on yield losses due to pests and diseases of sweet potato, farmers estimated that the SPB was the most damaging pest being responsible for a diminution of the yield of sweet potato by 70% (Table 1.3) (Tardif-Douglin and Rwalinda, 1993). In other African countries, artificial defoliation has been used to assess the impact of defoliation on the sweet potato. In defoliation experiments in Uganda, only repeated defoliation had a

significant effect on sweet potato tuber yield (Lugojja, 1996) whereas in Ethiopia and Nigeria, a single complete defoliation of sweet potato at eight weeks after planting caused 36 and 50% reduction in tuber yield respectively (Anioke, Echendu, Emehute, and Agbo, 1995, Azerefegne, 1999). The differences of the effect of sweet potato defoliation between these three sites were most likely due to soil, eco-climate and variety differences.

In a greenhouse study of the effect of the SPB on sweet potato, an initial load of 20 second instar larvae per plant, resulted in a foliage yield reduction of between 20-37%, although without significant tuber yield loss (Anioke *et al.*, 1995). However, in a four year field experiment (three consecutive cropping seasons) in Ethiopia, where two successive generations of the SPB were observed, Azerefegne (1999) reported that in addition to sweet potato above ground biomass losses of 32-47%, tuber yield losses varied between 31-53% for sweet potato harvested five months after planting. It is very important to underline that sweet potato biomass losses affect not only tuber yield through a reduction of plant photosynthetic activity, but also result in reduction of ground cover, an important means of soil protection against rainfall erosion. In the overpopulated regions of Eastern DRC, Burundi, Rwanda and Uganda (the leading producers of sweet potato in Africa) where severe outbreaks of the SPB are frequently reported, rainfall erosion, as in many other parts of Africa, is a major problem (Yates and Kiss, 1992). Most of the estimated 10,000 t/ha/year of soil erosion losses are due to rainfall erosion (Beets, 1990) and factors that expose the soil to direct rainfall impact by removing plant cover accelerates soil degradation by water erosion.

Taking into account the qualitative data on sweet potato losses due to the SPB, the effect of artificial defoliation on sweet potato tuber production and the yield losses recorded in farmers' fields in Ethiopia reported above, one may suggest that the level of sweet potato yield losses in countries where the butterfly has been reported (Plate 1.1) is likely to average 15-20% of total African production, equivalent to between 900, 0000 tonnes to 1,200,000 tonnes/year. On average, this is more than the annual

production of Rwanda, the second largest producer of sweet potato in Africa (Table 1.1).

Table 1.3 Effects of severe attacks of pests and diseases on the yield of sweet potato (farmers' views) in Rwanda.

Pests/diseases	Percentage of yield losses	Number of families who responded
Most damaging pests		
Sweet potato butterfly	70	532
Sweet potato weevils	50	269
Others	50	173
All pests together	60	974
Most severe diseases		
Viral diseases	50	105
Alternaria spp	50	652
Aceria spp (Erinose)	40	140
Fungi	50	45
All diseases together	50	963

Adapted from:Tardif-Douglin and Rwalinda, 1993

1.4 Control methods of the sweet potato butterfly

Today, the control methods of the SPB consist of hand-picking and destroying webs of larvae when they are still gregarious, cultural control and use of insecticides. Table 1.4 presents a comparison of the available pest control methods of the SPB by cost, feasibility, level and limitations of application.

Legislative control methods are put into place by governments and are enforced by laws. Government officers scrutinise import/export of plants and/or seeds to ensure that they are pest - and disease - free. However, cross-borders exchanges of infested planting material from one country to another outside the official import/export channels are possible. For instance, the cassava mealybug (*Phenacoccus manihoti*:

Pseudococcidae), a pest of cassava reported for the first time in Africa in 1973, in the DRC, was reported 10 years later in the neighbouring country of Rwanda. This pest might have been brought in by the cassava planting material exchanged between farmers near the borders (Ndamage, Ntawuruhunga and Mulindangabo, 1992). Furthermore when countries or regional entities are not separated by large non vegetated areas such as seas and/or oceans, it is very easy for insects especially flying insects like the SPB to cross borders. The presence of the Indian Ocean between Madagascar, one of the major producers of sweet potato in Africa, and continental Africa may explain why the SPB has not yet been reported in Madagascar (Plate 1.1).

Physical control methods are mostly mechanical -like hand-picking and destruction of webs of larvae and infested plant material- and are applied to small areas at early stages of the pest attacks whereas when large areas are attacked and/or at late larval growth stages, hand-picking becomes practically impossible.

Table 1.4 Comparison of sweet potato butterfly control methods

Control methods	Cost to farmer	Feasibility	Level of application	Limitations
Legislative	Low	Easy	Government	Limited to official imports
Physical	Low	Easy	Farmer	Timing of intervention Small scale
Cultural	Low	Easy	Farmer	Only preventive
Biological	High	± complex	Regional	Delicate balance
Chemical	High	± complex	Farmer	Effects on environment Commercial farming only
IPM	Moderate	± complex	Farmer	Holistic approach which needs interdisciplinary inputs

Cultural control methods include achieving optimal growing conditions, timing of planting and harvesting to avoid high populations of insects, crop rotation, fallowing, deep ploughing and sowing, weeding, crop sanitation, trap cropping, destruction of alternate hosts, intercropping and use of resistant varieties. These cultural control methods are very easy and cheap, apart from breeding programmes for resistant varieties which are normally mid- or long term research programmes and therefore costly. Furthermore the probability of finding a resistant variety to the SPB in a classic breeding programme seems remote as one outbreak of the pest at a research station in Rwanda attacked without discrimination a germplasm accession of more than 24000 different genotypes of sweet potato (Ndamage, 1987). Nevertheless, there is still a possibility of finding resistant varieties with the advances of genetic engineering, provided that research funding is available.

Biological control methods require well funded, well staffed mid- to long term research programmes and their application requires a minimum understanding of the interactions between host plant-insect pests and beneficial organisms (predators, parasitoids and pathogens). They are mostly government funded. *Glyptapanteles acraea* (Wilkinson) (Hymenoptera: Braconidae), *Zenillia* vara Curran, *Charops* spp. (Hymenoptera: Ichneumonidae), *Mesochorus* spp, *Caricelia normula* (Diptera: Tachinidae), *Brachymeria albicrus* (Klug) (Hymenoptera: Chalcidoidae) and the fungi *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) and *Isaria* spp are some of the parasitoids and pathogenic fungi of the SPB which might be involved in biological control or as part of an integrated pest management (IPM) programme, but thus far their effects on all developmental stages of the SPB seem limited (Lefèvre, 1948, Subukino, 1987, Smit *et al.*, 1997, Azerefergne, 1999). There is therefore a need for an extensive, in depth study on biological control of the SPB using known predators/parasitoids/pathogens and/or searching for new ones.

Chemical control methods, although very efficient in controlling the SPB, are not, simply, fitting in a low-input subsistence farming and with the negative impact of

some insecticides on the environment, their use is now being questioned and better alternatives are being sought.

IPM seeks to combine the use of the different control methods to keep a given pest under the economic threshold and/or acceptable level of losses. In the case of the SPB, preventive methods (cultural control methods) can be combined with affordable curative methods like hand-picking and destroying webs of larvae. All the reports on sweet potato production in Africa state that sweet potato growing conditions are far from the optimum. Marginal lands, no fertilisation, varying planting times, no or very infrequent weeding, piecemeal harvesting and complex mixed cropping are some of the key reasons why sweet potato production systems in Africa are low yielding (Ndamage, 1987, IITA, 1992, Ewell, 1993, Kapinga *et al.*, 1995, Tardif-Douglin and Rwalinda, 1993, Smit and Matengo, 1995). Comparing the available pest control methods for the SPB (Table 1.4), it appears that one of the best ways of reducing sweet potato damage consists of applying cultural control methods. They are simple, preventive cultural techniques, affordable by all sweet potato growers and they fit very well within their production systems. Of particular interest is intercropping which could be specifically designed and implemented to control the butterfly. Stating the various drawbacks for large-scale agriculture, Hill (1983) said that 'intercropping can certainly reduce a pest population on a crop, and without doubt reduces the visual and olfactory stimuli that attract insects to a particular crop'. However, not all types of intercropping will reduce a pest population on a crop. All reviews on intercropping agree that some types of intercroping can reduce one pest and not another (Altieri, 1994). It is therefore crucial to understand how the insect pest uses its host plant visual and olfactory stimuli to locate its host plants and thereafter seek ways of interfering with these stimuli in order to disrupt the behaviour of the host-seeking insect. It is only at this stage that an appropriate intercrop can reduce a pest population on a crop.

A review of studies on the SPB reveals that it has not received enough attention from researchers. Only two major studies have been carried out. The pioneering study by

Lèfevre (1948) was on its biology, its parasitoids and its control methods. The second major study about five decades later, in addition to the aspects investigated by Lèfevre, looked at the population dynamics of the SPB and the yield loss due to the insect in Ethiopia (Azerefegne, 1999). Meanwhile, Subukino (1987) carried out a study similar to Lèfevre's in Rwanda. Anioke and co-workers (1995) were the first to try to quantify sweet potato yield loss due to the SPB in Nigeria, and Lugojji (1996) did a similar study in Uganda but relying on artificial defoliation. Studies on interactions between the SPB and its host plants have never been carried out leaving researchers without key information which could be used to transform some of the sweet potato agricultural practices into important components of a strategy to manage the SPB.

1.5 Aims of the research project

The aims of this project are to understand how the SPB finds its host plants and to use that knowledge to elaborate and test a SPB management strategy which could be adopted by subsistence farmers. This will involve the study of visual and olfactory cues involved in host plant finding by the SPB and seeking ways of interfering with these cues to protect sweet potato from the insect attacks.

Chapter 2

Review on host plant-Lepidoptera interactions

Chapter 2 Review on host plant-Lepidoptera interactions

2.1 Introduction

In phytophagous insects, provisions for food, mating and oviposition sites, and shelter are generally met by plants. Moreover some plant species need insects as pollen transporters. Therefore, the interactions between phytophagous insects and host plants are vital not only for insects but also for some plants. Being normally mobile, insects have developed relatively efficient ways of searching for and finding their host plants. In particular, Lepidoptera species do walk and/or fly to locate their host plants. The latter do not passively let themselves be fed on by herbivores; on the contrary, they have developed mechanisms of auto-defence and do 'call for help' when they are attacked. In this chapter, a review of how phytophagous insects in general and Lepidoptera in particular use plant olfactory and visual cues to find their host plants is presented. The reaction of host plants to lepidopteran attacks (host plant defence mechanisms) and the subsequent interactions (lepidopteran counterdefence mechanisms) are also explored and, finally, the evolutionary ecological implications of the ensuing seemingly conflicting relationships are discussed.

2.2 General considerations on host plant finding by phytophagous insects

Visser (1988) distinguished between host plant finding and host plant recognition by phytophagous insects. He related host plant finding by insects to plant characteristics such as spatial distribution. Host plant recognition, on the other hand, he defined as the insect's final decision to feed and/or to oviposit on host plants and to leave non host plants. Host-finding therefore, like host-recognition, involves plant stimuli, insect sensory systems and a functional mechanism to link them, the major difference between host plant finding and host plant recognition being the kind of sensory modalities involved and the distance separating insects from their host plants.

Depending on the breadth of their diet, phytophagous insects use more or less specific cues to find and select their host plants. Kogan's models for insect host-

finding quoted by Ramaswamy (1988) and adapted to moths by the same author, classify insects into four categories with two extremes: the highly polyphagous species and the highly specialised monophagous species. The former are more likely to be non-selective, almost all plants being potential hosts, whereas the latter are more likely to be very selective and specialists. The two other categories are oligophagous species which are directional and polyphagous/oligophagous species which are selective and non-directional in their host-location. The central nervous systems of all these categories of insects integrate, as underlined by Dethier (1982) and Miller and Strickler (1984), not only varying levels of inputs from insect sensory systems responding to external stimuli but also inputs from within the insect due most notably to its physiological state. The closer an insect is to its host, the more sensory modalities are likely to be involved in host plant recognition.

Olfaction and vision are mostly used by mono- and/or oligophagous insects in directional search to locate host habitats and patches whereas mechanoreception and gustation (chemoreception) are almost solely involved in assessing host suitability of plants once physical contact has already been made between an insect and its potential host (Miller and Strickler, 1984, Ramaswamy, 1988, Renwick and Radke, 1988, Bell, 1990, van Loon, 1996). Concluding their study on visual cues used by *Lygus lineolaris*, a polyphagous plant bug, *Rhagoletis pomonella*, an oligophagous fly and *Hoplocampa testudinea*, an apparently monophagous sawfly, to locate their common host plant, the apple, Prokopy and Owens (1978) suggested that monophagous and/or oligophagous insects are likely to be more visual specialists than polyphagous insects (visual generalists). In random search, mostly used by highly polyphagous insects, tactile cues are used to differentiate between host plants and non-host plants and chemoreception (gustatory) cues play a preponderant role in determining their suitability. Therefore, unlike specialist insects, they do not generally need 'accurate, specific, long-distance orientation mechanisms' (Lance, 1983).

Long distance orientation towards host plants is essentially elicited by specific host plant volatiles mostly for specialist monophagous insects which will take off (if they were resting) and move or continue their movement (if they were already moving) in the direction of the odour source (Kennedy, 1977, Visser, 1986, Renwick, 1989, Bernays and Chapman, 1994). This mechanism has been termed odour-induced positive (upwind) anemotaxis (Kennedy, 1977, Visser, 1986, Bernays and Chapman, 1994). Visual cues may also stimulate insects' movement towards host plants (Miller and Strickler, 1984). Long distance visual attraction of phytophagous insects is assumed to be very broad (e.g: attraction to areas covered by plants instead of water) with increasing perception of details as distance decreases (Prokopy and Owens, 1983, Calvert and Hanson, 1983).

The assessment of host plant acceptability by phytophagous insects might imply an involvement by all sensory systems (but not all insects need all sensory systems to select their hosts). For some insects visual and chemosensory systems (cues) may be predominant, for others olfactory and chemosensory and/or tactile systems (cues) may be mostly involved. Comparing these senses individually however, most information about the form of the plant is given by vision with gustation collecting most information about plant composition (Miller and Strickler, 1984). Nevertheless, as gustation implies contact with the host plant, it incurs more cost to the insect including energy for locomotion, time and increasing risks of predation (Bell, 1990). These costs should be relatively less important for specialist monophagous and oligophagous insects which may refer first to distant cues (olfaction and vision) to narrow their search and then decide whether or not it is worthwhile to invest contact sensory systems.

2.3 Host plant finding by Lepidoptera

2.3.1 Olfactory cues

All examined insects that feed or lay eggs on leaves such as adult Lepidoptera are able to smell, using olfactory receptors generally found on the antennae, and to respond to common green leaf volatile components like hexanol and hexanal

(Bernays and Chapman, 1994). Common green leaf volatiles may be used by Lepidoptera to orient towards plant habitats which may or may not contain resource items. Some insects respond to specific host plant volatiles but others do not. Bernays and Chapman (1994) give some examples of insects that fly, walk or crawl towards the sources of host plant odours or host related chemicals. Among Lepidoptera, some species like the leek moth *Acrolepiopsis assectella* (Yponomeutidae), a monophagous insect and the cabbage semilooper *Trichoplusia ni* (Noctuidae) a polyphagous moth, fly towards the host specific compound or host plants while others like the larva of the citrus butterfly *Papilio demoleus* (Papilionidae) an oligophagous insect, walk towards compounds produced by host plants.

In his review on sensory modalities involved in host-finding by moths, Ramaswamy (1988) quotes eighty-five references indicating sensory modalities involved in host plant finding by moths. Only thirty-eight studies tested responses to olfactory cues and all of them reported positive responses to these cues. These results are however to be taken with caution as more than 1/4 of these studies were based on electroantennogram (EAG) responses which do not necessarily denote a specific host plant odour attraction. Such attraction has been observed in some species such as *Cidalia albulata* (Geometridae), *Antheraea pernyi* (Saturniidae), the cotton leafworm *Spodoptera littoralis* (Noctuidae), *Hypsipyla grandella* (Pyralidae) and the potato tuber moth *Phthorimaea operculella* (Gelechiidae) (Ramaswamy, 1988, Lecomte and Thibout, 1981).

In butterflies, the role of host plant volatiles in host plant finding, unlike the case of moths, has been underestimated in favour of the rather more obvious involvement of visual and gustatory cues (Feeny, Städler, Ahman and Carter, 1989). Nevertheless, some interesting experiments revealed that olfactory cues play an important role in host-finding by butterflies. The level of response of *Papilio demoleus* to host plant attractants was increased by smelling the host plant odour in addition to seeing its leaves (Saxena and Goyal, 1978). Feeny *et al.* (1989) found that the black

swallowtail butterfly *Papilio polyxenes* (Papilionidae) laid more eggs on model plants when the host plant volatiles were present. Hern, Edwards-Jones and McKinlay (1996) summarised evidence that host plant volatiles were involved in host plant finding by some species of Pieridae. In wind tunnel experiments, Hern (1997) found that the small white butterfly *Pieris rapae* (Pieridae) was responding to host plant volatiles.

In Nymphalidae, Mackay (1985) found that the searching behaviour of *Euphydryas editha* butterflies differed when the butterflies were searching in an area dominated by either one host plant, *Collias torreyi* or by another host plant, *Pedicularis semibarbata*. Though being genera of the same plant family of Scrophulariacae, *C. torreyi* is annual and *P. semibarbata* is perennial. In the *Collias* sp. area, although being more suitable for larval survival, the search mode was random except for bare ground which was avoided. Conversely, a nonrandom searching mode was exhibited by *Euphydryas* sp. butterflies in *Pedicularis* sp. area, strongly suggesting the involvement of olfactory and/or visual cues. Furthermore, the few pieces of available evidence tend to suggest that host plant finding in butterflies may involve both olfactory and visual cues acting synergistically.

2.3.2 Visual cues

In general, insects perceive visual cues mostly through their compound eyes. Plant characteristics used by phytophagous insects as visual cues to locate their host plants are particularly the size, the shape and the colour of plant structures (Prokopy and Owen, 1983, Bernay and Chapman, 1994). Plant structures such as leaves and fruits, which are principally used by some insects to discriminate their hosts, present a huge variety of sizes and shapes sometimes even within the same plant species. On the other hand, the colour of plants does not seem to vary much and the insect's compound eyes contain three visual pigments with maximum spectral sensitivity in ultraviolet (350 nm), blue (450 nm) and green (560 nm). In addition to this, many species of Lepidoptera have been found to have a fourth visual pigment with a maximum spectral sensitivity in red (600 nm) (Bernays and Chapman, 1994). One

may think that insects do not have too much choice in plant colours as most of them are green but there are three parameters that define a colour as given by Hern *et al.* (1996): 'the hue (wavelength in the spectrum); the tint (the amount of white added to the hue) and intensity (% reflectivity of the peak of the curve as compared to the white standard, or more exactly, the total area under the reflective curve)'. These parameters are of course a function of the source and intensity of the light, the nature and dimension of the viewed object, the optical properties of the milieu crossed by the light, and the background composition in addition to the organ of vision and its relative position (Prokopy and Owen, 1983). This means that there are many combinations of these parameters producing many different shades within different colours distinguishable by phytophagous insects.

Bernays and Chapman (1994) affirmed that all Lepidoptera are able to discriminate colours and all studied ovipositing butterflies seem to use visual cues to locate their host plants. Interestingly, Kinoshita, Shimada and Arikawa (1999) demonstrated, for the first time, in experiments using a coloured disk associated with the food of *Papilio xuthus* (Lepidoptera: Papilionidae), that butterflies have a true colour vision i.e 'the ability to discriminate visual stimuli solely on the basis of their chromatic content irrespective of their brightness'(Goldsmith, 1990). The importance of prior experience in discriminating visual cues has also been underlined (Prokopy and Owen, 1983) and some examples of butterflies which use the learned host plant specific cues such as leaf size and shape are given in the section about learning (section 2.3.4). In moths, of the seventeen studies which investigated the involvement of visual cues, only four did not reveal any use of visual cues (Ramaswamy, 1988). In fact, Ramaswamy's review on moth host plant finding reveals a general imbalance of studies on olfactory and visual cues in Lepidoptera: he found thirty eight studies which tested olfactory cues against seventeen which tested the involvement of visual cues and only seven which tested both cues. Nevertheless, as most of the moths are nocturnal, one may then wonder if it is not rather the problem of how to test with reliability moths' visual sensory involvement in host-finding which unbalances the evidence of visual cues involvement in favour of

olfactory ones. There may be a similar but opposite pattern in butterflies where overwhelming evidence of visual cues involvement in host-finding generally overshadows olfactory sensory involvement. However, case studies where both cues have been investigated suggest that in most of the Lepidoptera, both visual and olfactory cues are involved in host plant finding (Saxena and Goyal, 1978, Ramaswamy, 1988, Hern *et al.*, 1996).

Examples of Lepidoptera that use visual cues to locate their host plants include the cases of *Papilio demoleus*, *Pieris brassicae* (Pieridae) and *P. rapae* which discriminate shades of host plant colour (Saxena and Goyal, 1978, Chew and Robbins, 1984, Bernays, 1995, Hern, 1997) and *Heliconius* sp. and *Battus* sp. butterflies which discriminate their host plants by leaf shape (Chew and Robbins, 1984, Papaj, 1986). The importance of background composition in contrasting the colour of the plant structures used for visual detection is illustrated in *B. philenor* and *P. rapae* which discriminate more easily host plants with a bare soil background or very little vegetation than host plants with a background covered with vegetation (Rausher, 1981, Prokopy and Owen, 1983).

2.3.3 Interaction between olfactory and visual cues

Interactions between olfactory and visual cues in phytophagous insects can occur simultaneously or consecutively and at different stages in the behavioural repertoire involved in host plant finding. Saxena and Goyal (1978) found that *P. demoleus* was equally attracted by the green or yellowish-green colour of the glass-screened leaves of the host and non-host plants and the attraction became greater when the specific odour of the ether-soluble constituents of the citrus (host) leaves was combined with the visual stimuli. *P. rapae* which was believed to use mostly visual cues to locate its hosts, has recently been found to be responding to host plant derived volatiles by modifying its flight frequency, duration of flying time, motion orientation and frequency of alighting (Hern, 1997).

Stanton (1982) studied host plant selection of *Colias p. eriphyle* butterflies in the field. She observed egg-laying female *C. p. eriphyle* constantly flying upwind in search of oviposition sites and suggested that they may use host plant volatiles to orient to their hosts from a distance. She also noticed that *Colias* butterflies landed more on *Lathyrus leucanthus* and *Astragalus decumbes*, two legumes which have the same leaf shape as their apparently most preferred host plant, *Vicia americana*. However, the butterflies rarely accepted them for oviposition. Stanton suggested that *C. p. eriphyle* might be using leaf shape as a specific visual cue to identify *V. americana* but was misled by the two other legumes with the same leaf shape as *V. americana*. If this is confirmed by further studies, it would be a field illustration of interaction of both olfactory and visual cues in locating host plants.

Compared to vision some authors think that olfactory cues yield more specific information which can be used for preliminary decisions of landing or not landing on a plant (Calvert and Hanson, 1983) but other authors think that vision is crucial for landing insects (Bernays and Chapman, 1994). Nevertheless, whatever weight is put on olfactory cues or visual cues in host plant finding by phytophagous insects, it is now more widely accepted that both are involved to varying degrees. Some herbivorous insects may use them simply to avoid non host plants. This section is concluded with a quote from Prokopy and Owen (1983) who said that 'it is likely that many, if not most, herbivorous insects use combined visual and chemical information (including volatiles) to locate potential hosts'.

2.3.4 Effects of learning

Learning different cues associated with host plants allows some phytophagous insects to increase their host-finding efficiency (Papaj and Prokopy, 1989, van Loon, 1996). Learning ability has been reported in some butterflies and moths (Papaj and Prokopy, 1989, Thompson and Pellmyr, 1991, Bernays, 1995). Rausher (1981) proposed two types of learning: i) the associative learning (learning through prior experience to associate two stimuli or a stimulus with a response) reported in phytophagous insects particularly in some butterflies; and ii) the search image

formation where an insect learns to see and discover its search item especially in places where its visibility is more or less masked.

Papaj and Prokopy (1989) proposed three properties to characterise insect learning: i) the individual's behaviour changes in a repeatable way as a consequence of experience; ii) behaviour changes gradually with continued experience; and iii) the change in behaviour accompanying experience wanes in the absence of continued experience of the same type or as a consequence of a novel experience or trauma. If one or more of these criteria is/are met, then one may suggest a learning phenomenon. Categories of learning have also been defined: habituation, sensitisation, associative learning, food aversion learning, induction of preference, post-ingestive feedback and compulsive requirement for novelty (Papaj and Prokopy, 1989, Bernays, 1995).

In the case of host plant finding by phytophagous insects, associative learning is most likely to be used as other categories of learning require the initial involvement of contact sensory systems. There are some examples of associative learning in butterflies. Ovipositing *Battus* sp. butterflies find suitable hosts sometimes by learning the shape of the leaves of a host and sometimes by learning visual cues associated with the terminal leaf bud (Papaj and Rausher, 1983, Papaj, 1986). In his field study on shifts in foraging behaviour by *B. philenor*, Papaj (1986) observed *Battus* sp. butterflies landing predominantly on non host plants with leaf shapes similar to those of their host plants. It was discovered that *Battus* sp. butterflies were learning and relearning to search for their host leaf shapes as host plants varied from one season to another. In laboratory experiments, *P. rapae* was shown to be able to associate the colour of papers and leaf disks containing sinigrin, an ovipositing stimulant (Thompson and Pellmyr, 1991, Bernays, 1995, Hern *et al.*, 1996). *Spodoptera litoralis* (Lepidoptera: Noctuidae) was able to associate a particular odour to a food reward (Fan, Anderson and Hansson, 1997). *Helicoverpa armigera* (Lepidoptera: Noctuidae) has been found capable of using prior experience to enhance its host selection and acceptance efficiency (Cunningham, Jallow, Wright

and Zalucki, 1998). However, using naive and experienced insects, it was revealed that ovipositing *Euphydryas editha*, the checkerspot butterfly, lacks adaptive learning (Parmesan, Singer and Harris, 1995).

2.3.5 Disruption of distant sensory cues

Prokopy and Owen (1983) outlined four approaches which use visual cues to disrupt host plant location by herbivorous insects: use of ultraviolet reflecting materials to repel alighting insects; mixing hosts with weeds or to select spatial plant arrangements to reduce the contrasting effect of bare soil background or the appearance of the edge; use of fluorescent lamps to prevent nocturnal moths from alighting on hosts; and selection of plant cultivars with leaf pigmentation different to the normal one. Intercropping may be another approach used to reduce the visual contrasting effect of bare soil background/vegetation.

As discussed earlier, olfactory cues are involved in plant-host finding. In the case of intercropping, different wind patterns may mix specific host plant volatiles with non host plant volatiles with the effect of masking the specific volatile stimuli (Calvert and Hanson, 1983). Visser (1986) supported this assumption arguing that i) general compounds of plant odour overlap; ii) insect olfactory receptors are not solely tuned to specific compounds; and iii) plant odours are mixed as they are dispersed by the wind. Prokopy (1986) referred to some non host plant volatiles and visual structures as being able to mask or interfere with the detection of resources or initiate repulsion. In fact, as pointed out by Papaj and Rausher (1983) 'the crypticity of host plants' to searching phytophagous insects is a function of the characteristics (density, diversity, crop spatial orientation) of the surrounding non host plant community. It is then more likely that, if plant diversity is not only based on differences in taxonomy but also and more importantly on plant chemical dissimilarities (phytochemicals explain most of host plant preferences in phytophagous insects), specific visual and/or olfactory cues used by specialist phytophagous insects to locate their host plants may become disrupted or masked (Perrin and Phillips, 1978, Kareiva, 1983, Visser, 1986, Altieri and Liebman, 1986).

In Lepidoptera, the results of the study by Rausher (1981) on the susceptibility of *Aristolochia reticulata* to *B. philenor* butterfly attacks showed that the presence of natural vegetation around *A. reticulata* masked its discovery by searching butterflies. Karel (1993), studying the effect of intercropping on the mung moth *Maruca testularis* (Lepidoptera: Pyralidae) and the American bollworm *Heliothis armigera* (Lepidoptera: Noctuidae), two pod borers of common beans *Phaseolus vulgaris*, found that intercropping maize with common beans reduced the incidence of these two moths. He suggested that the reduction in the incidence of and damage by the pod borers might have been due to a restriction in the movement of the adult lepidopteran pests with maize acting as a physical barrier. However this does not exclude other kinds of interferences especially masking of host plant olfactory and visual cues. Intercropping sorghum with cowpeas reduced the incidence of stemborer *Chilo partellus* in sorghum resulting in an increase of sorghum grain yield (Ampong-Nyarko, Reddy, Nyang'or and Saxena, 1994). Altieri (1994) reported 30 selected examples of multiple cropping systems that prevented insect pest outbreaks of which half involved lepidopterous pests. Recently, a team of researchers from Kenya and UK found that intercropping maize with *Melinis minutiflora* reduced significantly the infestation of maize by the maize stalk borer *Bussolea fusca* (Noctuidae) and *C. partellus.* The volatiles of the non host plant repel the pests on the one hand and attract *Cotesia sesamiae* (Hymenoptera: Braconidae) a larval parasitoid of the stem-borers on the other hand (Khan, Ampong-Nyarko, Chiliswa, Hassanali, Kimani, Lwande, Overholt, Pickett, Smart, Wadhams and Woodcock, 1997a, Khan, Chiliswa, Ampong-Nyarko, Smart, Polaszek, Wandera, Mulaa, and Overholt, 1997b). It is clear that plant volatiles, as emphasised by some authors, may simply allow some insects to avoid non host plants (Renwick and Radke, 1988, Hern *et al.*, 1996).

2.4 Host plant defence against Lepidoptera

2.4.1 Direct defence

2.4.1.1 Physical defence

Plant surfaces are designed to more or less resist herbivore attacks. In particular, trichomes and leaf toughness are two plant traits that are used in physical defence

against herbivores (Southwood, 1986, Speight, Hunter and Watt, 1999). Trichomes which occur in almost all groups of plants may defend plants by simply hindering insect mobility or by trapping insects either by hooked hairs or sticky exudates from glandular trichomes (Southwood, 1986). Heliconiid butterfly larvae are reported to be caught by the hooked trichomes of *Passiflora adenopoda* whereas the speed of first instar larvae of *Pectinophora gossypiella*, the pink bollworm, is six times reduced on hairy leaves (Gilbert, 1971, Denno and Donnelly, 1981). There are however exceptions like the case of female *Heliothis zea* which lays more eggs on the hairy leaf surface of the corn. Sutherst and Wilson (1986) who reviewed how tropical legumes immobilize and kill cattle ticks showed a photograph of an unidentified adult lepidopteran insect trapped on *Desmodium uncinatum* (Leguminosae) by what is called 'Velcro® type of trichome hooks'. The exudate of glandular trichomes of *Datura wrightii* was found to be responsible for significantly reducing the development of the larvae of *Manduca sexta* reared on sticky leaves compared to those reared on velvety leaves (Van Dam and Hare, 1998). Glandular and non-glandular trichomes of tomato (*Lycopersicon esculantum*) were found to have an impeding effect on food searching by caterpillars of *M. sexta* (Wilkens, Shea, Halbreich and Stamp, 1996). A physical defensive effect of trichomes against the legume borer *Maruca testulalis* (Lepidoptera: Pyralidae) was demonstrated in experiments using pubescent wild and cultivated cowpeas (*Vigna vexillata and V. unguiculata*) (Oghiakhe, 1995). In choice and no-choice experiments, female *Chilo partellus* (Swinhoe) did not lay eggs on a maize cultivar (ICZ-T) which was covered by trichomes on both upper and lower leaf surfaces (Kumar, 1992). Lepidoptera have therefore to overcome and/or adapt to their host plant physical defence.

2.4.1.2 Chemical defence

Plant chemical defence might be detected by herbivores which use plant specific volatiles as cues to locate their host plants but also to avoid non host plants (Renwick and Radke, 1988). It might be that non host plants for herbivorous insects are those plants which have physical and chemical defences too 'strong' to be overcome by them. Many plant chemical compounds have been identified as secondary plant

metabolites and they are found in different plant families. Bernays and Chapman (1994) listed the major classes of plant secondary metabolites: non-protein amino acids, amines, alkaloids, cyanogenic compounds, betacyanins, phenols and phenolic acids, phenylpropanoids, flavonoids, quinones, tannins, terpenoids, organic acids, lipids and related compounds and sulfur-containing compounds. Terpenoids (more than 15,000 characterized) and alkaloids (more than 6,500 characterized) are more widespread (Metacalf, 1987, Bernays and Chapman, 1994). One of the roles played by plant secondary metabolites is to defend plants against herbivores with repellent, deterrent and/or toxic compounds.

2.4.2 Indirect defence

2.4.2.1 Attraction of predators and parasitoids by herbivore-induced volatiles.

Herbivore-induced volatiles are reported to be used by predators and parasitoids to locate their herbivorous prey. Dicke (1994) reviewed the evidence for the plant involvement in producing herbivore-induced terpenoids, a major class of herbivore-induced plant volatiles used by foraging predators and parasitoids. It has been shown that the production of herbivore-induced volatiles is not only restricted to the damaged plant part but also all other parts of the infested plant (Turlings and Tumlison, 1992, Dicke, van Baarlen, Wessels and Dijkkman, 1993, Potting, Vet and Dicke, 1995). This systemic response of the damaged plant leads to a systemic emission of herbivore-specific volatiles which are more detectable by carnivores (Dicke, 1999). Studies using caterpillar regurgitants on artificially injured plants showed that the elicitor of the systemic emission of carnivore attractants is contained in caterpillar's oral secretions (Turlings and Tumlinson, 1992, Turlings, McCall, Alborn and Tumlinson, 1993, Mattiacci, Dicke, Posthumus, 1994, Potting, Vet and Dicke, 1995, Alborn, Turlings, Jones, Stenhagen, Loughrin and Tumlinson, 1997). Herbivore-induced plant volatiles play an important role in plant indirect defence and seem to be a suitable solution to the 'reliability- detectability' problem (i.e. problem of prey location by natural enemies which are faced with highly detectable but less reliable information from herbivore host plants; and highly reliable but less detectable information from herbivores) of foraging carnivores (Vet and Dicke, 1992,

Vet, Lewis and Cardé, 1995, Dicke, 1999). The induced chemical signals were found to be highly clear and highly specific (if the host plants are fed on by herbivores of a single species), and were released at the right time for foraging herbivore natural enemies (Turlings, Loughrin, McCall, Rose, Lewis, Tumlinson, 1995). One of the most studied cases of indirect induced defence is that of corn plants attacked by *Spodotera exigua* (Lepidoptera: Noctuidae). The damaged plant emits volatiles which attract the parasitoid *Cotesia marginiventris* (Hymnoptera: Braconidae) (Turlings, Tumlinson and Lewis, 1990, Turlings, Tumlinson, Eller and Lewis, 1991, Turlings, Tumlinson, Heath, Proveaux and Doolittle, 1991).

Apart from corn, evidence has been produced to show that the following plants produce herbivore-induced volatiles which attract carnivores as a means of defence against Lepidoptera: apple (*Malus domestica*); cotton (*Gossypium hirsutum*), cowpea (*Vigna unguiculata*), cabbage (*Brassica oleracea*), hawthorn (*Crataegus* sp.), yellow cress (*Rorippa indica*), potato (*Solanum tuberosum*), sesame (*Sesamum indicum*), and nasturtium (*Tropaeolum majus*). A recent compilation of cases of attraction of carnivores to infested plants (Dicke, 1999) revealed that more than 50% of the cases involve Lepidoptera species mainly Pieridae and Noctuidae which feed on a variety of plant species from the Rosaceae, Poaceae, Malvaceae, Fabaceae, Brassicaceae, Solanaceae, Scrophulariaceae and Tropaelaceae families. The involvement of the third trophic level in the interactions between the first and second trophic levels brings more interesting but complex aspects to consider when one looks at plant-insect interactions especially in pest management strategies.

2.4.2.2 Plant and Lepidoptera -ant mutualisms

Some plants species produce extrafloral nectar, food bodies and/or domatia ('little houses') used by ants as food, feeding or nest sites. In return, ants form an 'army' which strongly defends these plants against predators (Huxley, 1986, Jeffree, 1986). Some plant-ant associations have even evolved in obligatory mutualisms. Such cases have been observed in the families of Boraginaceae, Euphorbiaceae, Leguminosae, Moraceae, Passifloraceae, Polygonaceae and Verbenaceae. The most reported case is

the ant-acacia mutualism found in Central America and East Africa (Janzen, 1981). Many Lepidoptera species were found to be prevented from feeding on ant-tended acacia or kept at a low density by the protecting action of ants (Janzen, 1981).

Ants do not trade their defence services to plants solely but also to butterflies that can reward them with food. The larvae of many species of the family Lycaenidae possess exocrine glands which secrete droplets of foods for ants (Axén and Pierce, 1998). The indirect but important involvement of host plants in ant-Lepidoptera relationships was highlighted by an experiment that compared the attractiveness of the larvae of *Polyommatus icarus* (Lepidoptera: Lycaenidae) fed on a high quality food (*Trifolium repens*) and those fed on a low quality food (*T. pratense* L.): larvae fed on *T. repens* attracted more ants than those fed on *T. pratense* (Fiedler, 1990). A feeding experiment with *Jalmenus evagoras* (Lepidoptera: Lycaenidae), another ant-tended butterfly, revealed that larvae fed on fertilised plants survived better and attracted more ants than those fed on unfertilized plants (Baylis and Pierce, 1991). Both examples demonstrate the importance of host plant quality in providing indirect defence to Lepidoptera through the attraction of ant guards.

2.5 Lepidoptera counterdefence against host plant defence

2.5.1 Introduction

Some herbivores especially specialist feeders have developed mechanisms to avoid, metabolise or store plant secondary metabolites which are toxic. A number of those plant toxins is known to be sequestered by some insects which use them to build up their own defence. Rothschild (1973) listed 43 species of insects from six different orders (Lepidoptera, Neuroptera, Hemiptera, Coleoptera, Diptera and Orthoptera) which sequester and store plant toxins to use them as means of defence against predators. More than half of the listed species belong to the following families of Lepidoptera: Papilionidae, Nymphalidae, Pieridae, Arctiidae, Ctenuchidae, Noctuidae. In addition to most of the Lepidoptera families mentioned above, Bowers (1988) reported other cases of plant toxin sequestration in the lepidopteran families of Lycaenidae and Zygaenidae. The toxic compounds sequestered are used as

acquired chemical defence compounds which render insects unpalatable to some predators and, more interestingly, these insects have aposematic colourations to help their predators to learn that they are not edible. Some palatable species have also evolved to mimic the same warning colourations as a defence strategy against predators (Ackery and Vane-Wright, 1984).

2.5.2 Sequestration of pyrrolizidine alkaloids (PAs)

Wink and Nickisch-Rosenegk (1997) listed the plant families which produce PAs: Asteraceae (Eupatorieae, Senecioneae), Apocynaceae, Boraginaceae, Celastraceae, Convolvulaceae, Leguminosae (*Crotalaria* sp.), Orchidaceae, Poaceae, Ranunculaceae, Rhizophoraceae, Santalaceae and Sapotaceae. There are about 400 different PAs already identified (Schulz, 1998). PAs are ingested by adult Ithomiinae (Nymphalidae) and Danainae (Nymphalidae) whereas Arctiidae sequester them during larval feeding (Bowers, 1988, Lamunyon, 1997). The male of the arctiid moth *Uthethesia ornatrix* pass on to the female a nuptial gift of PAs during mating. When eggs are laid, they already contain PAs from both female and male *U. ornatrix*. PAs have been reported to be used by males in the Nymphalidae and Arctiidae families as male pheromone precursors; in particular, female *U. ornatrix* uses male pheromone to assess the male PAs sequestering ability and how much PAs 'gift' it might receive from its mate and makes its choice accordingly (McNeil and Delisle, 1989, Lamunyon, 1997). Adult *Aeria olena* (Ithomiini) has been reported to acquire PAs by phamacophagy when it visits PA-containing plant species of Asteraceae (*Senecio* spp and *Eupatorium* spp), Apocynaceae and Boraginaceae (*Heliotropium* spp) (Trigo, Brown, Witte, Hartmann, Ernst and Barata, 1996). The same phenomenon is reported for other species of Danainae, Ithomiinae and Arctiidae (larvae of the arctiid *Creatonatos transiens* ingest pure PA) (Schulz, 1998).

2.5.3 Sequestration of iridoid glycosides (IGs)

Plant species from the families Scrophulariaceae, Plantaginaceae, Caprifoliaceae and Bignoniaceae contain iridoid glycosides (IGs) used as chemical defence but sequestered during larval feeding by Lepidopteran species especially the buckeye

butterfly, *Junonia coenia* (Nymphalidae), *Euphydryas* sp. (Nymphalidae) and larvae of some artiid moths (Bowers, 1988, Bowers and Stamp, 1993, Bowers and Stamp, 1997). In their experiments with *Plantago lanceolata* (Plantaginaceae), an IG-containing plant species, Bowers and Stamp (1993) found that *P. lanceolata* damaged by a specialist herbivore contained more IGs than undamaged plants. Moreover, of the two IGs (catalpol and aucubin) contained in *P. lanceolata*, catalpol, the more toxic, had a higher concentration and higher catalpol/total IGs ratio in damaged plants than undamaged plants. This plant defence strategy to increase the level of toxic compounds when it is being attacked profits IG-sequestering insects which may prefer high levels of chemical defense over nutritional quality as suggested by Camara (1997). Comparing the IG contents of two different populations of *Euphydryas gillettii*, Bowers and Williams (1995) showed that the concentration of the sequestered chemicals depended greatly on the host plant chemistry and its variation.

2.5.4 Cardiac glycosides (CGs)

Cardiac glycosides have been reported in some plant species of Scrophulariaceae, Apocynaceae, Asclepidiaceae, Ranunculaceae, Brassicaceae, Liliaceae, Celastraceae, Convolvulaceae and Moraceae (Marsh, Clarke, Rothschild and Kellett, 1977, Boppré, 1978, Bowers, 1988, Rowell-Rahier, Pasteels, Alonso-Mejia and Brower, 1995, Wink and Nickish-Rosenegk, 1997). CGs inhibit enzymatic activity of Na^+ and K^+ ATPase resulting in disruption of neural activity, secondary active transport, muscle contraction and many other cellular functions (Wink and Nickish-Rosenegk, 1997). In acting this way, CGs, which are bitter and noxious to vertebrates, exercise a defensive role against herbivores in CG-containing plant species. However, some insect herbivores have learned to sequester CGs from host plants in order to use them for their own defence. Among them, some species of Lepidoptera e.g. *Danus plexippus*, the American Monarch (Nymphalidae), one of the best studied unpalatable insects, stores CGs from larval food plants (Boppré, 1987, Rowell-Rahier *et al.*, 1995). *Hypolimnas bolina* (a mimic of *Euploea core* which stores CGs) was also reported to sequester CGs when it feeds on *Ipomoea batatas* (Convolvulaceae)

(Marsh *et al.*, 1977). It was also suggested that the SPB (Nymphalidae), an aposematic butterfly the larvae of which feed almost exclusively on *I. batatas* may store CGs (Azerefegne, 1999). Furthermore, *I.batatas* contains glycoresins, indole derivatives and dendrolasin which are toxic substances (Marsh *et al.*,1977). Apart from species of Nymphalidae, some arctiid moths such as *Ctenuchid* sp. also sequester CGs when they feed for instance on *Nerium oleander* (van Edem, 1973).

2.5.5 Sequestration of cyanogenic glycosides

Plants species of the following families produce cyanogenic glycosides which are toxic to most generalist insect herbivores: Caricaceae, Rosaceae, Fabaceae, Poaceae, Araceae, Compositae, Euphorbiaceae, Passifloraceae, Turneraceae, Rutaceae. Like the other groups of plant secondary metabolites used by plants for their defence, cyanogenic glycosides are sequestered by a number of specialist herbivores. In Lepidoptera, species of Zygaenae, Heliconiini and Acraeinae groups contain cyanogenic compounds (van Emden, 1973, Raubenheimer, 1989). *Zygaena trifolii* (Esper) has been found capable of sequestering the cyanogenic gylcosides, linamarin and lotaustralin, as well as synthesising them *de novo* (Nahrstedt and Davis, 1986). Raubenheimer (1989) demonstrated that *Acraea horta* (Lepidoptera: Acraeinae) contained gynocardin, a cyanoglycoside of the larval food plant, *Kiggelaria africana* (Flacourtiaceae) with or without feeding on cyanogenic-containing plants. These are some examples showing that cyanogenic glyosides are sequestered from host plants and/or produced *de novo* by some lepidopteran insects which they might use for defence purposes against predators (Bowers, 1988).

2.5.6 Other avoidance mechanisms

Some herbivorous insects have learned to avoid parts of their host plants with high concentrations of toxic substances or simply to metabolise them. Generalist insect herbivores which feed on celery, *Apium graveolens* may avoid the toxic effect of furanocoumarins (which become very toxic when they are activated by UV light) by making silken webs for early instars and by avoiding feeding on certain parts of the plant to sidestep photoactivation of the furanocoumarins. For instance, to avoid

photoactivation, the noctuid *Peridroma saucia* feeds in the heart ('leaves and young petioles in which leaves are not folded or which had been unfolded for at the most one week') of celery, *Trichoplusia ni* on the underfoliage, *Heliothis zea* 'between overlapping leaves webbed together', and *Platynota stultana* (Tortricidae) and *Udea profundalis* (Pyralidae) feed in webbed rolled leaves (Jones and Granett, 1982 and Berenbaum, 1990). First instar larvae of *H. virescens* feed on leaves of cotton avoiding glands containing gossypol, a secondary chemical metabolite which is a deterrent to them. Early instars of the SPB feed on leaves of sweet potato leaving major veins probably to avoid latex and/or high concentrations of cyanogenic glycosides. Some other insect herbivores which feed on latex and resin-containing plants start by cutting the main leaf veins or simply cutting a trench through a part of or the whole leaf to reduce the flow of latex or resin before they start feeding. *T. ni* exhibits this behaviour when it feeds on resin or latex-containing plants (Berenbaum, 1990, Bernays and Chapman, 1994, Smit *et al.*, 1997).

P. polyxenes (Lepidoptera: Papilionidae) and *Spodoptera frugiperda*, the black swallowtail butterfly, metabolise furanocoumarins by cytochrome P450 monooxygenases (Berenbaum, 1990) (Umbelliferae or Apiaceae) whereas *Eumaeus* sp. (Lepidoptera: Lycaenidae) and *Seirarctia echo* (Arctiidae) sequester or metabolise cycasin when feeding on plant species of Cycadaceae. The larvae of *Battus* sp. and *Parides* (Lepidoptera: Papilionidae) sequester aristolochic acids when feeding on plant species of Aristolochiaceae (Bowers, 1988).

2.6 Host plant-Lepidoptera interactions: plant induced defence

Reports of plant induced resistance against herbivory have recently been gathered and analysed (Karban and Baldwin, 1997). More than a hundred plant species belonging to thirty four families were found to induce resistance when fed on by herbivores. About half of the herbivores listed were lepidopteran species. However, the same authors collated reports on an opposite phenomenon where herbivory induces plant susceptibility and only slightly less than one third of the plant families was not represented in both categories. In this case also lepidopteran species were

well represented. It appears difficult to draw a general conclusion or make a general prediction as to whether a herbivore will induce resistance against or susceptibility to further herbivory on the plant it feeds on. A systemic release of feeding deterrents was induced by the feeding of beet armyworm, *Spodoptera exigua* on cotton (Alborn, Rose, MacAuslane, 1996, McAuslane, Alborn, Toth, 1997, McAuslane and Alborn, 1998). Agrawal (1998) reported that early induction of plant resistance by *Pieris rapae* (Lepidoptera: Pieridae) larvae resulted in increased concentrations of defensive glucosinolates and densities of setose trichomes in wild radish. The induced defence halved the feeding of chewing herbivores and reduced the abundance of phloem-feeding aphids compared to controls. Interestingly, using wild radish and *P. rapae* larvae, Agrawal, Leforsch and Tollrian (1999) demonstrated that induced plant defence was transmitted from one generation to the next one. The same effect, referred to as 'transgenerational induction of defence' was reported in *Daphnia cacullata*, a crustacean and its carnivorous predator *Chaoburus flavicans* (Agrawal *et al.*, 1999). Haukioja (1999) highlighted it with more dramatic wording: 'bite the mother, fight the daughter'. However, in another experiment, Agrawal (1999) found that specialist herbivores such as *P. rapae* did not appear to be affected by induced defence to herbivory. This suggests that the reaction of herbivores to induced response to herbivory may be of three types: induced defence, induced susceptibility and status quo.

Compared to constitutive defence (i.e. always expressed in the plant (Karban and Baldwin, 1997)), induced defence has been justified by the cost-benefit model which favours the latter simply because plant allocations to defence are made only when needed. However few experimental data support that model. Based on the results of field experiments involving *Bucculatrix thurberiella* (Lepidoptera: Lyonetiidae) and its host plant *Gossypium thurberi*, Karban (1993) questioned the appropriateness of that model in explaining the advantages of induced defence over constitutive defence. Some years later, Karban and his co-workers suggested a new explanation which considers induced resistance as providing increased variability in defence. Due to variability, a herbivore's performance is reduced (Karban, Agrawal and Mengel,

1997). More experiments are also needed to test this new 'increased variability' model.

2.7 Evolution towards three trophic level interactions

In summarising host plant-Lepidoptera interactions, Table 2.1 reveals three levels of interactions between plants and phytophagous insects: long range distance, short range distance and contact with the plant. At long range distance, insects come into contact with plant volatiles mostly 'green leaf volatiles' carried around by wind. At this first level of contact, plant odours might trigger a movement away or towards the source of odours by the insect. When the plant odours trigger a movement away from the source of the odours this is seen as an avoidance mechanism, a reaction to the information contained in specific plant odours. The information meant 'not suitable' to the host-seeking insect and corresponds to the first plant defence barrier against phytophagous insects. However, although some insects 'read' the message in plant odours as 'not suitable' for others the message means 'possible suitable host plant' or may be using a random searching mode and therefore move closer to the source of odours.

At short range distance, insects may receive more information about the source of plant odours because of the high frequency of odour bursts containing information about the nature of the plant but also some insects might be able to use their vision to learn about the colour, size and shape of the plant's leaves (Prokopy and Owen, 1983, Miller and Strickler, 1984, Visser, 1986, Nottingham, 1988, Degen and Städler, 1997). With more information from volatiles and the visual appearance of the plant, the central nervous system of some insects might tell them that the plant in their vicinity is 'not a suitable host plant' and the insect moves away. This second level of interaction between phytophagous insects and potential host plants constitutes the second defence barrier for the plant against insects. As with the first level of interactions, some insects will get the message 'possible suitable host' and will move to land and/or walk on the plant.

Table 2.1 Insect-plant interactions: different levels of plant defence

Long range distance			Short range distance		Contact with plant	
Plant stimuli	Insect behaviour	Type of plant	Insect behaviour	Type of plant	Insect behaviour	Type of plant
Plant volatiles	Move away	**non host**				
	Indifferent	may be host	move away	**non host**		
			indifferent	may be host	move away	**non host**
					feeding/oviposition	host
			move towards	may be host	move away	**non host**
					feeding/oviposition	host
	Move towards	may be host	move away	**non host**		
			move towards	may be host	move away	non host
					feeding/oviposition	host
Plant appearance	Move towards	may be host	move away	non host		
			indifferent	maybe host	move away	non host
					feeding/oviposition	host
			move towards	maybe host	move away	non host
					feeding/oviposition	host

Table 2.1 Insect-plant interactions: different levels of plant defence (continued)

Long range distance			Short range distance		Contact with plant	
Plant stimuli	Insect behaviour	Type of plant	Insect behaviour	Type of plant	Insect behaviour	Type of plant
Plant surface structure	n/a	n/a	n/a	n/a	move away	non host
					feeding/oviposition	host
Plant surface chemistry	n/a	n/a	n/a	n/a	move away	non host
					feeding/oviposition	host
Plant chemistry	n/a	n/a	n/a	n/a	move away	non host
					feeding	host

n/a = not applicable

The contact of the insect and the plant constitutes the third level of interactions between the host-seeking insect and its possible host plant. This is the ultimate level of interactions between insects and plants. Insects use their tactile and gustatory senses to learn more about the plant surface morphology, structure as well as its chemistry. Spiny or spineless, hairy or not, trichomes or not, tough surface or tender, deterrent or stimulant chemical, presence or not of any other kind of protection: all these plant characteristics are expressed at the plant surface. While some insects are immediately put off by such information and move away, others are sufficiently attracted by the information to feed on the plant or to oviposit. Once the feeding has started, the insect is subject to three different types of plant defence: physical defence, constitutive chemical defence, and the induced chemical defence which is switched on by the feeding insect with the eventuality of facing predators and/or parasitoids informed by herbivore-induced volatiles emanating from the injured plant.

Apart from intrinsic defence factors, plants have probably been building up and diversifying their defence mechanisms against insect attacks since they encountered plant feeding insects for the first time, most likely in the Carboniferous period (3×10^8 years BC) when the first insects appeared (Smart and Hughes, 1973, Metcalf, 1987). Different plant populations are believed to have evolved different defence traits and/or strategies to withstand parasitic relationships with herbivorous insects which inhibit plant population growth without necessarily decreasing it (Marquis, 1991). Table 2.2 shows the different types of interactions between species of two different populations and how they are affected by them. The hypothesis of plants evolving defence traits in relation to the risk of herbivory was supported by the results of the study of the insular endemic plants of the Santa Cruz Island (California) which, because they had evolved without mammalian herbivore pressures, had larger leaves, shorter and lesser number of spines than the same plant species or their closest relatives on the adjacent mainland. Moreover when they were offered to hungry sheep in a choice assay, island shrubs were eaten more than mainland ones (Bowen and Van Vuren, 1997, Van Varun and Bowen, 1999). The lack of difference

between plant defence chemicals of the two localities was attributed to a large proportion of herbivorous insects among the 1000-4000 insect species living on Santa Cruz Island. There were, therefore, reasons for the plants on the island to invest in chemical defence.

Mutualistic relationships which profit species of both populations in interactions (Table 2.2) by increasing their fitness would be the most suitable in terms of evolutionary ecology between plants and phytophagous insects. Such a type of stable relationships was suggested by Strauss and Agrawal (1999) in their conclusion reviewing the ecology and evolution of plant tolerance to herbivory as a possible outcome of the evolution of plant tolerance in contrast to plant resistance which would lead to a 'coevolutionary arms race' between plant resistance and herbivore counterdefence. However the recent discovery of the attraction of herbivorous insect's predators and/or parasitoids by herbivore-induced plant volatiles has widened the thinking of insect/plant interactions from two to three trophic level interactions.

The relationships between predators and/or parasitoids (which respond to the 'call for help' made by herbivore injured plants) and insect host plants could evolve into mutualistic interactions with insect host plants. The plant could tolerate a certain level of feeding by insects which will be preyed on by predators and/ or parasitoids to keep the overall level of plant population injury tolerable and constant. This could be a more natural regulating coevolution. And it might be not surprising to find that such plant/insect/parasitoid relationships where plants and parasitoids are in mutualistic associations to regulate herbivorous insects exist already in undisturbed natural ecosystems.

The human intervention with agricultural systems to increase crop productivity in monocropping and, to a lesser extent, in mixed cropping have greatly contributed to an instability of such natural systems with the emerging of new insect pests each time

that tri-trophic level relationship for a given population of plant, herbivorous insect and parasitoid species is significantly destabilised.

Table 2.2 Analysis of interactions between populations of two species, A and B.

Type of interaction	Effect on population growth of A and B				General result of interaction
	When not interacting		When interacting		
	A	B	A	B	
Neutralism (A and B independent)	0	0	0	0	Neither population affects the other
Competition (A and B competitors)	0	0	–	–	Population most affected eliminated from niche
Mutualism (A and B competitors)	–	–	+	+	Obligatory for both
Protocooperation (A and B cooperators)	0	0	+	+	Non-obligatory but favorable to both
Commensalism (A commensal; B host)	–	0	+	0	Obligatory for A; B not affected
Amensalism (A amensal; B inhibitor)	0	0	–	0	A inhibited; B not affected
Parasitism (A parasite; B host)	–	0	+	0	Obligatory for A; B inhibited
Predation (A predator; B prey)	–	0	+	–	Obligatory for A; B inhibited

\+ Population growth increased,

– Population growth decreased

0 Population growth not affected

Source: Cheng, 1991

Without human interventions or other destabilising events, plants, phytophagous insects and, predators and parasitoids would most probably coexist in a non-conflicting relationship.

2.8 Conclusion

Phytophagous insects use their senses to find and recognise their host plants. In particular, Lepidoptera species depend on olfaction and vision to locate their potential host plants. At relatively long distance, both common green leaf and specific host plant volatiles were found to be crucial in inducing the movement of host-seeking Lepidoptera (and other phytophagous species) towards the source of volatiles. Lepidopteran vision which acts generally in synergy with olfaction plays a major role in landing on host plants. This is particularly true with the group of butterflies which were recently found to have genuine colour vision. Evidence of improved searching efficiency by learning of host plant visual as well as olfactory cues and the disruption of host plant finding by interfering with host plant cues was also found in the literature. Plant defence mechanisms against Lepidoptera and their counterdefence revealed that host plant-Lepidoptera interactions are dynamic. The attraction of parasitoids and/or predators by herbivore-induced plant volatiles brought a third trophic level into host plant/phytophagous insect interactions. The evolution from bi- to tri-trophic level relationships where parasitoids and/or predators would continue to use the highly reliable and highly detectable herbivore-induced plant volatiles to locate their prey would probably lead to a more stable relationship in which one trophic level would be playing a balancing role by keeping the herbivore population to a tolerable level.

This review revealed that there was not a published study on how the SPB, a pest of sweet potato in East Africa, finds its host plants. However, evidence from studies of host plant finding by other butterflies suggests that the SPB, a specialist feeder on *Ipomoea batatas* (Convolvulaceae) and very few other related species of the Convolvulaceae family, might rely on plant olfactory and visual cues to locate its host plants. Hence, a study to investigate this assumption is of prime importance. It

would provide useful information which could be used to build up management strategies against the SPB compatible with resource-poor subsistence farming in East Africa.

Chapter 3

Attraction of *Acraea acerata* (Lepidoptera: Nymphalidae) to sweet potato plants (*Ipomoea batatas*, L. (Lam.))

Chapter 3 Attraction of *Acraea acerata* (Lepidoptera: Nymphalidae) to sweet potato plants (*Ipomoea batatas*, L. (Lam.))

3.1 Introduction

Female SPBs have been seen laying eggs on sweet potato plants in the field as well as in laboratory conditions. In studies of host plant acceptability, captive female SPBs were offered potential host plants within a few centimetres inside their rearing cages (Lefèvre, 1948, Subukino, 1987, Lugojja, 1996, Azerefegne, 1999). During these bioassays, the interest was not so much about knowing the sequence of behavioural events leading to accepting or rejecting of potential host plants, rather it was about answering the question as to whether or not a life cycle of the SPB would be completed on each of the potential host plants tested. The review of the available literature on the SPB (Chapter 1) revealed that the whole area of insect-host plant interactions has never been investigated before. In an attempt to start the study of host plant finding by the SPB, a wind tunnel bioassay was planned and implemented.

The aim of the bioassay was to test if the SPB was attracted to lay eggs on sweet potato plants in wind tunnel conditions. Wind tunnels are commonly used in researching insect's olfactory behaviour as well as orientation and migration (Baker and Lin, 1984, Wyatt, 1997). They allow the researcher to create experimental conditions which are closer to that of the natural environment of the insect by controlling the lighting, temperature, relative humidity and wind speed. These environmental conditions are critical in the lives of insects (Gossard and Jones, 1977, Wyatt, 1997). The odour-induced movement of insects towards or away from odour sources (positive or negative anemotaxis) are best studied in wind tunnels (Kennedy, 1986). The wind tunnel used in this experiment was used by Hern (1997) to study the effect of host plant volatiles on *Pieris rapae* (Lepidoptera: Pieridae) and Kirkland (1999) to identify semiochemicals which attract *Episyrphus balteatus* Deager (Diptera: Syrphidae).

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Sweet potato butterflies

Eggs of SPBs collected from sweet potato fields were obtained from Uganda (Scottish Office Agriculture Environment and Fisheries Department Import licences no. PH/27/1997, PH/10/1998 and PH/20/1999). They were kept in a mesh-covered cage of 50 × 50 × 50 cm in the insectary of the Scottish Agricultural College in Edinburgh. The temperature in the insectary was kept between 23-27 °C with a photoperiod of L12:D12 under full light spectrum (fluorescent tubes from Sylvania Activa 172 professional 58 watt, supplier: Lightbox Scotland Ltd, Glasgow, UK) and 40-60% relative humidity. When eggs started hatching, they were moved to another cage with a whole sweet potato plant to feed on. Early instars could feed on a plant for a week whereas late instars needed new sweet potato plants or cuttings every day until all started to pupate. Pupation occurred all over the walls of the cage, the pots (which contained sweet potato plants) and sweet potato plants. About a week after pupation, butterflies started to emerge and were allowed some time to get their wings strengthened before being transferred to another cage where they were offered a Petri dish containing a 10% sugar (sucrose) solution absorbed in a yellow sponge for feeding and a sweet potato plant for oviposition. The length of the SPB life cycle (eggs to eggs) under these rearing conditions was 37-42 days.

Butterflies used in all bio-assays were not offered sweet potato plants (to prevent them from getting any prior experience of the host stimuli). Each day, at the same time, newly emerged butterflies both males and females were transferred to a new cage. Captive SPBs started to mate during their first day after emerging from pupae. Preliminary observations revealed that females were losing parts of their wings as they struggled to escape mate hunting males when few females were caged together with a large number of males. To prevent this from happening, attention was paid to placing a similar number of males (slightly higher) and females in the same cage. The mating was natural and could take some hours. With the laboratory culture of sweet potato butterflies, it was observed that two day old females started to lay eggs.

This observation prompted the decision to use two day old mated females in all bio-assays. At this age, they were assumed to be physiologically ready to oviposit and therefore would display host-searching behaviour for oviposition.

3.2.1.2 Sweet potato plants

Sweet potato plants used to feed the larvae were grown in a glasshouse under a photoperiod of D16:L8 and an average temperature of 20 °C. The first vine cuttings planted were obtained by growing sweet potato tubers bought from a local supermarket. Sweet potato tubers were first sprouted by horizontally half submerging them in water and keeping them at 20 °C in an incubator. Once they started to sprout, they were transferred into potted peat in the glasshouse. From time to time, to prevent the shortage of food for SPB larvae, sweet potato was grown in Fison cabinets where the temperature and the duration of light were slightly increased to quicken the growth of the plants. Occasionally, fertilisers (20 ml/9 litres of water/20 plants of Plant liquid feed, NPK 5-5-10 from Premier Way Ltd, Falkirk, UK) were applied. Pesticides against aphids were also sometimes used (Aphox: 0.5 g/l litre of water of pirimicarb/Zeneca/UK). Sprayed plants were left for at least two weeks before they could be fed to SPB larvae.

Sweet potato plants used in all bioassays (not for feeding the butterfly larvae) were all grown in a glasshouse without agro-chemicals. They were all from the same clone. The clone was obtained by sprouting one sweet potato tuber as described earlier. To keep sweet potato plants clean from insects and diseases, vine cuttings were planted in peat contained in pots (150 mm) and put on a tray made of a waterproof black plastic and covered with a cage of 1 × 0.5 × 0.5 m. The tray was constantly covered by water which, not only watered the plants but also stopped insects especially aphids from walking onto the plants. The cage served as a physical barrier to flying insects. All sweet potato plants used in bioassays were 6-8 weeks old.

3.2.1.3 Wind tunnel

The wind tunnel was described by Hern (1997) and Kirkland (1999). Figure 3.1 shows a diagrammatic representation of the wind tunnel. Its experimental area measured 2.0 m wide, 1.75 m long and 1.0 m high; and was lengthwise, arbitrarily subdivided into six equal sections each just under 0.3 m long (section 1 to section 6). The walls (upwind and downwind) of the experimental area were made of steel chicken wire and a nylon mesh (1mm) was fixed on them to prevent insects from escaping but allowing at the same time a free air flow into the chamber. A small window was made in the middle of the downwind wall to allow the introduction of butterflies onto the releasing point . The releasing point was made of a cotton thread hanging from the top of the chamber in the middle of section 1 down to a height of 0.35 m from the floor of the chamber and at about 0.10 m from the small window. To allow the monitoring of the insects, the side walls of the chamber were made of clear PVC. The wooden floor of the experimental area painted in alternate black and white stripes (to provide a uniform optomotor stimulus for the orientation of flying insects) was slippery to the butterflies and they could hardly walk. To help the butterflies walk freely, a clear plastic mesh was fixed on the floor of the chamber. Lighting, by eight evenly spaced fluorescent strip lights, provided full spectrum light close to daylight. The intensity of light that reached the experimental area of the wind tunnel was not evenly distributed. The middle sections of the wind tunnel received more light intensity (approximately 1700 lux) which decreased evenly towards down and upwind sections of approximately 50 lux (Hern, 1997, Kirkland, 1999). The transition section and settling chamber of the wind tunnel were built to provide a laminar airflow at the entrance of the experimental chamber. A centrifugal blower fan and an electric heating system allowed the control of the wind speed and the temperature of the ambient air drawn from outside. The wind speed and the temperature were both monitored by a combined wind speed and temperature probe (Dantec 9054R0102) linked to a low velocity flow analyser (DISA 54N50). They were recorded as means over a period of three minutes.

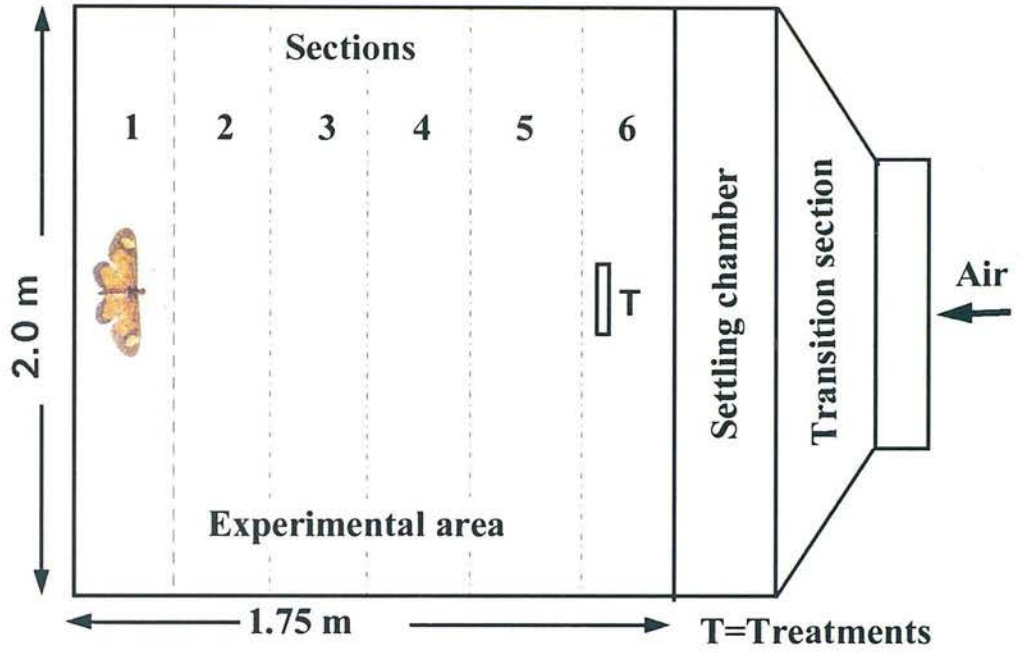


Figure 3.1 Diagrammatic representation of the wind tunnel (modified from Hern, 1997)

3.2.2 Methods

Treatments were first introduced into section 6 (upwind section) of the experimental area to allow the wind speed and the temperature to stabilise before releasing butterflies. There were two treatments: i) the control or ambient air made of three pots with peat only (notice that the control was not made of odour filtered air: in addition to odours from the pots and peat, the ambient air contained odours from all the odour sources surrounding the building in which the wind tunnel was housed. In particular, the building was surrounded by trees and different kinds of vegetation from which emanated, in addition to their specific volatiles, plenty of common green leaf volatiles) and ii) three pots of sweet potato plants. They were positioned in section 6 across the width of the experimental area. One pot was placed in the middle; in a straight (or almost straight) line with the releasing point and the other

two pots were placed on either side of the middle one 0.40 m apart. For each treatment, a different group of ten butterflies was released onto the thread and observations made for a 180 minute period. Every 15 minutes, the position of butterflies (S1 to S6) and the activities of butterflies (resting, walking, flying and egg laying) were recorded. Landing on treatments was recorded whenever it happened during the observation period. There were four replicates: one replicate per day and treatments were randomised within a day. In all bioassays a 10% sucrose solution was always positioned in the middle of section 1 to allow butterflies to feed *ad libitum*. The observations were made between 11h00 min and 17h30 min. During the bioassay, the mean wind speed and the mean temperature were respectively 30. 03cm/s (± 0.23) and 28. 37 ^{o}C (± 0.08).

The experiment was designed as a completely randomised block with day as a blocking factor. The numbers of butterflies resting, walking, flying and the average distances moved towards treatments (average distances were calculated using the recorded positions of butterflies at each observation time) were analysed using Genstat 5 Second Edition (for Windows (Genstat 5, Release 3.2 (PC/Windows/Win32s), Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)). The model for the analysis of variance was as follows: General Analysis of Variance; BLOCK : Day/Treatment/Time; TREATMENTS: Treatment * POL (Time; 2); COVARIATE : No Covariate; ANOVA [PRINT = aovtable, information, mean, contrast; FACT=3; FPROB=yes; PSE = diff].
The normality of the data was checked using the Genstat command: DAPLOT fitted, normal, halfnormal, histogram. The number of landings on treatments and the number of butterflies which laid eggs did not require statistical analysis as butterflies landed and laid eggs only on sweet potato plants.

3.3 Results and Discussion

3.3.1 Results

The data on average distances were normally distributed. Although the residuals had a normal distribution for the number of butterflies resting and walking, they were larger for low values of response in resting (opposite for walking). Consequently, the standard errors were only approximately correct (Hunter, *personal communication*).

The analysis of variance of the number of butterflies resting, walking and flying did not reveal any statistically significant difference (Appendix 3.1, $P > 0.05$) between the numbers of butterflies resting, walking and flying with/without the presence of sweet potato plants. Nevertheless, there was a highly significant linear relationship between time and the mean number of butterflies resting ($P< 0.01$, Figure 3.2), a significant linear relationship between time and the mean number of butterflies walking and flying ($P < 0.05$, Figure 3.3, Figure 3.4) and a very highly significant curvilinear relationship between time and the mean average distances moved by butterflies ($P < 0.001$, $P < 0.005$ (quadratic), Figure 3.6).

The mean average distances moved by butterflies towards treatments were significantly different with and without the presence of sweet potato plants ($P< 0.05$, Figure 3.5). There was also a significant effect of time on the movement of butterflies with and without the presence of sweet potato plants ($P < 0.05$, Figure 3.7). Landings on treatments were recorded as they occurred during the observation time. Each time sweet potato plants were present, at least one butterfly among a group of ten landed on sweet potato plants and there was no landing at all in ambient air (Table 3.1).

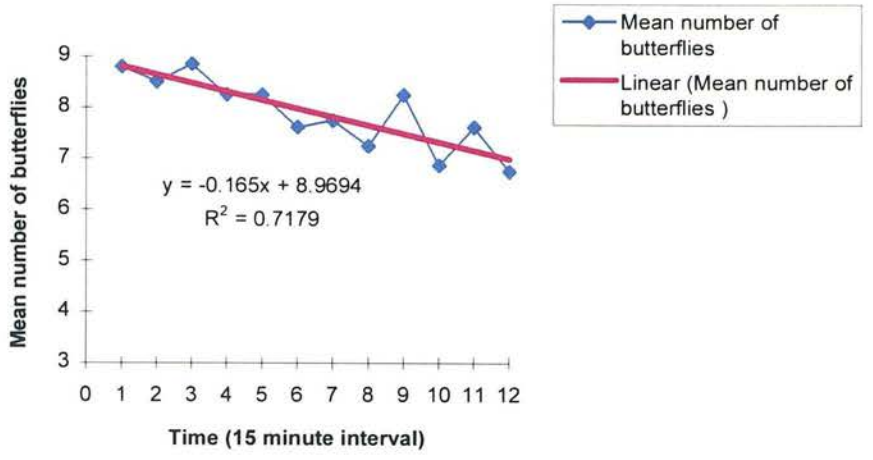


Figure 3.2 Linear relationship between time and the mean numbers of butterflies resting in wind tunnel with/without the presence of sweet potato plants (n = 80)

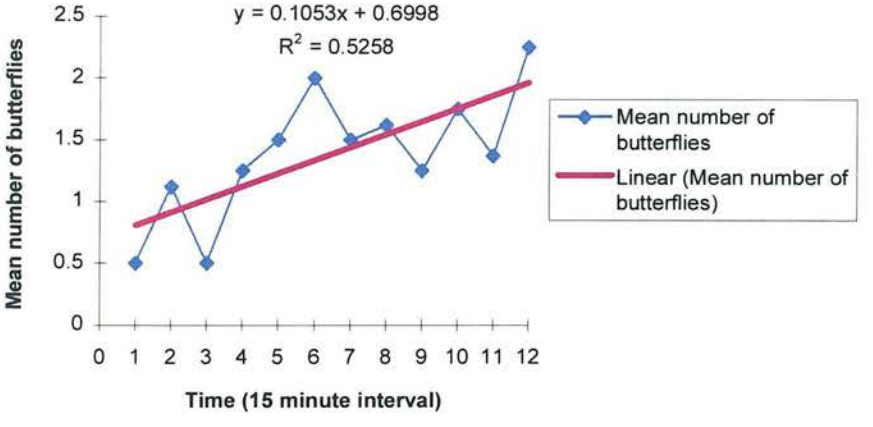


Figure 3.3 Linear relationship between time and the mean number of butterflies walking in wind tunnel with/without sweet potato plants (n = 80)

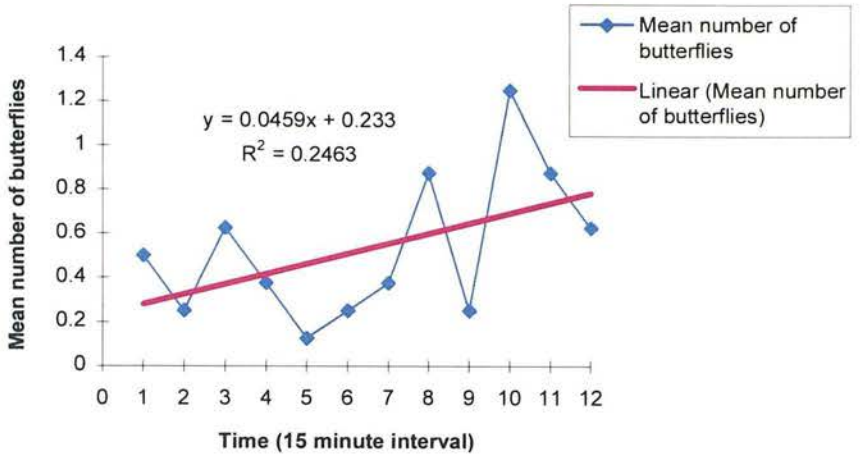


Figure 3.4 Linear relationship between time and the mean number of butterflies flying in wind tunnel with/without the presence of sweet potato plants (n = 80)

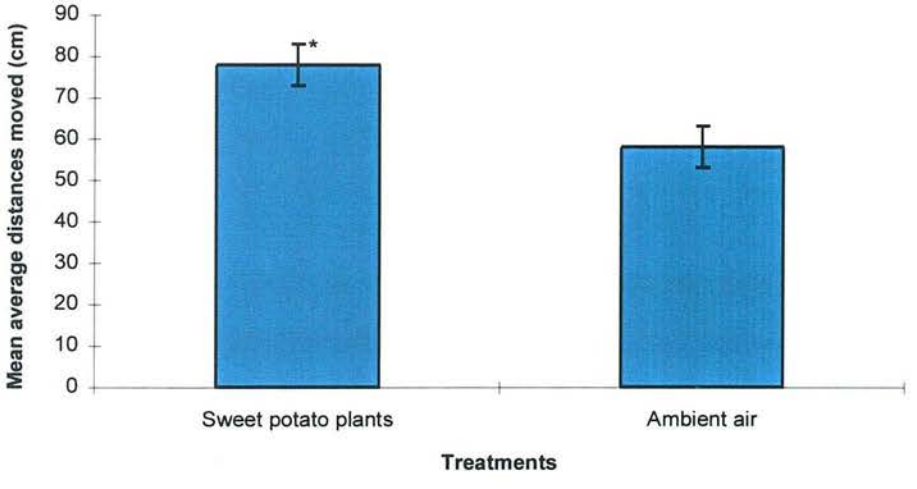


Figure 3.5 Comparison between mean average distances (cm) (± SED) moved by butterflies in wind tunnel with and without the presence of sweet potato plants (n = 40) (* $P < 0.05$)

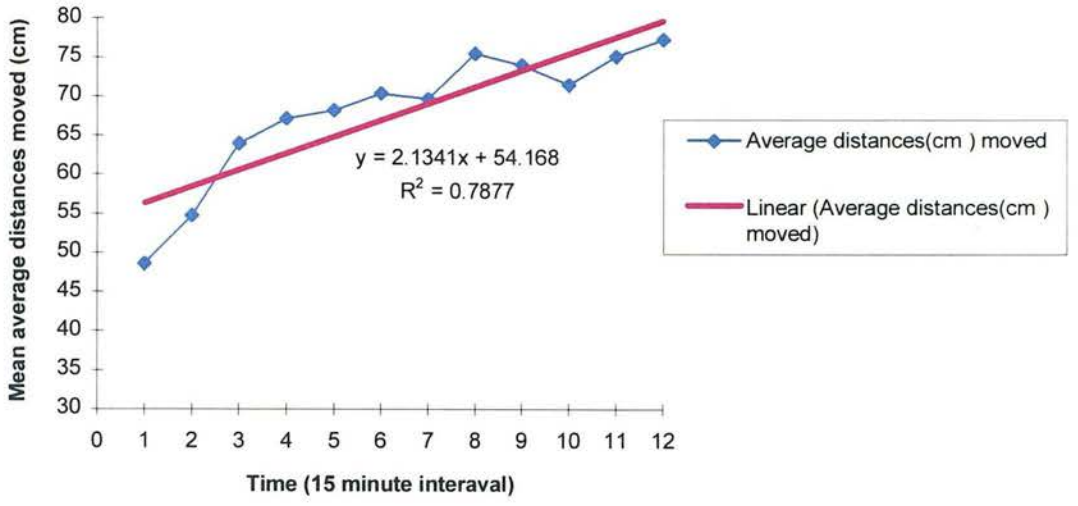


Figure 3.6 Linear relationship between time and the mean average distances moved by butterflies in wind tunnel with/without the presence of sweet potato plants (n = 80)

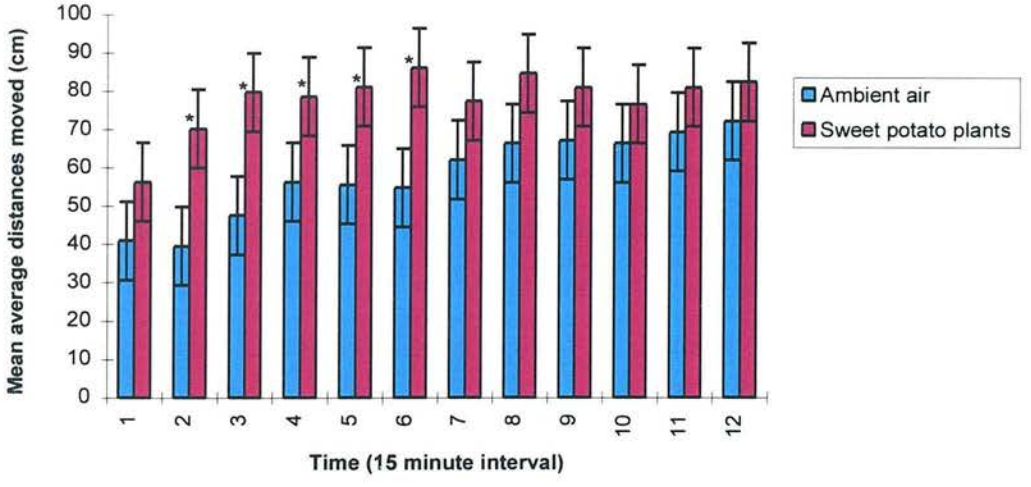


Figure 3.7 Comparison of mean average distances (cm) (± SED) moved by butterflies in wind tunnel with and without the presence of sweet potato plants for each recording time (n = 40) (There are statistically significant differences between means with * for the two treatments, $P < 0.05$)

Table 3.1 Number of groups (/4) of butterflies of which at least one butterfly landed

Treatments	Landing	No landing
Ambient air	0	4
Sweet potato plants	4	0

Egg laying was only observed when butterflies were offered sweet potato plants. Each time one out of ten butterflies laid eggs but not a single butterfly laid eggs in presence of ambient air alone (Table 3.2).

Table 3.2 Number of butterflies (/40) which laid eggs in a wind tunnel

Treatments	Egg laying	No egg laying
Ambient air	0	40
Sweet potato plants	4	36

3.3.2 Discussion

The number of butterflies walking or flying was not statistically different whether sweet potato plants were present or not ($P > 0.05$). However the analysis of variance of the average distances (which measure the distances moved (net movement) from the releasing point of butterflies to the treatments) revealed a significant difference between the mean average distances moved by butterflies with and without the presence of sweet potato plants in the wind tunnel ($P < 0.05$, Figure3.5). This suggested that at least a certain number of butterflies which walked and/or flew in the presence of sweet potato plants moved closer to sweet potato plants or even reached them. This is clearly confirmed by the number of landings which occurred only in the presence of sweet potato plants (Table 3.1). The longer the distances moved, the closer the butterflies came to treatments and consequently the more butterflies seemed to be attracted to sweet potato plants. Compared to ambient air, sweet potato plants appeared to be more attractive to butterflies (Figure 3.5 and Figure 3.7). The attractiveness of sweet potato plants seemed to be greater during the first half of the

observation period (Figure 3.7). This suggests that butterflies responded relatively quickly to sweet potato plant stimuli. The maximal distance beyond which SPBs can not visually distinguish their host plants from other plants is yet to be determined. The butterflies might have been able to access both host plant olfactory and visual stimuli at their releasing point (about 1.40 m away from the plants). The fact that not a single butterfly landed on pots with peat alone in spite of the presence of green leaf volatiles in the ambient air whereas at least one butterfly landed (there were 49 landings in total) on sweet potato plants each time a group of them was offered sweet potato plants (Table 3.1) suggests that landings were solely due to the presence of sweet potato plants in wind tunnel. Therefore, the results of this assay show that female sweet potato butterflies responded to sweet potato plant olfactory and visual stimuli by moving (walking or flying) upwind, landing on the host plants and laying eggs. From a distance, butterflies use olfaction as well as vision to locate their host plants (Bernays and Chapman, 1994). It seemed consequently more likely that the SPB used both specific host plant odours as well as host plant visual stimuli (Miller and Stricker, 1984) to locate sweet potato plants.

There was a linear trend decrease of butterflies resting (Figure 3.2, $P < 0.01$) with a consequent linear trend increase of butterflies moving (Figure 3.3, $P < 0.05$; Figure 3.4; $P < 0.05$; Figure 3.6, $P < 0.001$). Time is a factor which stimulates the behaviours of butterflies in relation to circadian rhythms but it also embodies a number of other internal factors such as length of time since the last meal, need of male mates, individual versus group behaviour, genetic variability and prior experience which might have stimulated the butterflies to move. The use of naive, mated females, all from the same laboratory culture and the presence of the sugar solution in the wind tunnel (not a single butterfly was observed feeding!) helped to control all these factors except the individual versus group behaviour. Nevertheless, it was clear that landing and egg laying were not group behaviours (Table 3.1, Table 3.2). External stimuli such as temperature, wind and lighting were also controlled. Although, efforts were made to ensure even lighting, the middle sections (sections 3&4) of the wind tunnel received more light intensity which decreased evenly

towards S1 and S6. This might have been the reason why most butterflies were located in the middle sections of the wind tunnel where the light intensity was maximal (Hern, 1997, Kirkland, 1999).

Egg laying, like landing, was observed only when butterflies were offered sweet potato plants (Table 3.2). In the presence of sweet potato plants, one butterfly in a group of ten laid eggs on a sweet potato plant. In Ethiopia the majority of adult SPBs were found to emerge before noon and in the field, mating was observed only in the afternoon (Azerefegne, 1999). In this assay, it was observed that egg laying started always after 14h00 and only one finished laying eggs before the end of the observation period. As the butterfly rearing conditions had fixed the beginning of the day of the butterflies at 8h00, they all seemed to prefer to lay eggs in their 'afternoon' which might explain why the percentage of egg laying butterflies was relatively low (10 %) because the observation time extended only into the first half of their 'afternoon'.

All the butterflies which laid eggs flew upwind, landed on the upside of a sweet potato leaf and walked to the underside of the leaf where eggs were laid. This confirmed that the upwind movement and the landing of female butterflies on sweet potato plants were not random behaviours but responses to sweet potato plant stimuli. Egg laying being the final stage of host plant selection by ovipositing insects, it could be argued that this experiment has showed that adult female SPBs are attracted to sweet potato plants on which they land and oviposit.

3.4 Conclusion

Female SPBs have been observed laying eggs on sweet potato plants both in field and in laboratory cultures. There was no doubt as to whether the SPB was attracted to sweet potato plants or not. However, there was no experimental proof to support that fact. The results of the bioassay discussed in this chapter show, for the first time, that the SPB is attracted to sweet potato plants in a wind tunnel. This attraction resulted in butterflies moving upwind, landing and laying eggs on sweet potato plants. The

involvement of both sweet potato plant olfactory stimuli in a mechanism termed odour-induced upwind anemotaxis (Kennedy, 1977) and visual structures in attracting female SPBs can no longer be questioned; however the extent to which these two types of stimuli affected the attraction needs further investigation.

Chapter 4

Effects of olfactory and visual cues on the attraction of *Acraea acerata* to sweet potato plants

Chapter 4 Effects of olfactory and visual cues on the attraction of *Acraea acerata* to sweet potato plants[1]

4.1 Introduction

The review of host plant-Lepidoptera interactions has gathered information showing that phytophagous insects in general and lepidopteran species in particular, searching for their host plants, can use olfactory and/or visual stimuli to orientate and move to discover their host plants (Kennedy, 1977, Prokopy and Owens, 1983, Miller and Strickler, 1984, Visser, 1986, Feeny *et al.*, 1989, Bernays and Chapman, 1994). In particular, butterflies, generally known for the predominance of their vision in host plant finding, were also found to use the information contained in both common green leaf volatiles as well as specific host plant volatiles in the process of host plant finding (Saxena and Goyal, 1978, Mackay, 1985, Feeny *et al.*, 1989, Bernays and Chapman, 1994, Hern, 1997).

Lepidopteran insect species (like all other phytophagous insects) are presumed to respond more importantly to the combination of host plant olfactory and visual stimuli than when each type of stimulus is offered separately. They have also been reported as able to learn host plant olfactory or visual stimuli and use prior experience to increase their host-searching efficiency (Saxena and Goyal, 1978, Papaj and Rausher, 1983, Papaj, 1986, Stanton, 1982, Thompson and Pellmyr, 1991, Fan *et al*, 1997, Cunningham *et al.*, 1998). This description abbreviates the general knowledge of host plant finding by Lepidoptera built up from reported studies carried out on particular species which normally present common as well as particular behaviours. The more insect species studied, the more information is gathered and the more consistent knowledge about common and particular behaviours in host plant finding is acquired. Before this study was started, no information on host plant finding by the SPB had been published.

[1] Some of the results in this chapter were presented at a conference. See Published paper 1

Knowing that the information from olfactory and visual stimuli is that which can be accessed by host-seeking phytophagous insects over relatively long distances and, having demonstrated that the SPB was attracted to lay eggs on sweet potato plants in a wind tunnel (Chapter 3), it was decided to examine the role of host plant olfactory and visual stimuli in attracting the SPB.

4.2 Materials and Methods

4.2.1 Materials

Apart from the muslin and glass-screens used to offer sweet potato plant volatiles alone and sweet potato plant visual stimuli alone respectively, other materials used were the same as described in section 3.2 of chapter 3. The muslin-screen was made of a white muslin fabric attached to a wooden rectangular frame, 2 m long and 1 m high. When it was needed, it was fitted between sections 6 and 5 of the experimental area of the wind tunnel. The role of the muslin fabric was to hide the visual structures of sweet potato plants but at the same time allow a free air flow. Conversely, the role of the glass-screen was to prevent the volatiles emanating from sweet potato plants from reaching the butterflies but allowing them at the same time to see sweet potato visual structures. The dimensions of the glass-screen were 30 × 60 × 30 cm and made of clear glass (Aquarium type). The glass-screen was placed in the middle of section 6 to cover the treatments when it was needed.

4.2.2 Methods

The bioassay was a 2 × 2 factorial experiment. The two factors were: sweet potato plant olfactory stimuli (muslin-screened plants) and sweet potato plant visual stimuli (glass-screened plants) with two levels for each factor (presence or absence). For each treatment, three pots were placed at about 0.25 m from the upwind wall of the tunnel and arranged as described in point 3.2.2 of chapter 3. Individual, two-day-old naive and mated female butterflies were introduced onto the releasing point. The main behavioural events recorded were resting, walking, flying and landing on the treatment for a total observation time of 30 minutes per individual butterfly. The positions of butterflies in the wind tunnel were also recorded. Twenty different

butterflies were observed per treatment. Treatments were randomized within a day. The observations were made between 10h 30 min and 17h00 min; and the mean wind speed and temperature were respectively 28.2 cm/s (± 0.3) and 28.5 °C (± 0.1).

The statistical analysis was carried out using Genstat 5, Release 3.2 (PC/Windows/Win32s) for the analysis of variance of the average distances moved by butterflies towards treatments and the time allocated to resting and moving (walking + flying) (Appendix 4.1). A randomisation test was carried out to confirm the effects of treatments (Appendix 4.2). The normality of the data was checked using the Genstat command: DAPLOT fitted,normal,halfnormal,histogram. The data on the number of butterflies landing were analysed as proportions landing and not landing using a Chi-square test with MINITAB for Windows. The data on the number of landings did not require statistical analysis.

4.3 Results and Discussion

4.3.1 Results

Both host plant olfactory cues, visual cues and their interaction had significant effects on the mean average distances moved by butterflies for the first ten minutes of the observation time. Olfactory cues had very highly significant positive effects on the distances moved by butterflies ($P < 0.001$, Table 4.1) whereas the visual cues and the interaction between olfactory and visual cues had highly significant negative effects ($P < 0.01$, Table 4.1). In the second 10 minutes of the observation time, the effect of olfactory cues was still very highly significant and positive ($P < 0.001$, Table 4.2) while only the interaction between olfactory and visual cues had significant but negative effects on the distances moved by butterflies ($P< 0.05$, Table 4.2). In the last ten minutes of the observation time, only the effect of olfactory cues remained very highly significant and positive ($P < 0.001$, Table 4.3). However the trend of the effects of visual cues and consequently that of the effects of the interaction remained negative (Table 4.3).

The randomisation test which used the grand means of the experimental data to randomly build a larger random sample of N= 1000 which had the same means (i.e. grand means) revealed that throughout the observation time (1-10, 11-20 and 21-30 minutes), the mean average distances moved by butterflies in the presence of host plant olfactory cues were outside the 1000 numbers drawn randomly (Table 4.4). The same test revealed that it was only during the first 10 minutes of the observation time when the mean average distances moved in the presence of host plant visual cues and consequently the interaction were found to be equal to or smaller than ten out of the thousand numbers of the random sample (Table 4.4).

The differences between the proportions of butterflies that landed on different treatments was very highly significant ($\chi^2_{df=3} = 22.07$, $P < 0.001$). There was a very highly significant difference between the number of butterflies landing in response to olfactory cues than landing in response to visual cues ($\chi^2_{df=1} = 13.73$, $P < 0.001$). The Chi square test revealed also a significant difference between the number of butterflies that landed in response to olfactory cues and the interaction between olfactory and visual cues ($\chi^2_{df=1} = 6.065$, $P < 0.05$). Figure 4.1 and Figure 4.2 show that both the percentage of landed butterflies and the mean number of landings per butterfly on olfactory cues were very high compared to visual cues and the interaction of the two factors.

Table 4.1 Effects of host plant olfactory cues, visual cues and their interaction on the average distances (cm) moved by butterflies in a wind tunnel for the observation period from 1-10 minutes

Olfactory cues	Visual cues		Olfactory cues means (cm)
	No	Yes	
No	24.5	25	24.8
Yes	78.1	39.1	58.6
Visual cues means	51.3	32	41.7
Effects (± SE)			
Olfactory cues effect	33.8 ± 6.9 (n=40)		
Visual cues effect	-19.3 ± 6.9 (n=40		
Olfactory × Visual cues effect	-39.5 ± 13.9 (n =20)		

Table 4.2. Effects of host plant olfactory cues, visual cues and their interaction on the average distances (cm) moved by butterflies in a wind tunnel for the observation period from 11-20 minutes

Olfactory cues	Visual cues		Olfactory cues means (cm)
	No	Yes	
No	29.6	36.2	32.9
Yes	100	67.6	83.8
Visual cues means	64.8	51.9	58.4
Effects (± SE)			
Olfactory cues effect	50.9 ± 8.7 (n=40)		
Visual cues effect	-12.9 ± 8.7 (n=40)		
Olfactory × Visual cues effect	-39.0 ± 17.4 (n=20)		

Table 4.3 Effects of host plant olfactory and visual cues on the average distances (cm) moved by butterflies in a wind tunnel for the observation period from 21-30 minutes (n = 40)

Treatments	Mean average distances (cm) moved towards treatments	Treatment effects (±SE)
No olfactory cues	39.8	
Olfactory cues	91.3	51.5 ± 9.0
No visual cues	69.5	
Visual cues	61.6	-7.9 ± 9.0

Table 4.4 Results of a randomisation test using the means of the main effects and interactions for the average distances moved by butterflies in a wind tunnel

Observation period	i for olfactory cues	i for visual cues	i for interaction
1-10 minutes	1000/1000	990/1000	992/1000
11-20 minutes	1000/1000	914/1000	964/1000
21-30 minutes	1000/1000	756/1000	815/1000

i = the probability of getting a real treatment effect (which is not due to random variation).

Host plant olfactory cues had a significant negative effect on the mean time butterflies spent resting ($P < 0.05$, Table 4.5). Consequently, they had a significant positive effect on the mean time butterflies allocated to moving (walking + flying) ($P < 0.05$, Table 4.6).

Table 4.5 Effects of host plant olfactory and visual cues on the time of resting (min) of butterflies in wind tunnel (n = 40).

Treatments	Mean time (min) spent resting	Treatment effects (± SE)
No olfactory cues	22.62	
Olfactory cues	18.92	-2.70 ± 1.57
No visual cues	20.60	
Visual cues	20.95	0.35 ± 1.57

Table 4.6 Effects of host plant olfactory cues and visual cues on the time of movement (min) (walking + flying) of butterflies in wind tunnel (n = 40)

Treatments	Mean time (min) spent moving	Treatment effects (± SE)
No olfactory cues	6.85	
Olfactory cues	10.17	3.32 ± 1.40
No visual cues	9.40	
Visual cues	7.63	-1.77 ± 1.40

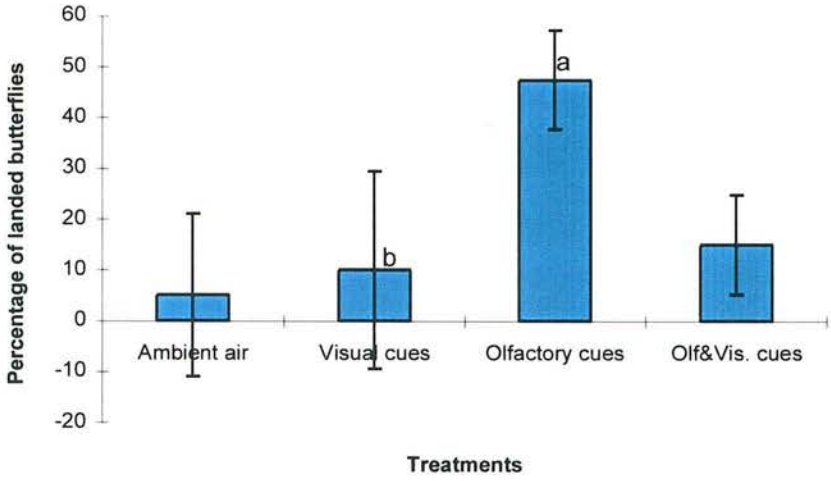


Figure 4.1 Landing of female *Acraea acerata* in response to the presence of host plant olfactory cues, visual cues and their interaction (olf&vis.cues) in a wind tunnel bioassay (There are statistically significant differences ($P < 0.05$) between percentages of landed butterflies on treatments with different letters; 95% confidence intervals are shown).

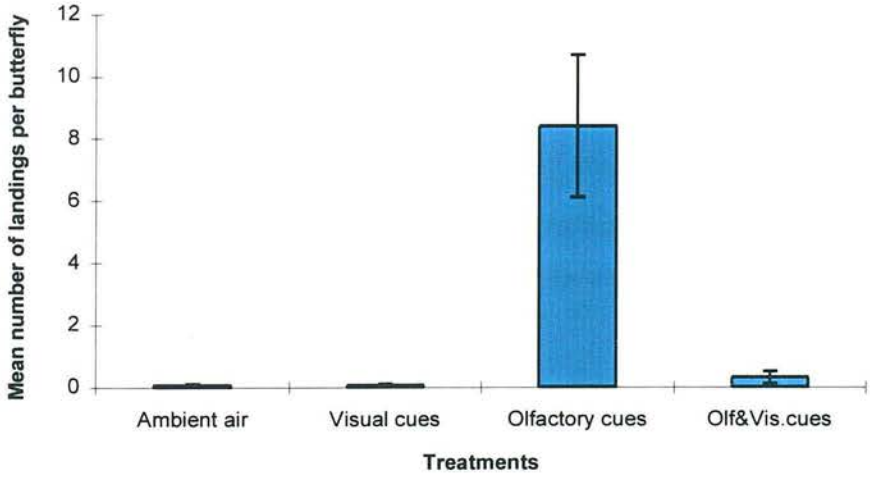


Figure 4.2 Mean landings (± SE) of female *Acraea acerata* in response to the presence host plant olfactory cues, visual cues and their interaction (olf&vis.cues) in a wind tunnel bioassay (n = 20).

4.3.2 Discussion

The analysis of variance of the average distances (cm) moved by sweet potato butterflies towards the treatments suggests a strong effect of sweet potato volatiles ($P < 0.001$, Table 4.1, Table 4.2, Table 4.3) in attracting the butterflies towards their host plant. The effect of visual cues ($P < 0.01$, Table 4.1) and subsequently the effect of the interaction of both visual and olfactory cues ($P < 0.01$, Table 4.1) on movement towards the host plant seemed to be negative for the first 10 minutes of the observation period. The negative trend of the effect of visual cues and hence the effect of interaction between olfactory and visual cues (significant interaction effect for the period 11-20 minutes, $P < 0.05$, Table 4.2) has been consistent during the whole observation period (Table 4.3).

The effect of host plant olfactory cues in attracting the SPB was supported by the time butterflies allocated to 'searching' (walking and flying) in the presence of sweet potato plant volatiles ($P < 0.05$, Table 4.5, Table 4.6). This was further confirmed by the high percentage of landed butterflies and the mean number of landings per butterfly in the presence of sweet potato plant volatiles alone compared to visual stimuli alone or the interaction of olfactory and visual stimuli (Figure 4.1, Figure 4.2). The results strongly suggested that female sweet potato butterflies are stimulated by host plant volatiles to orient and move upwind towards their host plants. The attractive effect of sweet potato odours was confirmed by the results of the randomisation test (Table 4.4).

Lepidoptera are said to be able to discriminate colours and to use visual stimuli to locate their host plants (Bernays and Chapman, 1994). This is supported by many experimental proofs like the cases of *P. demoleus*, *P. rapae*, *Heliconius* and *Battus* butterflies, and *P. xuthus* (Rausher, 1981, Prokopy and Owens, 1983, Saxena and Goyal, 1978, Chew and Robbins, 1989, Hern, 1997, Kinoshita *et al.*, 1999). Evidence for the widespread use of plant odours in host plant finding by Lepidoptera is also accumulating (Ramaswamy, 1988, Feeny *et al.*, 1989, Hern, 1997, Khan *et al.*, 1997a, Khan *et al.*, 1997b). Thus, it would be reasonable to expect, when both host

plant olfactory and visual stimuli are simultaneously offered to a lepidopteran insect, a response at least equal to the more predominant stimulus as in the case of *P. demoleus* (Saxena and Goyal, 1978). The SPB, however, did not respond to the interaction of olfactory and visual stimuli according to that expectation. Visual cues seemed to have a negative effect on the response of SPBs to sweet potato olfactory stimuli. But this was not true because, as discussed in Chapter 3, female SPBs used host plant visual structures to land at the time of egg-laying. One might therefore argue that, in this bioassay, the SPB could have been able to use vision to locate its host plants in the wind tunnel at the releasing point (as there was less than 1.5 m between the releasing point and the experimental plants (Figure 3.1). This might have stopped the butterflies from moving closer to their host plants unless it was time to lay eggs as discussed in the previous chapter. Conversely, when butterflies were offered host plant volatiles alone, they could not see the plants and could not know at what distance they were located. Consequently, they would orient to the source of the volatiles and move upwind towards the volatile sources (Kennedy, 1977). This might explain why there was a very highly significant effect of olfactory stimuli (distance moved, time of searching and landing) as butterflies seemed to try to reach a point where they could see and identify their host plants. This strongly suggested that female SPBs use host plant volatiles to orient to the volatile sources from a long distance and move upwind to locate the source of the volatiles.

Unlike some other butterflies like *Pieris* sp. in which the use of vision appeared to be predominant in the pre-contact phase of host-finding and alightment (Chew and Renwick, 1995), the SPB seemed to use olfaction as the predominant sensory modality in its host plant finding. Purseglove (1968) described the leaves (the most visible parts of the plant) of sweet potato (the main host plant for the SPB) as 'very variable, even on the same plant; lamina mostly ovate in outline, entire to deeply digitately-lobed, base usually cordate in first leaves, tip acute or obtuse, glabrous or with variable pubescence, 5-15 × 5-15 cm, green to purple in colour, sometimes with purple stain at base; veins palmate, green or purple beneath'. With such variability in shape, size and colour of the leaves of sweet potato plants, it appears that though the

detectability of sweet potato plant visual stimuli might be high for butterflies which are said to have true colour vision, the reliability of host plant visual stimuli in finding the host is very low. Vet and Dicke (1992) and Vet *et al.* (1995) noted the same problem of reliability-detectability with long-distance host searching by parasitoids. The stimuli from herbivores (hosts for parasitoids) are highly reliable but less detectable (because stimuli will be lost in the wider complex surroundings) by foraging parasitoids and the stimuli from the food of hosts (plants) are highly detectable but less reliable (because of variation in plant volatiles and presence of food does not mean the herbivore is also present). This dilemma is solved by parasitoids which learn to associate the hosts with the food of their hosts through the use of the 'herbivore-induced plant volatiles' as olfactory stimuli. The herbivore-induced plant volatiles combined the high detectability of plant volatiles and the high reliability of herbivore volatiles. By analogy with the long-distance host-finding by parasitoids which used herbivore-induced plant volatiles, it is presumed that sweet potato plant volatiles might have specific reliable volatiles which are followed by the SPB up to near their source where the volatiles are associated with host plant visual stimuli. Vision of the SPB has most probably an important role in landing on host plants.

4.4 Conclusion

The results of the bioassay revealed a very strong effect of host plant volatiles in attracting the SPB to its host plants. It appeared that, from a long distance, host plant volatiles might be playing a predominant role in leading the SPB closer to its host plants. At that stage of host plant finding, host plant visual stimuli seemed to affect negatively the movement of the SPB towards its host plants. It would now be interesting to investigate how female SPBs respond to the presence of volatiles collected from host plants.

Chapter 5

Attractiveness of host plant volatiles to *Acraea acerata*

Chapter 5 Attractiveness of host plant volatiles to *Acraea acerata*[2]

5.1 Introduction

The response of phytophagous insects to plant volatiles has been largely documented (Visser, 1986). In particular, lepidopteran insect species have been found to respond positively to common green leaf volatiles as well as to some specific chemical compounds of their host plant volatiles. Lecomte and Thibout (1981) showed that the leek moth *Acrolepiopsis assectella* (Yponomeutidae) was attracted by volatile compounds from *Allium porrum*, a host plant. More evidence for host plant volatile attraction to moths was gathered by Ramaswamy (1988): European corn borer, *Ostrinia nubilalis* (Pyralidae), the cabbage semilooper *Trichoplusia ni* (Noctuidae), *Cidalia albulata* (Geometridae), *Antheraea pernyi* (Saturniidae), the cotton leafworm *Spodoptera littoralis* (Noctuidae), *Hypsipyla grandella* (Pyralidae) and the potato tuber moth *Phthorimaea operculella* (Gelechiidae) are some examples of moths reported to be attracted by host plant volatiles or some of the specific chemical compounds of the host plant volatiles.

In butterflies, *Papilio demoleus* (Papilionidae), the citrus butterfly increased its level of response to host plant visual stimuli in the presence of host plant volatiles (Saxena and Goyal, 1978). The black swallowtail butterfly *Papilio polyxenes* (Papilionidae) responded to the presence of host plant volatiles from carrot leaves by increasing their landing rates and the number of eggs laid on model plants (Feeny *et al.*, 1989). The small white butterfly *Pieris rapae* (Pieridae) responded to its host plant volatiles by moving upwind and flying more often (Hern, 1997).

A previous bio-assay using screened sweet potato plants had suggested that the SPB was mostly attracted to its host plant by host-volatiles (Chapter 4). Plant volatiles which affect the behaviour of phytophagous insects are mostly emitted by intact plant organs mainly leaves, fruits and flowers. In chemical ecology, headspace volatile

[2] Some of the results in this chapter were presented at a conference. See Published paper 2.

sampling and analysis are commonly used. Comparing different methods of headspace sampling, Agelopoulos and Pickett (1998) found that, in addition to being able to collect all the chemical compounds present in the headspace sample as with the other methods, the sampling method which uses porous polymers as volatile traps with solvent desorption had the advantages of resulting in liquid samples which can be used for quantitative chemical analysis (addition of an internal standard) and/or can be stored and used as needed in other studies such as electrophysiological studies (GC-EAD: coupled gas chromatography-electroantennographic detection) and bioassays. Thus, the headspace sampling using porous polymer with solvent desorption was chosen to collect sweet potato plant volatiles to use in bioassays and identification of sweet potato volatile compounds.

The aim of the bioassay was to confirm or not the attractiveness of sweet potato plant volatiles to the SPB by studying the behaviour of female butterflies in the presence of headspace sweet potato plant volatiles in a wind tunnel. Using coupled gas chromatography-mass spectrometry (GC-MS) method, the main chemical compounds in sweet potato plant volatiles were identified and some were used for electroantennogram (EAG) recordings.

5.2 Materials and Methods

5.2.1 Materials

Apart from the materials used to collect sweet potato plant volatiles, GC-MS and EAG recordings, all other materials were described in Chapter 3 (section 3.2). Figure 5.1 shows a simplified diagrammatic representation of the entrainment system used to collect plant volatiles and Figure 5.2 shows the set up for recording EAGs. The chemicals used to record electrophysiological responses of SPBs were *cis*-3-hexen-1-ol, ethylbenzene, (-)-*trans*-caryophyllene and (+)-3-carene. Mineral oil was used as a solvent. All these chemicals were ordered from Sigma-Aldrich Chemical Co. Ltd, Gillingham, UK).

5.2.2 Methods

5.2.2.1 Volatile collection

Sweet potato plant volatiles used in this bioassay were collected using the headspace entrainment system similar to that described by Robertson, Griffiths, MacFarlane-Smith and Butcher (1993). Ambient air was drawn by a vacuum pump (Pump B85 SE, Ref. X41/215, Charles Austin Pumps, 100 Royston Road, Weybridge, surrey KT14 7PB, UK) through stainless steel puritubes filled with activated charcoal (to clean air from odours) (Cat. No. 900058, Phase Separations Ltd, Deeside Industrial Estate, Clwyd CH5 2NU, UK) and molecular sieve (to regulate the humidity of the air) (Cat. No.900046, Phase Separations Ltd, Deeside Industrial Estate, Clwyd CH5 2NU, UK) into a 2 litre airtight flask. The flask contained vine cuttings of sweet potato plants placed in water in a small glass container. Six to eight week old plants were used. A glass column filled with 0.3 g of Tenax Ta ([C6H4 0]X: poly (2,6-diphenyl-p-phenylene oxide)), a porous polymer, used as a volatile trap was placed at the exit port of the flask to collect volatiles given off by the sweet potato plants. The column was connected to an air flow meter (Platon Air Products, UK) and set at 300 ml/min.

The Tenax-Ta was conditioned by passing through the column 3.5 ml (2 volumes of the column) of diethyl ether (HPLC grade). The columns were then dried by passing through clean dry air for 30 minutes. The columns were heated in an oven at 180 °C (heating rate: 8 °C/minute) for 3 hours with a stream of helium (BOC grade A) passing through at a rate of 20 ml/minute. To eliminate odour contamination, Teflon (PTFE) tubing was used and the sealing was done by a Teflon tape (PTFE tread seal tape, BS 7786: 1995 Grade L). The collection was done for 24 hours with a photoperiod of L16:D8 under sodium light (same conditions that existed in the glasshouse where the plants were grown). After 24 hours, the columns were removed and the trapped volatiles were eluted with 3.5 ml of diethyl ether in a glass sample tube in a bath of ice/methanol.

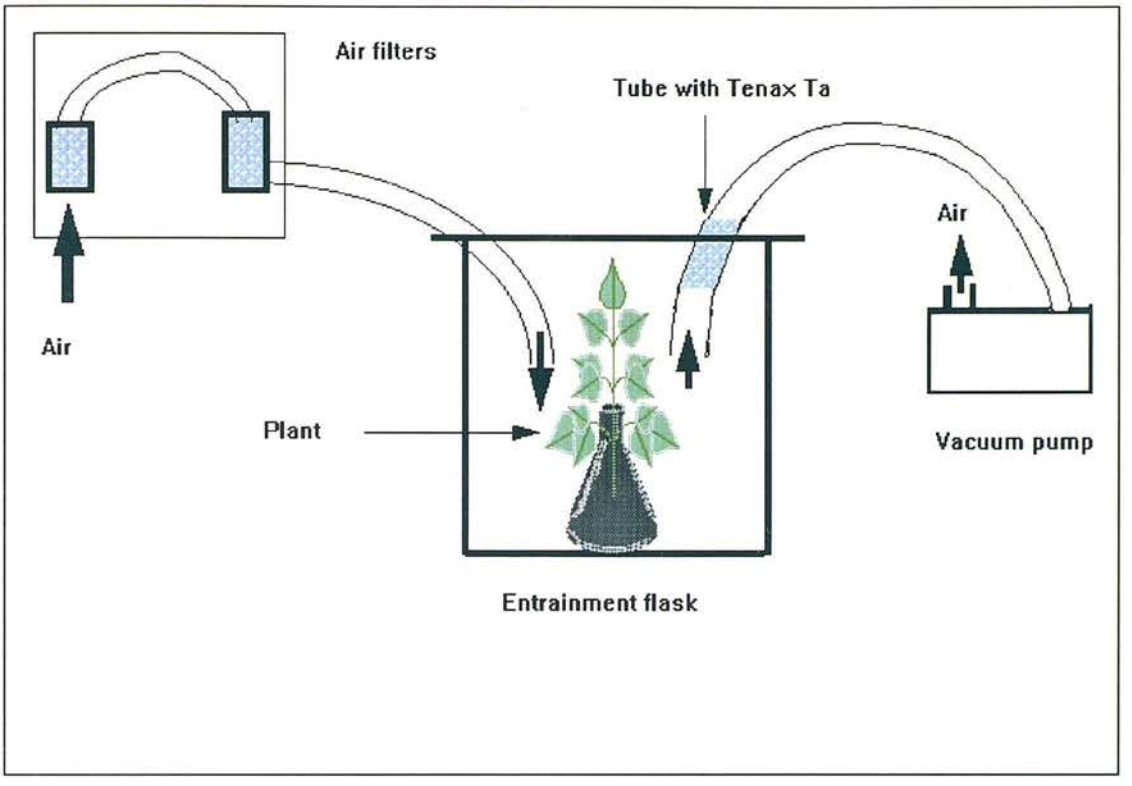


Figure 5.1 Simplified diagrammatic representation of the headspace entrainment system

The volatiles were diluted to 1 gle (gram leaf equivalent) before being used in bioassays. The Tenax-Ta columns used for identification of volatile chemical compounds by GC-MS were put in an airtight plastic container and kept at –20 °C in a freezer until they were sent for analysis. Four samples were analysed.

5.2.2.2 Wind tunnel bioassay

The assay was carried out in the same way as described in chapter 3. Treatments were first introduced into the experimental area of the wind tunnel 3-5 minutes before the butterflies were released. There were 20 butterflies per treatment. There were three treatments: ambient air, solvent alone (diethyl ether) and solvent + sweet potato volatiles. A 50 ml vial with a wick placed in the middle of section 6 of the wind tunnel (Figure 3.1) behind a muslin screen was used to offer the solvent and the

sweet potato volatiles to butterflies. Individual two day old naive and mated female butterflies were released onto a thread through a small window at a height of 35 cm in the downwind wall of the tunnel and observed for a 30 minute period. The main behavioural events recorded were resting, walking, flying and landing on the treatment. The positions of butterflies along the length of the wind tunnel were also recorded. The bioassay was carried out during the day between 10h30 min and 17h00 min with a mean wind speed of 26.91 cm/s (± 0.20) and a mean temperature of 29.3 °C (± 0.1).

5.2.2.3 Identification of the main chemical compounds of sweet potato plant volatiles (see Acknowledgements)

Volatiles were eluted from the Tenax-Ta column using 1000 µl of diethyl ether (GC Grade: purity > 99.5%) with 5-10 ppm butylated hydroxy toluene as a stabiliser. Samples were stored in glass vials with a PTFE-silicon septa at -20 °C until the analysis was carried out. The instrument used was a Hewlett Packard gas chromatograph (HP 6890 + Series) fitted with an auto-sampler (HP 7683 series) and mass selective detector (HP 5973). The column used was a Hewlett Packard HP 1 (cross linked methyl silicone gum) column length 30 m; id 0.25 mm; film thickness 0.25 mm; and phase ratio 250. The GC was run in constant pressure mode with an injection pressure of 9.00 torr. This pressure produced a column flow of helium (Purity 99.999%) of 1.1 ml/min at 50 °C. The injector was set at 250 °C; splitless, and the oven held at 50 °C for 2 minutes and then the temperature was increased by 5 °C per minute up to 300 °C (held for two minutes). The column effluent was transferred to the GC-MS detector via a transfer line (280 °C). The mass spectrometer used was a Hewlett Packard (HP 6795). The MS detector was used in the scan mode. The scan parameters were: low mass 25 and high mass 750 with the threshold set at 150. The MS quad was operated at 150 °C and the MS source at 230 °C. Putative identifications were made by searching the National Institute of Standards and Technology mass spectra library (NIST 98) and a user created mass spectra library from known compounds.

5.2.2.4 EAG recordings (see Acknowledgements)

The chemicals used for EAG recording (*cis*-3-hexen-1-ol, ethylbenzene, (-)-*trans*-caryophyllene and (+)-3-carene) were among the main chemical compounds identified from sweet potato headspace volatiles (Figure 5.11) which were commercially available. For each of the four sweet potato volatile chemical compounds tested, three different concentrations were used: 0.1, 1 and 10%. Light mineral oil (paraffin oil) was used as a solvent to prepare the chemicals at different concentrations. At each concentration, ten two-day-old butterflies of each sex were used to record their EAG responses to the test chemicals.

To prepare the butterfly's antennae for EAG recording, the head of the butterfly was excised and placed on a 1.5 mm diameter glass electrode (Haematocrit tubes, Denly Instruments, Daventry, UK) partly filled with the electrolyte Beadle-Ephrussi ringer solution [made of 7.5 g NaCl, 0.35 g Kcl and 0.29 g $CaCl_2.H_20$ in 1 litre of distilled water] (Ephrussi and Beadle, 1936). A silver wire (o.d. 0.5mm, FSA Laboratory Supplies, Loughborough, Leicestershire, UK) was inserted through the electrode holder into the glass electrode to connect the preparation to the recording instruments. A recording electrode was prepared in the same way as the indifferent electrode on which the head (with antennae) of the butterfly was placed. Two micromanipulators (Gallenkamp, Loughborough, Leicestershire, UK) secured to a metal plate by magnetised base were holding the electrodes in place.

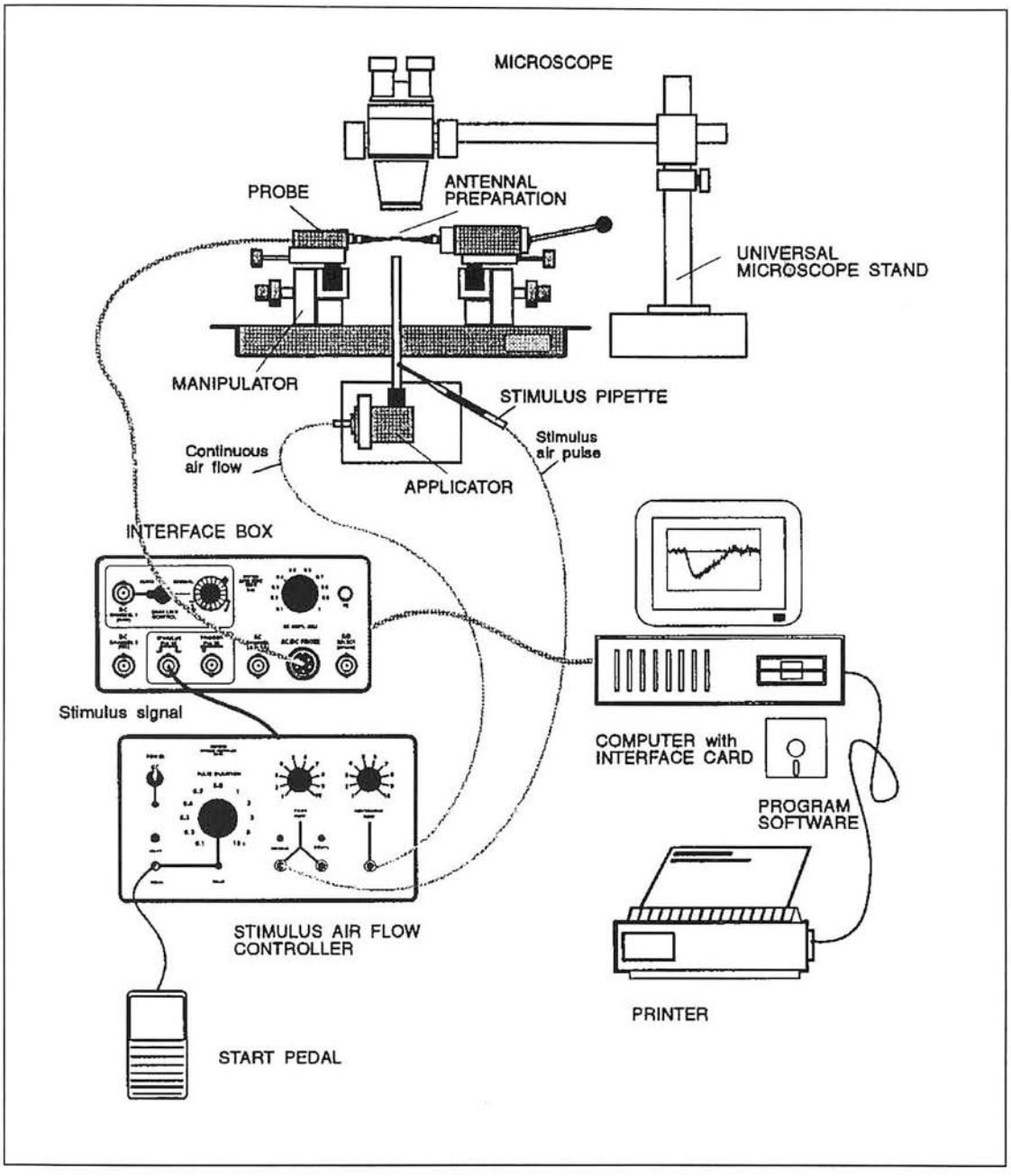


Figure 5.2 EAG recording system (modified from Syntech, 1996)

The recording electrode was connected to a P-01 universal probe joined to the AM-05 EAG amplifier (Synchem, Hilversum, The Netherlands). Electrography (Synchem, Hilversum, The Netherlands), a Windows software package running on an IBM computer connected to the amplifier allowed the display of the antenna's response on the screen of the PC monitor. To establish an electric circuit, the

recording electrode was manoeuvred to touch one of the butterfly's antennae and the signal was displayed on the PC monitor screen.

Test chemicals were carried by air delivered from a metal tube (o.d. 1cm) positioned at 0.5 cm from the antenna. The air which was flowing over the mounted antenna at a rate of 600 ml/min was filtered through activated charcoal which retained any kind of odours found in the ambient air (Stimulus Controller C5-05/b, Syntech, Hilversum, the Netherlands). 10µl of the test chemical was placed on to a glass microfibre filter paper (GF/C, Whatman, Maidstone, Kent, UK) cut into pieces of about 2 ×1 cm each and inserted into a 5ml disposable glass Pasteur pipette (John Poulton Ltd, Barking, Essex, UK). After leaving the pipette for 30 seconds (to allow the test chemical to evaporate and equilibrate), it was connected to the air pump by rubber tubbing with its tip inserted into a hole in the air delivery metal tube. By depressing the start pedal (Figure 5.2) for one second, a pulse of air at 600 ml/min was redirected down the Pasteur pipette over the antenna. The response of the antenna was automatically recorded and stored on the computer. At each stimulation, a fresh filter paper and a fresh pipette were used.

Within a sequence, a chemical test to start with was selected randomly but it had to meet the criterion of provoking a substantial EAG response. At 10% concentration (high concentration) the choice of a standard was between *cis*-3-hexenol, ethylbenzene and 3-carene and, at 1 and 0.1% (low concentration) only *cis*-3-hexenol met the above criterion of being used as standard. The sequence of test chemicals was randomly selected. EAG amplitudes were recorded and the relative amplitudes were compared to test for any amplitude differences by test chemical, sex or concentration.

To correct data for antennal fatigue and subsequent drop of EAG response over time, EAG recorded responses to the test chemicals were adjusted using the percentage decline at each stage of the sequence (obtained by substracting the EAG response to the final standard stimulus from the EAG response to the initial standard stimulus,

divided by the number of test chemicals in the sequence) as shown in the formula below (Brockerhoff and Grant, 1999).

$C = E/(1-rk)$

C = Corrected EAG value (mV)

E = Recorded EAG value (mV)

$k = (Si-Sf/Si)\times 1/n$

Si = EAG value for the standard at the beginning of the sequence

Sf = EAG value for the standard at the end of the sequence

r : rank of the test chemical in the sequence (r = 1....n)

n = number of test chemicals in the sequence (including the solvent)

Initial standard does not need to be corrected.

When the final standard initiated a larger response than the initial standard, there was no need to adjust the data as it was assumed that there had been no decline in the antenna's responses over time. Absolute corrected EAGs were calculated by substracting the corrected control response from the corrected responses acquired from the test chemicals to negate the effect of the control on the EAGs. An example of one sequence of recording is given below (Table 5.1).

Table 5.1 Sequence of test chemicals

1.Initial standard (e.g *cis*-3-hexenol)

2. Control (mineral oil)

3. Test stimulus (e.g. 3-carene)

4. Test stimulus (e.g. *trans*-caryophyllene)

5. Test stimulus (e.g. ethylbenzene)

6. Standard (*cis*-3-hexenol)

5.2.2.5 Statistical analyses

Data on the average distances moved by butterflies towards treatments (calculated as in Chapter 3), the time allocated by butterflies to resting, moving (walking + flying),

the number of landed butterflies and the number of landings per landed butterfly were analysed using Genstat 5 Second Edition (for Windows (Genstat 5, Release 3.2 (PC/Windows/Win32s), Copyright 1995, Lawes Agricultural Trust (Rothamsted Experimental Station)). The analysis of variance was perfomed as follows: General Analysis of Variance; BLOCK: "No Blocking"; TREATMENTS: Treatments; COVARIATE: "No Covariate"; ANOVA: [PRINT = aovtable, information, effects, mean; FACT = 3; FPROB = yes; PSE = diff, means]. The normality of the data was checked using the Genstat command: DAPLOT fitted,normal,halfnormal,histogram. Time spent by butterflies in different sections of the experimental area of the wind tunnel was analysed using the χ^2 test. The sections S1 + S2, S3 + S4 and S5 + S6 were arbitrarily called downwind, middle and upwind sections respectively.

Concerning EAG data, at each concentration, mean EAG responses of male and female SPBs to test chemicals were compared to the response to the control using paired t-test (Minitab for windows, Release 11.1) when the conditions of normality and similar variance were met. The normality of the data was tested using the Anderson-Darling test for normality (Minitab for windows, Release 11.1) and the table of *F*-distribution was used to test the similarity of variance between two samples (Fowler, Cohen and Jarvis, 1998). When the conditions of using a t-test were not met, the Wilcoxon test for matched pairs was used (Fowler *et al.*,1998).

The ANOVA using Genstat 5 Second Edition (as above) on log-transformed data [log10(data + 1)] was performed to compare EAG response of males and females to each test chemical, different chemical concentrations and their interactions. The analysis was done as follows: Analysis of Variance; BLOCK: "No Blocking"; TREATMENTS: Sex*Concentration; COVARIATE: "No Covariate"; ANOVA: [PRINT = aovtable, information, effects, mean; FACT = 3; FPROB = yes; PSE = diff,lsd,means] test chemical. The normality of the data was checked using Genstat command: DAPLOT fitted,normal,halfnormal,histogram.

5.3 Results and Discussion

5.3.1 Results

5.3.1.1 Wind tunnel bioassay

The mean average distances moved by butterflies towards treatments were not statistically different between different treatments ($P > 0.05$). However there was a consistent trend for female SPBs to move closer to the source of sweet potato plant volatiles than that of ether or ambient air during the whole time of the observation period. Moreover, there was also a consistent trend for female SPBs to move closer to the source of ether than that of ambient air (Figure 5.3, Figure 5.4, Figure 5.5).

There was a very highly significant difference between the time butterflies spent resting or moving (walking + flying) in the presence of sweet potato plant volatiles compared to solvent alone (ether) ($P< 0.001$, Figure 5.6, Figure 5.7). Furthermore, in the presence of ambient air or solvent alone, butterflies spent more than 50% of their time in the downwind section whereas in the presence of sweet potato plant volatiles, they spent about 70% of their time in the middle and upwind sections (Figure 5.8; $\chi^2_{df=4} = 167$, $P < 0.001$). Furthermore, there was a highly significant difference between the proportion of landed butterflies in the presence of sweet potato plant volatiles compared to solvent alone ($P < 0.01$, Figure 5.9). There was also a very highly significant difference between the mean number of landings per landed butterfly in presence of sweet potato plant volatiles compared to solvent alone ($P = 0.001$, Figure 5.10).

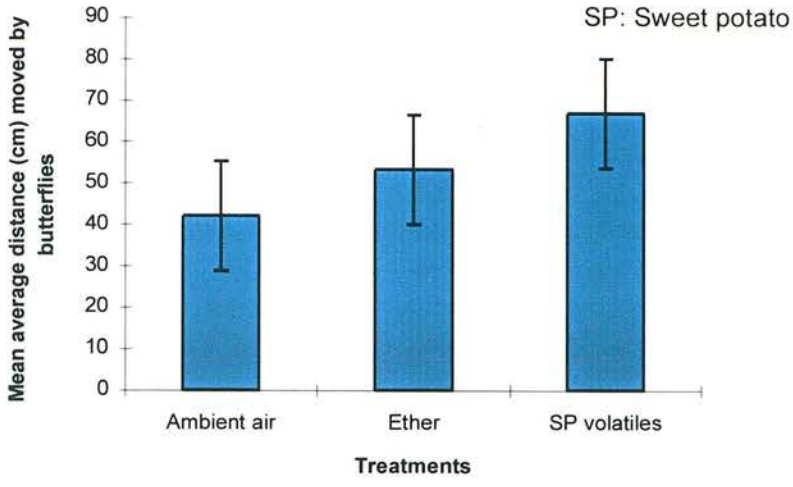


Figure 5.3 Mean average distance (cm) (± SED) moved by female *Acraea acerata* towards the sources of different volatiles in a wind tunnel (n = 20) during the time 1-10 minutes of the observation period

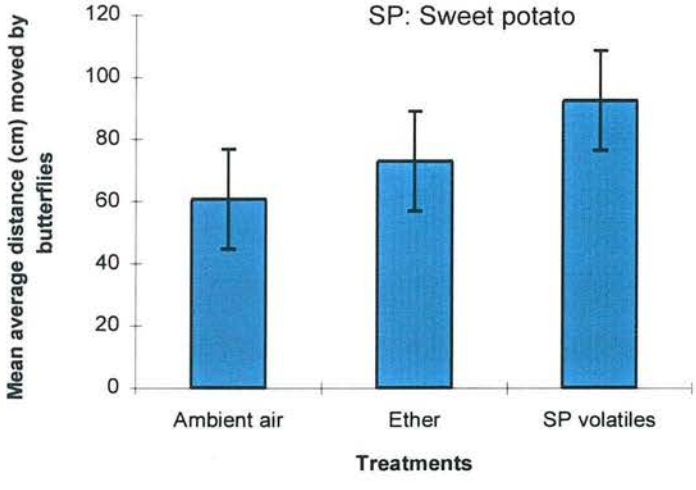


Figure 5.4 Mean average distance (cm) (± SED) moved by female *Acraea acerata* towards the sources of different volatiles in a wind tunnel (n = 20) during the time 11-20 minutes of the observation period.

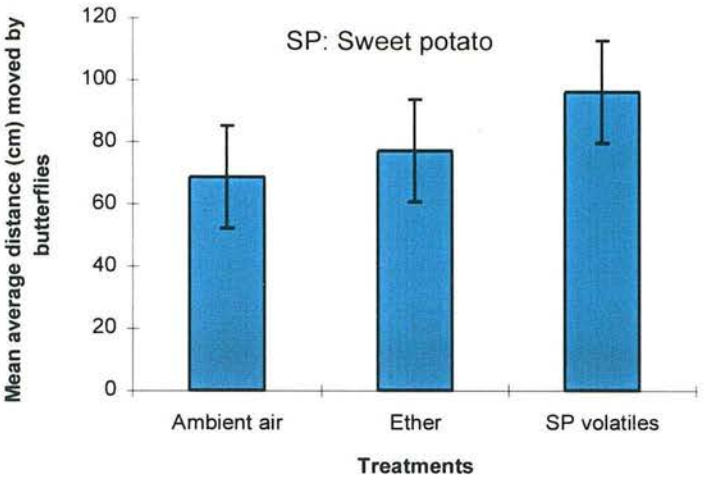


Figure 5.5 Mean average distance (cm) (± SED) moved by female *Acraea acerata* towards the sources of different volatiles in a wind tunnel (n = 20) during the time 21-30 minutes of the observation period.

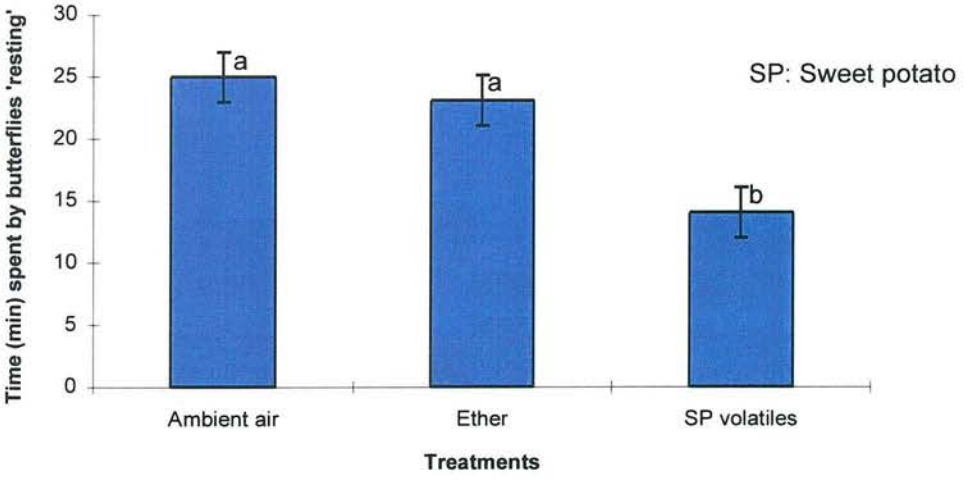


Figure 5.6 Comparison of the mean average time (min) (± SED) allocated to resting by butterflies in presence of different treatments (n = 20). Means with different letters have statistically significant differences ($P < 0.001$)

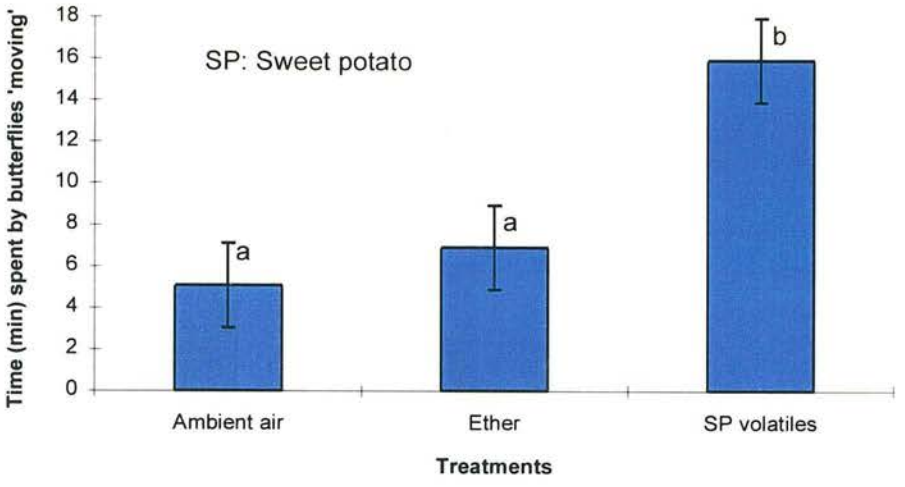


Figure 5.7 Comparison of mean average time (min) (± SED) allocated to moving (walking + flying) by butterflies in presence of different treatments (n = 20). Means with different letters have statistically significant differences ($P < 0.001$)

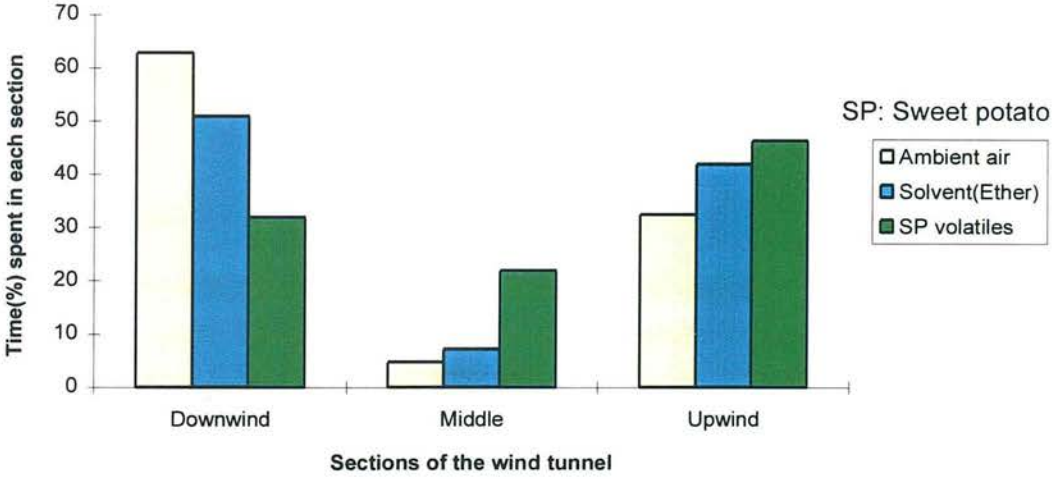


Figure 5.8 Percentage of time butterflies spent in different sections of the wind tunnel in presence of the different treatments (n = 20).

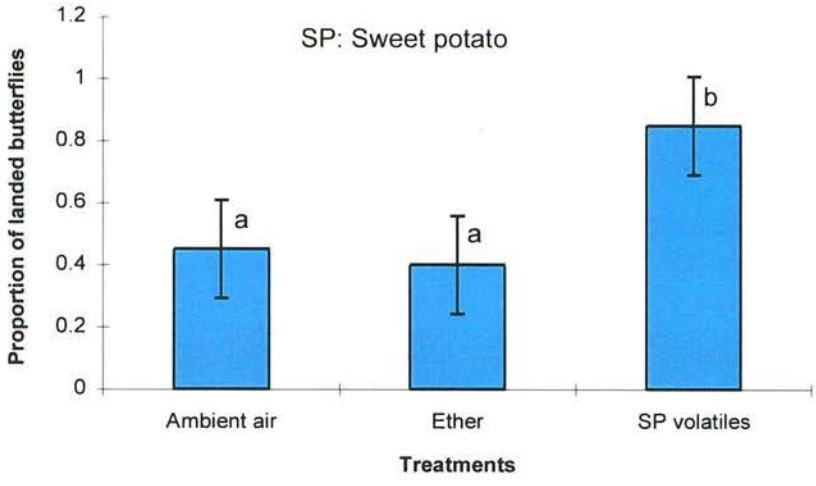


Figure 5.9 Comparison of mean proportions (± SED) of landed butterflies in presence of different treatments (n = 20). Means with different letters have statistically significant differences ($P < 0.01$)

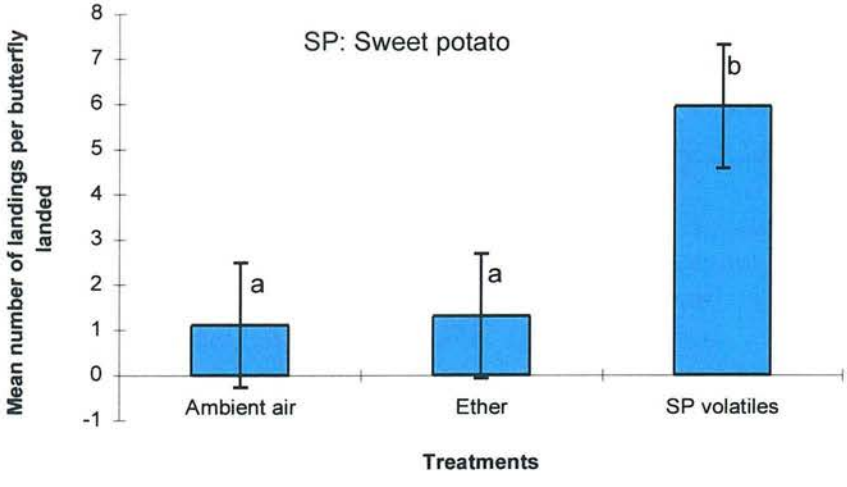


Figure 5.10 Comparison of mean numbers (± SED) of landings per landed butterfly in presence of different treatments (n = 20). Means with different letters have statistically significant differences ($P < 0.01$)

5.3.1.2 Identification of chemical compounds of sweet potato plant volatiles

Sweet potato plant volatile compounds were tentatively identified by coupled GC-MS analysis and they are listed in Table 5.2. The major compounds which include esters, alcohols, terpenoids, hydrocarbons and aromatics are shown in Figure 5.11.

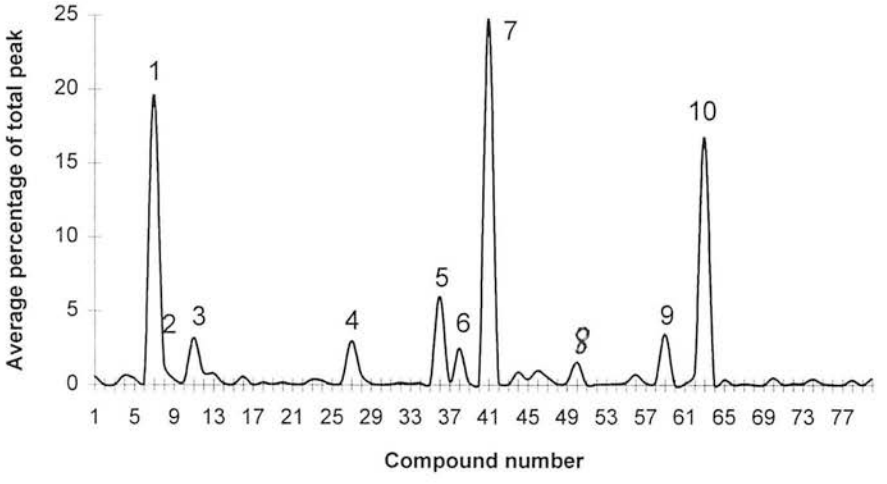


Figure 5.11 Average percentage of total area of GC peaks for compounds detected in headspace samples of sweet potato plants. Compound number corresponds to the compound indicated with the same number in Table 5.2

Peak 1 3-Hexen-1-ol, (Z)- (compound number 7), alcohol
2 Ethylbenzene (compound number 8), hydrocarbon
3 p-Xylene (compound number 11), hydrocarbon
4 3-Hexen-1-ol, acetate, (Z) (compound number 27), ester
5 3-Carene (compound number 36), terpenoid
6 1,6-Octadien-3-ol, 3,7-dimethyl- (compound number 38), alcohol
7 (E)-2- Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester (compound number 41), ester
8 Indole (compound number 50), aromatic
9 (-)Trans-Caryophyllene (compound number 59), terpenoid
10 Butylated hydroxytoluene (compound number 63), added stabiliser.

Table 5.2 Retention time (min), percentage (± SE) of total GC areas for compounds detected in headspace of sweet potato plants and the percentage of matching between the sample and library spectra.

Peak	Compounds[3]	Retention time(min)	Mean % (± SE) of total peak area	Quality of matching (%)
1	Octane	4.42	0.64 ± 0.213 $^{++++}$	72
2	Cyclohexane, 1,2-dimethyl-, trans-	4.47	0.02 ± 0.02 $^{+}$	86
3	2,2'-Bioxirane	4.87	0.0325 ± 0.0325 $^{+}$	28
4	2-Hexenal	5	0.64 ± 0.45 $^{++}$	97
5	Propene	5.06	0.463 ± 0.193 $^{+++}$	9
6	3-Hexen-1-ol, (E)-	5.22	0.11 ± 0.0734 $^{++}$	64
7	3-Hexen-1-ol, (Z)-	5.29	19.64 ± 7.86 $^{++++}$	95
8	Ethylbenzene	5.48	1.425 ± 0.21 $^{++++}$	94
9	2-Hexen-1-ol, (E)-	5.56	0.428 ± 0.164 $^{+++}$	78
10	1-Hexanol	5.64	0.25 ± 0.25 $^{+}$	83
11	p-Xylene	5.69	3.205 ± 0.721 $^{++++}$	97
12	Bicyclo[4.2.0]octa-1,3,5-triene	6.12	0.855 ± 0.052 $^{++++}$	95
13	Benzene, 1,2-dimethyl-	6.24	0.78 ± 0.252 $^{++++}$	94
14	Heptane, 3-methyl	6.85	0.1375 ± 0.0807 $^{++}$	64
15	Benzene, (1-methylethyl)-	7.13	0.0375 ± 0.0375 $^{+}$	90
16	Cyclohexanecarboxaldehyde	7.2	0.57 ± 0.13 $^{++++}$	64
17	Benzoyl isothiocyanate	7.61	0.0325 ± 0.0325 $^{+}$	9
18	Cyclopropene, 1-bromo-2,3,3-trifluoro-	7.67	0.185 ± 0.0644 $^{+++}$	1
19	Benzene, propyl-	7.96	0.05 ± 0.05 $^{+}$	83
20	Benzene, 1-ethyl-2-methyl-	8.18	0.165 ± 0.0974 $^{++}$	90
21	Benzene, 1-ethyl-4-methyl-	8.24	0.05 ± 0.05 $^{+}$	90
22	Benzene, 1,2,3-trimethyl-	8.41	0.0475 ± 0.0475 $^{+}$	9
23	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	8.71	0.378 ± 0.17 $^{+++}$	90
24	Benzene, 1,3,5-trimethyl-	9.13	0.315 ± 0.186 $^{++}$	94
25	1-Hexene	9.21	0.0425 ± 0.0425 $^{+}$	35
26	Acetaldoxime	9.27	0.21 ± 0.146 $^{++}$	9
27	3-Hexen-1-ol, acetate, (Z)-	9.41	3.02 ± 0.691 $^{++++}$	90
28	Heptane, 2,2,4,6,6-pentamethyl-	9.6	0.755 ± 0.0922 $^{++++}$	72
29	Nonane	9.8	0.11 ± 0.0636 $^{++}$	74
30	1H-Pyrrole-2,5-dione	10.03	0.025 ± 0.025 $^{+}$	2

[3] All the compounds detected in more than one sample have the same retention time but in some cases, compounds with the same retention time were identified with different chemical names. In such cases, the name of the compound given in this table is the one with the highest percentage of quality of matching.

Table 5.2 Retention time (min), percentage (± SE) of total GC areas for compounds detected in headspace of sweet potato plants and the percentage of matching between the sample and library spectra (continued).

Peak	Compounds	Retention time(min)	Mean % (± SE) of total peak area	Quality of matching (%)
31	Benzene, 4-ethyl-1,2-dimethyl-	10.1	0.0525 ± 0.0525 $^{+}$	90
32	1-Propanol, 2,2-dimethyl-	10.23	0.15 ± 0.106 $^{++}$	9
33	Oxirane, (2-methylpropyl)-	10.3	0.0575 ± 0.0575 $^{+}$	9
34	R(+)-Limonene	10.39	0.145 ± 0.104 $^{++}$	90
35	Cyclopropene, 1-bromo-2,3,3-trifluoro-	10.63	0.0875 ± 0.0613 $^{++}$	1
36	3-Carene	10.96	6.02 ± 3.29 $^{++++}$	97
37	2,3,4,5-Tetrahydropyridazine	11.06	0.275 ± 0.0312 $^{++++}$	17
38	1,6-Octadien-3-ol, 3,7-dimethyl-	12.38	2.512 ± 0.379 $^{++++}$	91
39	1,5-Heptadiene, 3,3-dimethyl-, (E)-	12.47	0.17 ± 0.114 $^{++}$	9
40	Butane, 2,2-dimethyl-	12.9	0.02 ± 0.02 $^{+}$	39
41	(E)-2-Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester	13.07	24.86 ± 9.64 $^{++++}$	53
42	Pentane, 3-bromo-	13.72	0.22 ± 0.131 $^{+++}$	9
43	Butane, 2,2-dimethyl-	14.79	0.0225 ± 0.0225 $^{+}$	2
44	Butanoic acid,3-hexenyl ester,(E)-	14.96	0.885 ± 0.474 $^{++++}$	83
45	Decanal	15.45	0.39 ± 0.164 $^{+++}$	64
46	Spiro[2.9]dodeca-4,8-diene	16.15	0.982 ± 0.403 $^{++++}$	47
47	3-Hexen-1-ol,propanoate, (Z)-	16.39	0.543 ± 0.333 $^{++++}$	78
48	Propanenitrile, 2-hydroxy-	16.45	0.0825 ± 0.0825 $^{+}$	1
49	2,6-Dimethyl-1,3,6-heptatriene	16.59	0.2 ± 0.137 $^{++}$	53
50	Indole	17.36	1.57 ± 0.482 $^{++++}$	64
51	6,7-Diazabicyclo[3.2.2]non-6-ene, 2-methylene-	17.88	0.065 ± 0.065 $^{+}$	47
52	1,2-Propadiene	19.11	0.05 ± 0.0314 $^{++}$	2
53	1,2-Propadiene	19.15	0.06 ± 0.0505 $^{+}$	2
54	5-Hexanoic acid	20.15	0.0875 ± 0.0875 $^{+}$	4
55	Copaene	20.85	0.172 ± 0.116 $^{++}$	98
56	7-Propylidene-bicyclo[4.1.0]heptane	20.97	0.715 ± 0.382 $^{+++}$	50
57	1,4-Heptadiene, 3-methyl-	21.19	0.15 ± 0.15 $^{+}$	38
58	Spiro[cyclopropane-1,2'-[6.7]diazabicyclo[3.2.2]non-6-ene]	21.27	0.183 ± 0.114 $^{++}$	25
59	(-)Trans Caryophyllene	21.96	3.47 ± 1.15 $^{++++}$	92

Table 5.2 Retention time (min), percentage (± SE) of total GC areas for compounds detected in headspace of sweet potato plants and the percentage of matching between the sample and library spectra (continued).

Peak	Compounds	Retention time(min)	Mean % (± SE) of total peak area	Quality of matching (%)
60	4H-1-Benzopyran-4-one, 2-amino-	22.33	0.0225 ± 0.0225 $^{+}$	2
61	Tricyclo[2.2.1.02,6]heptane, 1,3,3-trimethyl-	22.83	0.0825 ± 0.0825 $^{+}$	72
62	Germacrene D	23.5	0.845 ± 0.639 $^{++}$	98
63	Butylated Hydroxytoluene	23.99	16.88 ± 2.5 $^{++++}$	97
64	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	25.27	0.0325 ± 0.0325 $^{+}$	9
65	1,5-Heptadiene, 2,6-dimethyl-	25.88	0.403 ± 0.14 $^{+++}$	50
66	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	28.18	0.0325 ± 0.0325 $^{+}$	28
67	n-Decanoic acid	29.95	0.0875 ± 0.0875 $^{+}$	50
68	5-Methyl-7-amino-S-triazolo(1,5-A)pyrimidine	31.79	0.03 ± 0.03 $^{+}$	2
69	3,6-Dimethyl-triazolo(4,3-b)(1,2,4)-triazine	33.71	0.0325 ± 0.0325 $^{+}$	7
70	n-Hexadecanoic acid	34.23	0.505 ± 0.505 $^{+}$	97
71	.alpha.-D-Galactopyranoside, methyl 3,6-anhydro-	38.11	0.035 ± 0.035 $^{+}$	33
72	2,3-Dimethylenetricyclo[9.2.2.2(4,7)] heptadeca-1(14),4(17),5,7(16),11(15) ,12-hexaene	38.77	0.12 ± 0.0712 $^{++}$	83
73	(1'R,2S,3R)-1',3-Dimethyl-2-2'-spirobiindan-1-one	41.12	0.12 ± 0.12 $^{+}$	47
74	Phosphine	44.19	0.415 ± 0.304 $^{++}$	83
75	Phthalic acid, diisooctyl ester	44.45	0.1025 ± 0.0781 $^{++}$	9
76	6-Nitro-8-methoxy-2H-chromene	45.4	0.0175 ± 0.0175 $^{+}$	2
77	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole	45.49	0.0125 ± 0.0125 $^{+}$	3
78	4,7,9-Trihydroxy-2-methylnaphtho[2,3-b]furan-5,8-dione	45.54	0.362 ± 0.285 $^{++}$	72
79	Octadecane, 1-iodo-	47.47	0.025 ± 0.025 $^{+}$	25
80	Squalene	49.12	0.515 ± 0.277 $^{+++}$	59

$^{+}$ indicates the presence of the compound in one sample

$^{++}$ indicates the presence of the compound in two samples

$^{+++}$ indicates the presence of the compound in three samples

$^{++++}$ indicates the presence of the compound in four samples

Only compounds detected in more than one sample will be considered in the discussion.

5.3.1.3 EAG response of *Acraea acerata* to some chemical compounds of sweet potato plant volatiles.

Mineral oil which was used as solvent elicited similar EAG responses from the antennae of both male and female SPBs (0.2601 ± 0.014 mV for males and 0.258 ± 0.033 mV for females). In general, compared to the control (solvent), cis-3-hexenol provoked the highest EAG response in the antennae of both males and females, followed by ethylbenzene, 3-carene and *trans*-caryophyllene in decreasing order. EAG response to *trans*-caryophyllene was always lower than the the control (Figure 5.12). In males, *cis*-3-hexenol had a significantly higher EAG response than the control at both 10% ($T = 0$, $P < 0.002$, Wilcoxon's test for matched pairs) and 1% ($T = 4$, $P < 0.02$, Wilcoxon test for matched pairs). For ethylbenzene and 3-carene, only the concentration of 10% elicited a significantly higher EAG response than the control ($P < 0.01$, for ethylbenzene and $P < 0.05$ for 3-carene, paired t-test). In females only, EAG responses to *cis*-3-hexenol, ethylbenzene and 3-carene at 10% were significantly higher than the control ($T = 4$, $P < 0.02$, Wilcoxon's test for matched pairs for cis-3-hexenol, $P < 0.01$ for ethylbenzene and $P < 0.05$ for 3-carene, paired t-test). These test chemicals have therefore reached the threshold response (response which is significantly greater than the response to the control). Trans-caryophyllene always provoked significantly lower EAG responses than the control at all concentrations in both males and females ($P < 0.001$, paired t-test, $T = 0$, $P < 0.002$, Wilcoxon's test for matched pairs).

Considering each test chemical, males elicited always higher EAG responses than females. In particular, EAG response to ethylbenzene by male SPBs was significantly higher that that of females (Figure 5.13a, Figure 5.14a, Figure 5.15a, Figure 5.16a). EAG response of SPBs increased with the increase of the concentration of the chemical tests and the three different concentrations (0.1%, 1%, 10%) provoked statistically different EAG responses for all the chemicals ($P < 0.05$) except for *trans*-caryophyllene (Figure 5.13b, Figure 5.14b, Figure 5.15b, Figure 5.16b). However when the interaction of the concentration of the test chemical and the sex of the butterfly was considered, only males had consistently statistically significant responses to different chemical concentrations except for *trans*-caryophyllene (Figure 5.13c, Figure 5.14c, Figure 5.15c).

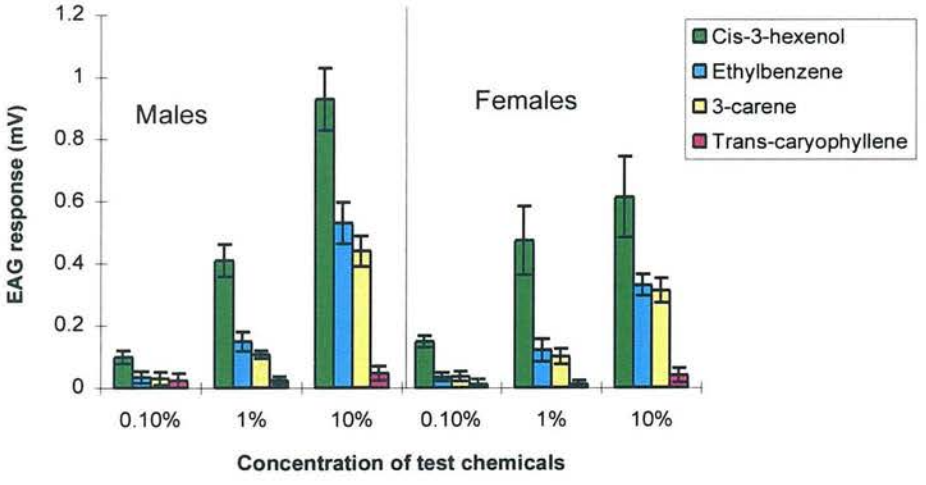


Figure 5.12 EAG responses (mV ± SE) of male and female *Acraea acerata* to some of the chemical compounds identified in sweet potato plant volatiles at three different concentrations (n = 10).

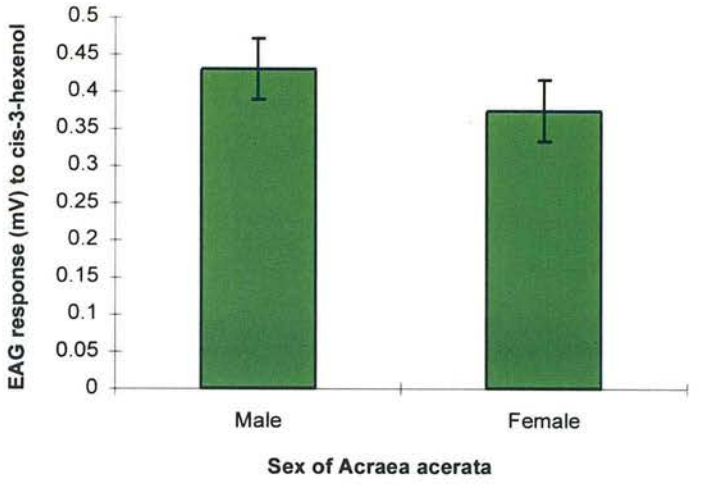


Figure 5.13a Comparison between the mean EAG response (mV ± SED) (n = 30) of male and female *Acraea acerata* to *cis*-3-hexenol.

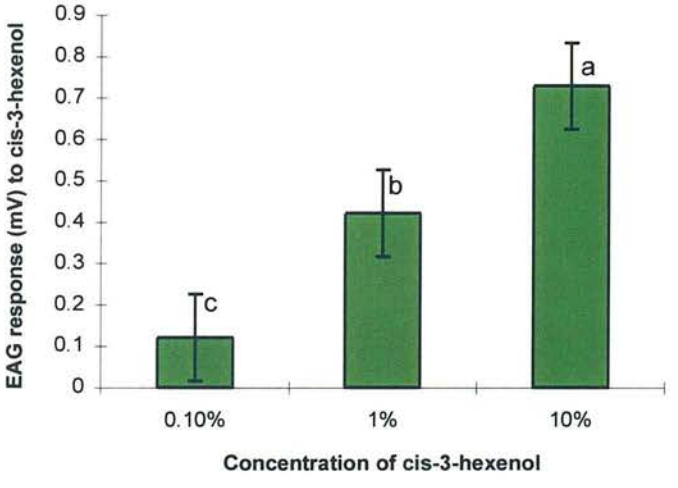


Figure 5.13b Comparison between the mean EAG response (mV ± SED) (n = 20) of *Acraea acerata* (male + female) to three different concentrations of *cis*-3-hexenol. Means with different letters are statistically different ($P < 0.05$, LSD test).

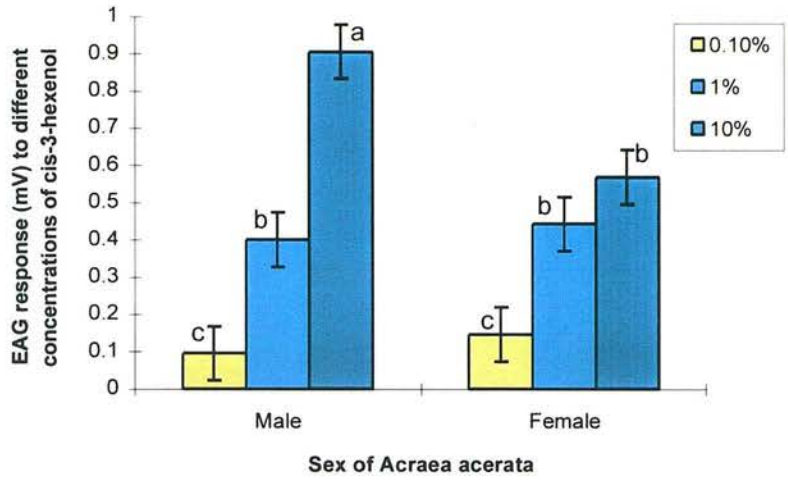


Figure 5.13c Comparison between the mean EAG response (mV ± SED) (n = 10) of male and female *Acraea acerata* to three different concentrations of *cis*-3-hexenol. Means with different letters are statistically different ($P < 0.05$, LSD test).

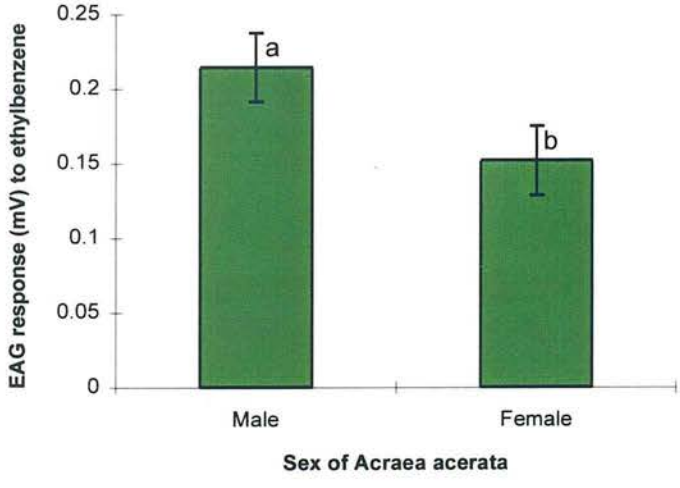


Figure 5.14a Comparison between the mean EAG response (mV ± SED) (n = 30) of male and female *Acraea acerata* to ethylbenzene. Means with different letters are statistically different ($P < 0.05$, LSD test).

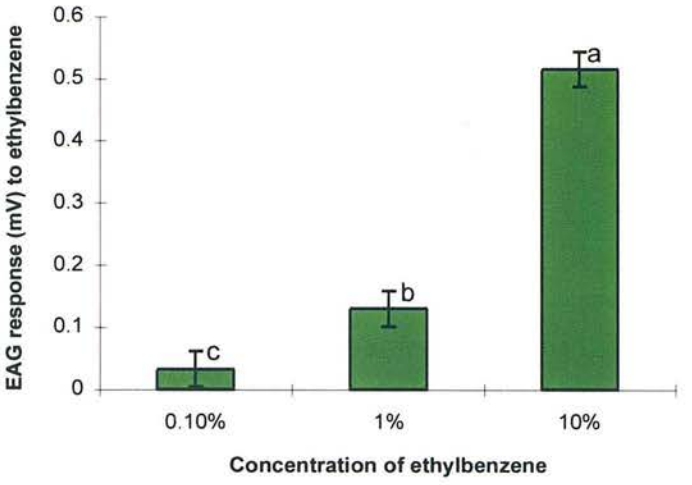


Figure 5.14b Comparison between the mean EAG response (mV ± SED) (n = 20) of *Acraea acerata* (male + female) to three different concentrations of ethylbenzene. Means with different letters are statistically different ($P < 0.05$, LSD test).

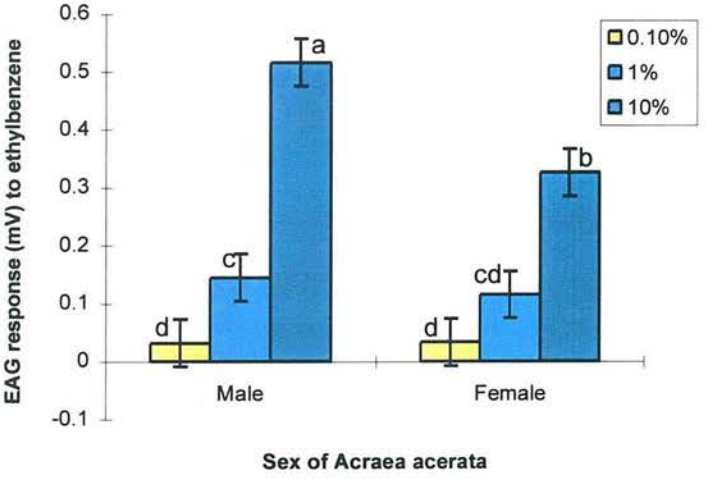


Figure 5.14c Comparison between the mean EAG response (mV ± SED) (n = 10) of male and female *Acraea acerata* to three different concentrations of ethylbenzene. Means with different letters are statistically different ($P < 0.05$, LSD test).

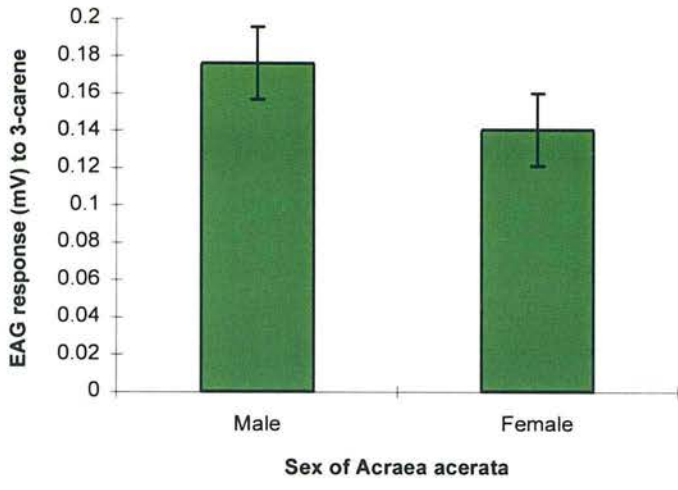


Figure 5.15a Comparison between the mean EAG response (mV ± SED) (n = 30) of male and female *Acraea acerata* to 3-carene.

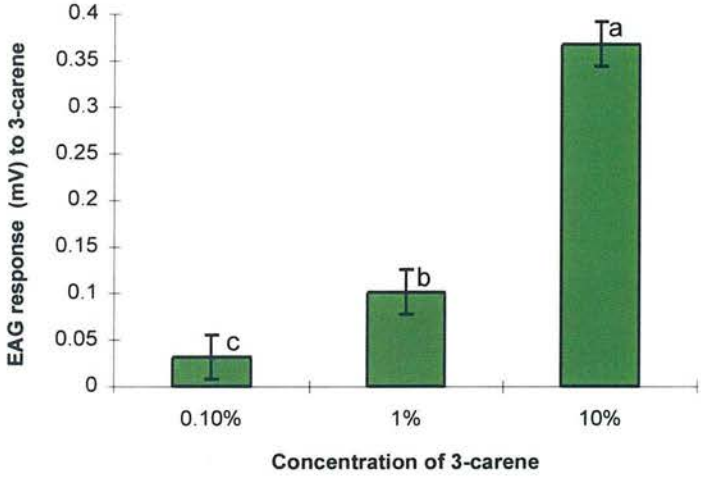


Figure 5.15b Comparison between the mean EAG response (mV ± SED) (n = 20) of *Acraea acerata* (male + female) to three different concentrations of 3-carene. Means with different letters are statistically different ($P < 0.05$, LSD test).

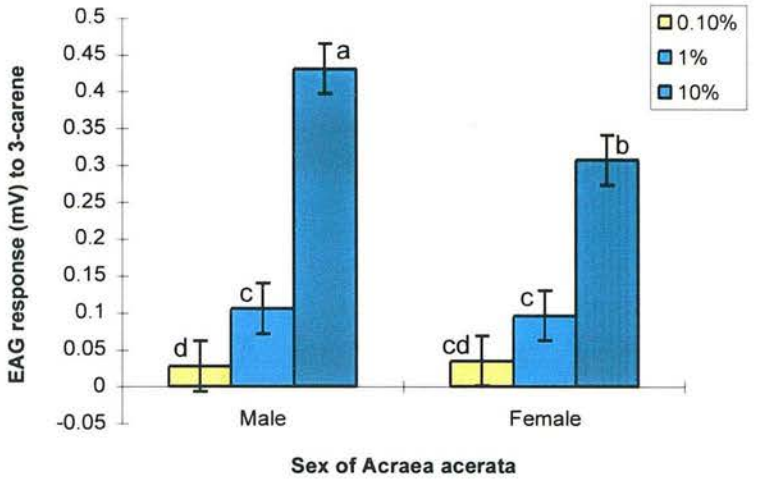


Figure 5.15c Comparison between the mean EAG response (mV ± SED) (n = 10) of male and female *Acraea acerata* to three different concentrations of 3-carene. Means with different letters are statistically different ($P < 0.05$, LSD test).

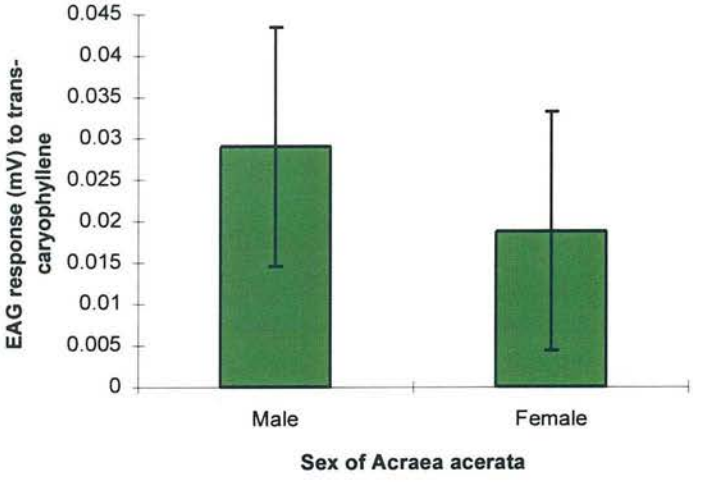


Figure 5.16a Comparison between the mean EAG response (mV ± SED) (n = 30) of male and female *Acraea acerata* to *trans*-caryophyllene.

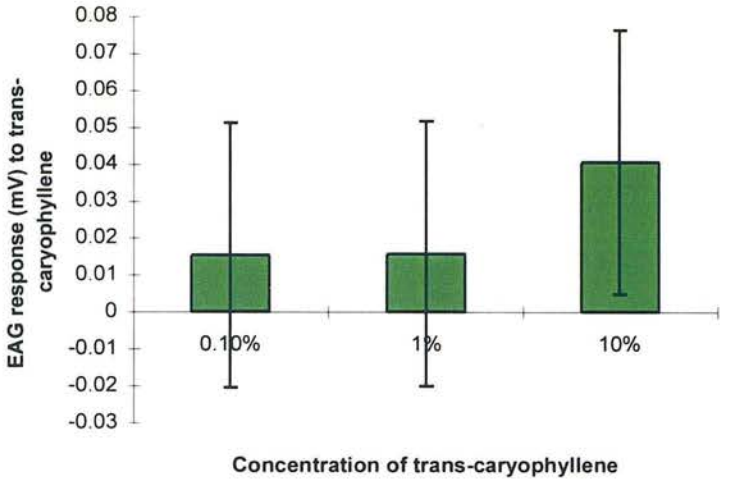


Figure 5.16b Comparison between the mean EAG response (mV ± SED) (n = 20) of *Acraea acerata* (male + female) to three different concentrations of *trans*-caryophyllene.

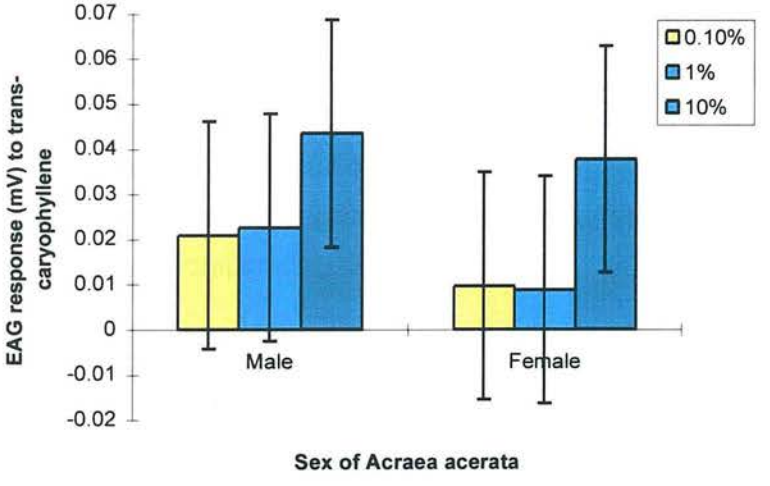


Figure 5.16c Comparison between the mean EAG response (mV ± SED) (n = 10) of male and female *Acraea acerata* to three different concentrations of *trans*-caryophyllene.

5.3.2 Discussion

The mean average distances moved by butterflies in response to the presence of sweet potato plant volatiles although consistently larger, did not differ statistically from the controls ($P > 0.05$, Figure 5.3, Figure 5.4, Figure 5.5). This was due to a relatively larger number of butterflies staying for some time in the middle section (S3 and S4) of the wind tunnel in the presence of sweet potato plant volatiles whereas for the other treatments they were mostly located either in the downwind section or in the upwind section (Figure 5.8). Nevertheless, when the time allocated to moving is considered, butterflies spent more than 50% of the observation time (min) moving (walking + flying) (Figure 5.7) in the presence of host plant volatiles whereas they spent the same amount of time resting in the presence of ambient air and the solvent (Figure 5.6). This was consistent with the results obtained with *Rhagoletis pomonella* flies which moved significantly longer periods of time in the presence of host plant odours in a wind tunnel compared to when flies were exposed to no odour (Aluja, Prokopy, Buonaccorsi and Cardé, 1993). The study of the effect of host plant volatiles on the pre-oviposition behaviour of *P. rapae* in a wind tunnel showed also similar results with female *P. rapae* responding to host plant volatiles by increasing the number of flights and the total duration of time spent in flight (Hern, 1997). Time allocated to movements (walking + flying) in this bioassay could be equivalent to time allocated to searching in a patch where time spent by a searching insect increases with high initial resource density (Bell, 1990). It seemed therefore that female SPBs responded to the presence of host plant volatiles by increasing the time spent moving compared to the controls.

Female SPBs responded also to the presence of host plant volatiles by spending most of their time in the middle or upwind sections of the wind tunnel while in the presence of ambient air or ether most of them stayed in the downwind section ($\chi^2_{df=4} = 167$, $P < 0.001$, Figure 5.8). The results seemed to indicate a clear trend of upwind movement of butterflies (from S1 where they were released) towards the source of host plant volatiles (S6). Although *R. pomonella* and *P. rapae* did manifest the same behavioural trend, other insects such as the cabbage seed weevil *Ceutorhynchus assimilis* Payk, the female brassica pod midge *Dasineura brassicae* Winn., or the hoverfly *Episyrphus balteatus* Degear exhibited the same upwind movement in the presence of their resource item odours (Evans, 1991, Aluja *et al.*, 1993, Hern, 1997, Kirkland, 1999).

Moreover the high proportion of butterflies which landed on the muslin-screen when sweet potato plant volatiles were offered ($P < 0.01$, Figure 5.9) suggested that butterflies were trying to reach the source of the sweet potato plant volatiles they were detecting. There were 2 to 3 times more landings per landed butterfly in the presence of sweet potato volatiles than in the controls ($P < 0.01$, Figure 5.10). In experiments conducted in laboratory cages, Feeny *et al.* (1989) found that the presence of host plant volatiles enhanced the landing rates of female *P. polyxenes* on plant models, accompanied by more egg laying, compared to plant models without the presence of host plant volatiles. This indicated that the landing of *P. polyxenes* butterflies involved an olfactory response to host plant odours as is being suggested for female SPBs.

The results of this wind tunnel bioassay with headspace collected host plant volatiles appeared to confirm the likelihood of attraction of female SPBs to their host plant volatiles. This is consistent with the results of the previous chapter and many other study reports which suggested that host plant volatiles play an important role in attracting host-seeking phytophagous insects (Visser, 1986, Metcalf, 1987, Renwick, 1989, Bernays and Chapman, 1994).

The identification of sweet potato volatile compounds by coupled GC-MS showed predominance of esters ((E)-2- butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester, 3-hexen-1-ol, acetate,(Z)), alcohols (3-hexen-1-ol,(Z), 1,6-octadien-3-ol, 3,7-dimethyl-), terpenoids (3-carene and (-) trans-caryophyllene), hydrocarbons (ethylbenzene, p-xylene) and aromatic hydrocarbons (indole) (Figure 5.11). 3-Hexen-1-ol, acetate,(Z), 3-hexen-1-ol,(Z), 1,6-octadien-3-ol, 3,7-dimethyl-, 3-carene, (-) trans-caryophyllene, ethylbenzene and p-xylene which represent more than 80 % (if the stabiliser is added) of the average percentage of the total peak area (of GC profile) were all detected in the four samples and their percentage of matching with the library spectra varied between 90 and 97 (Table 5.2). The quality of matching with library spectra of butylated hydroxytoluene, an authentic compound added to stabilise samples was 97%. These seven compounds which were consistently detected in sweet potato volatile samples and of which quality of matching with library spectra was very high (same or close to that of a known commercial compound) leave little doubt about their presence in sweet potato plant volatiles. Nottingham *et al.* (1989) using different methods of sampling (purge and trap of leaf volatiles; leaf volatile extraction by methylene chloride) reported only terpenoids of which trans-caryophyllene and copaene were detected in our headspace samples

(Table 5.2). They confirmed the identity of trans-caryophyllene by comparing its sample's mass spectral and GC retention time to the ones obtained from authentic samples. The use of authentic compound samples or other available methods to confirm definitively the identity of the major headspace sweet potato volatile compounds especially (E)-2- butenoic acid, 2-(methylenecyclopropyl) prop-2-yl ester and indole of which the quality of matching with the library spectra was not high (respectively 53% and 64%) is thus recommended.

In addition to the nine compounds identified as main chemical compounds of sweet potato plant volatiles, there were also other 39 minor compounds identified in sweet potato plant volatiles (Table 5.2 and Figure 5.11). They belong to the groups of esters, alcohols, terpenoids, hydrocarbons, aromatics as well as aldehydes, ketones and acids. Some of the compounds identified in the leaves of maize (*Zea mays*), sugar beet (*Beta vulgaris*) and cabbages (*Brassica oleracea*, *B. oleracea capitata* L. var. *alba* cv Langedijker de Waar or red cabbage, *B. oleracea capitata* L. var. *rubra* (DC) or red cabbage) were classified in the same chemical groups (Metcalf, 1987, Geervliet, Posthumus, Vet and Dicke, 1997). It would be very interesting to confirm the identity of the compounds detected in sweet potato plant volatiles and use them individually or as blends in bioassays to identify which elicits the response of the SPB to sweet potato plants.

Since the differences in test chemical volatility were not corrected for in the EAG experiments, relative comparisons between chemicals can be made only (Visser, 1979). Chemical volatility might have had an important role as the EAG response increased as the molecular weight (m.w) of the test chemical decreased with cis-3-hexen-1-ol (m.w = 100.16), eliciting the highest response, followed by ethylbenzene (m.w = 106.17), (+)-3-carene (m.w = 136.24) and (-)-trans-caryophyllene (m.w = 204.4) (Figure 5.12). Notwithstanding, the EAG response of SPBs to the four test chemicals revealed the selectivity of SPB antennal olfactory receptors and their ability to perceive changes in test chemical concentrations. This is in accordance with the general knowledge about the antennal receptors which are known to be chemical specific (Visser, 1986, Hansson and Anton, 2000). Like all other phytophagous insects, the antennae of SPBs showed a strong response to *cis*-3-hexenol, an alcohol which belongs to the 'general green leaf volatile' (Visser, 1986, Bernays and Chapman, 1994). This does not mean, however, that the SPB would necessarily manifest a behavioural response to *cis*-3-hexenol and none to *trans*-caryophyllene which elicited the lowest EAG response (next to nothing) as EAG responses measure

only the total receptor potential provoked by a chemical stimulus. The difference between male and female EAG responses to test chemicals in the SPB is also common to many other phytophagous insects which, like lepidopteran species, present a sexual dimorphism in the structure of the glomeruli of their antennal lobe (Hansson and Anton, 2000). Behavioural experiments would reveal more information about the role of these chemical compounds of sweet potato plant volatiles used in host plant finding by the SPB.

5.4 Conclusion

In the presence of sweet potato plant volatiles, butterflies spent more time moving, landed very often and were mainly located in middle and upwind sections of the wind tunnel. These results confirm the conclusion of a previous bio-assay which used screened sweet potato plants that sweet potato volatiles play a very important role in attracting the SPB to the sweet potato plants (Chapter 4).

The coupled GC-MS analysis of the sweet potato plant volatile samples revealed a rich blend of compounds of which the main components were tentatively identified as esters: (E)-2- butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester and 3-hexen-1-ol, acetate,(Z); alcohols: 3-hexen-1-ol,(Z) and 1,6-octadien-3-ol, 3,7-dimethyl-; terpenoids: 3-carene and (-) trans-caryophyllene); hydrocarbons: ethylbenzene and p-xylene; and the aromatic hydrocarbon indole. EAG response to cis-3-hexenol, ethylbenzene, 3-carene and *trans*-caryophyllene revealed that the antennae of the SPB are selective, react to test chemical concentration changes and respond differently according to the sex of the butterfly. Further studies could confirm the identity of the compounds identified and using them as individual compounds or blends in bioassays could help to identify which compound or blend is responsible for the specific attractiveness of sweet potato plant to SPBs. Such information could then be used to build up a pest management strategy to control the SPB by manipulating its behaviour using attractants.

Chapter 6

Initial screening of potential repellent/disorienting plants for *Acraea acerata*

Chapter 6 Initial screening of potential repellent/disorienting plants for *Acraea acerata*

6.1 Introduction

The results of the previous chapters revealed that host plant volatiles play a predominant role in attracting the SPB to sweet potato plants. The identification by GC-MS analysis of sweet potato volatile compounds pointed to seven major chemical compounds which constitute the sweet potato headspace volatiles. Among them are alcohols, hydrocarbons, esters, terpenes, and aromatics. The electrophysiological responses of the antennae of the SPB to four of these chemical compounds showed a relatively larger response to cis-3-hexenol (alcohol), a common green leaf volatile but a moderate response to 3- carene (terpene) and almost no response to *trans*-caryophyllene (terpene), the two terpenes which were suspected to be specific chemical compounds of sweet potato plant (*trans*-caryophyllene and other terpenes had been already identified by Nottingham and co-workers (1989)) to which the SPB might respond to locate its host plant. The SPB might behaviourally respond instead to another chemical compound of sweet potato plant volatiles which was not tested for electroantennogram responses [e.g. (E)-2- Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester, an ester which was the most abundant chemical compound of the sweet potato plant volatiles (Figure 5.11) but which was not commercially available] or a specific volatile mixture made up of different chemical compounds emanating from sweet potato plants rather than responding to one or two specific chemical compounds.

At this stage, it may have been possible to take one of the logical ways ahead by identifying the chemical compound or the specific mixture of sweet potato volatile chemicals which is most attractive to the SPB in order to use it as a lure to trap adult female SPBs. However, such an approach could have at least one major drawback: the chemical compound or the mixture -trap would have to be industrially formulated, produced and marketed. As the SPB is found mostly in East Africa where the sweet potato is grown in subsistence farming, this kind of pest control

method though ecologically friendly, would not be economically viable just like pesticides (Chapter one). That is why an alternative approach which could be suitable for subsistence East-African farming system was sought. It consisted of investigating the possibility of interfering with host-seeking female SPBs by repelling them and/or masking the specific sweet potato plant volatiles using strong smelling plants in an intercropping system with sweet potato plants. Intercropping which is an old, widely used agricultural practice in the tropics, is here referred to as growing two or more crops simultaneously in the same field during part or all of the life cycle of each crop (Papendick, Sanchez and Triplett, 1976, Francis, 1986, Vandermeer, 1989, Innis, 1997).

The aim of this experiment was to investigate whether some of the plants reported as having the potential of repelling herbivorous insects could be combined with sweet potato plants to interfere with the host plant finding process of the SPB. Initial screening was in a four-armed olfactometer. Linear track, Y or T, four- , six- even 8-armed olfactometers have all been widely used to study the response of insects to olfactory stimuli (Lecomte and Thibout, 1981, Vet, Van Lenteren, Heymans and Meelis, 1983, Liu and Sengonca, 1994, Beerwinkle, Shaver, Lingren, Raulston, 1996, Bartlet, Blight, Lane and Williams, 1997, Mboera, Knols, Takken and Huisman, 1997, Kirkland, 1999, Romeis, Shanower and Zebitz, 1999). Although they have been used to test olfactory stimuli attraction as well as repellency, they cannot differentiate between an inhibitor, a non stimulatory compound or a repellent (Dogan and Rossignol, 1999). Repellency is better tested in a repellometer which is made of a median chamber (where insects are introduced), a proximal chamber offering the possibility of moving towards the odour source and a distal chamber for insects which respond by moving away from the odour source (Dogan and Rossignol, 1999). In this experiment, the interest was in finding a plant or plants which, if mixed with sweet potato plants, would repel the SPB away from the mixture or mask the specific sweet potato plant volatiles or change the response of female SPBs towards sweet potato plant volatiles. A masking substance was defined by Yamaski, Sato and Sokoguchi (1997) as an agent inhibiting the locomotory

movements toward the attractant source. As olfactometers have been used to study olfactory responses of larger flying insects like the leek moth, *A. assectella* (Lecomte and Thibout, 1981), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Beerwinkle *et al.*, 1996), the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Yan, Bengtsson and Witzgall, 1999) and the worker bee, *Apis mellifera* (Hymenoptera: Apidae) (Phamdelegue, Trouiller, Bakchine, Roger and Masson, 1991) it was assumed that a four-armed olfactometer would be appropriate for the initial screening of repellent/masking plants for/from SPBs and would have the advantage of allowing the screening of more plants in a reasonable time.

6.2 Materials and Methods

6.2.1 Selection of odour masking/insect repellent plants

Table 6.1 summarises some of the plants which have been reported as insect repellent plants or masking volatiles given off by insect host plants. Repellency and attraction of a herbivorous insect away from or towards a host or non host plant is due to the plant volatiles or visual stimuli which convey to the insect's central nervous system information about the un/suitability of that plant as a host plant. Species from the family Umbelliferae with their aromatic scents are generally known as species belonging to an insect-free plant family and are thought to be insect repellent (Berenbaun, 1990). However there are species from other plant families which are also known as strong smelling insect repellents or capable of masking host plant specific volatiles .

Of the list of more than 20 plants reported in the literature as insect repellent or having a masking effect on insect host plant volatiles (Table 6.1), tomatoes, garlic, onions, molass grass were chosen to be pre-screened for the following reasons: i) reported in the literature as insect repellent or having a masking effect on host plant volatiles, in particular against some Lepidoptera species; ii) could be easily grown in eco-climatic conditions of East Africa; and iii) could be suitable for subsistence farming. Tomato, garlic and onions are all crops grown in East Africa (Le Pelley, 1959, FAO, 1999).

Table 6.1. Some of the reported insect repellent/masking plants

Repellent/masking plant	Plant family	Insect repelled/disoriented	Type of intercrop	References
Tomatoes	Solanaceae	*Plutella xylostella*	cabbage/tomato	Perrin and Phillips, 1978
		Phthorimaea operculla	potato/tomato	CIP, 1983
		Empoasca dolichi	cowpeas/tomato	Ofuya, 1991
		Pieris rapae	cabagge/tomato	Wu *et al*, 1999
Onions	Liliaceae	*P. operculla*	potato/onions	CIP, 1983
		P. operculla	potato/onions	Potts and Gunadi, 1991
		M. persicae	potato/onions	Potts and Gunadi, 1991
		Aphis gossypii	potato/onions	Potts and Gunadi, 1991
		Empoasca spp	potato/onions	Potts and Gunadi, 1991
		Henosepilachna sparsa	potato/onions	Potts and Gunadi, 1991
		Acanthoscelides obtectus	beans/onions	Mateeva *et al*.,1998
		Chilo infuscatellus	sugarcane/onions	Varun *et al*.,1994
		Psila rosae	carrot/onions	Uvah and Coaker, 1984
		Cavanella aegopodii	carrot/onions	Uvah and Coaker, 1984
Thyme	Labiatae	*P. xylostella*	cabagge/thyme	Doves, 1985

Table 6.1 Some of the reported insect repellent/masking plants (continued)

Repellent/masking plant	Plant family	Insect repelled/disoriented	Type of intercrop	References
Sage	Labiatae	*P. xylostella*	cabbage/sage	Doves, 1985
Garlic	Liliaceae	*P. xylostella*	cabbage/garlic	Talekar *et al.*,1986
		C. infuscatellus	sugarcane/garlic	Varun *et al.*, 1994
		P. operculla	tomato/garlic	Afifi, Haydar and Omar, 1990
		M. persicae	potato/garlic	Potts and Gunadi, 1991
		Aphis gossypii	potato/garlic	Potts and Gunadi, 1991
		Empoasca spp	potato/garlic	Potts and Gunadi, 1991
		Henosepilachna sparsa	potato/garlic	Potts and Gunadi, 1991
Beans	Leguminosae	*P. operculla*	potato/beans	CIP, 1983
Soyabeans	Leguminosae	*P. operculla*	potato/soyabeans	CIP,1983
Chillies	Solanaceae	*P. operculla*	potato/chillies	Lal, 1991
Peas	Leguminosae	*P. operculla*	potato/peas	Lal, 1991
Coriander	Umbelliferae	*C. infuscatellus*	sugarcane/coriander	Varun *et al.*, 1994
Molass grass	Graminaceae	*C. partellus* and *Bussolea fusca*	maize/molass grass	Khan *et al.*, 1997b

Table 6.1 Some of the reported insect repellent/masking plants (continued)

Repellent/masking plant	Plant family	Insect repelled/disoriented	Type of intercrop	References
Tagetes erecta L.	Asteraceae	Aphids	tomato/tagetes	Castro *et al.*,1990
Tobacco	Solanaceae	*P. rapae*	cabbage/tobacco	Wu *et al.*,1999
Cowpea	Leguminosae	*C. partellus*	maize/cowpea	Ampong-Nyarko *et al.*, 1994
			maize/cowpea	Skovgard and Pats, 1997
Peas	Leguminosae	*P. opercullela*	potato/peas	Lal, 1991
Dill	Umbelliferae	*P. xylostella*	cabbage/dill	Talekar *et al.*, 1986
Safflower	Compositae	*P. xylostella*	cabbage/safflower	Talekar *et al.*, 1986
Oats	Gramineae	*P. xylostella*	cabbage/oats	Talekar *et al.*, 1986
Carrot	Umbelliferae	*P. xylostella*	cabbage/carrot	Chelliah and Srinivasan,1986
Rosemary	Labiatae	*Neotoxoptera formosana*	rosemary/*Allium* sp.	Hori and Komatsu, 1997
Pennyroyal (*Mentha pulegium* L.)	Labiatae	*N. formosana*	Pennyroyal/*Allium* sp.	Hori and Komatsu, 1997

Molass grass is used as fodder in the same region (Khan *et al.*, 1997a, Khan *et al.*, 1997b). The silverleaf desmodium, *Desmodium uncinatum* (Jack.) DC, a leguminous plant used as fodder was suggested by Smit (*personal communication*) who worked on the SPB in Uganda (see Acknowledgements). Wild tomato is not grown but it has been reported as insect repellent and its repellency character could be integrated into the cultivation of tomato if it was found repellent or disorienting to SPBs. There are other plants in Table 6.1 which could also have been pre-screened if time had not been a limiting factor.

6.2.2 Initial screening of repellent/disorienting plants for *Acraea acerata* in an olfactometer.

6.2.2.1 Materials

6.2.2.1.1 Plants involved

Desmodium plants**:** seeds of *Desmodium uncinatum* from Uganda were sown in pots with peat in glasshouse. The lighting, temperature and photoperiod were the same as for sweet potato plants (Chapter 3). Two to three weeks after emergence, *D. uncinatum* seedlings were transplanted into other pots taking care to transplant one plant per pot.

Onion plants *(Allium sativum*): seeds of cv Bedfordshire Champion obtained from a local garden centre were sown in pots with peat in the glasshouse. The transplantation was done three weeks after sowing. They were grown in the same conditions as *D. uncinatum* plants.

Garlic plants (*Allium cepa*): garlic bulbs bought from a local supermarket were subdivided into cloves and planted in pots in the same conditions as *D. uncinatum* plants.

Molass grass plants: seeds of molass grass (*Melenis minutiflora*) obtained from ICIPE (International Centre of Insect Physiology and Ecology) in Kenya were sown

in pots with peat and grown under the same glasshouse conditions as for *D. uncinatum* plants.

Tomato plants: seeds of tomato (*Lycopersicum esculentum*) cv Moneymaker were bought from a local shop and seeds of a wild tomato *L. hirsutum* (Lyc.4/88, IPK-Germany) were kindly provided by G. Saavedra (concurrent University of Edinburgh PhD student who was working on genetic base broadening of tomato). They were also sown under the same conditions as for the other plants and transplanted two to three weeks after emergence.

Sweet potato plants: 15 cm vine cuttings were planted in the same pots as the potential repellent/masking plants in the ratio 1:1. The transplanting of different plants and the planting of sweet potato and garlic were done at different times in order to have relatively similar vegetative growth at the time of the assay. Thus, vine cuttings of sweet potato were planted at the same time as the transplantation of 3 three-week old tomato, wild tomato and onion plants. Garlic was planted 2 weeks before the planting of sweet potato. Seeds of molass grass were sown two weeks before the planting of sweet potato whereas seeds of *D. uncinatum* were sown four weeks in advance. Once the sweet potato was planted all the plants were placed in a large cage (to protect sweet potato from aphids) in the glasshouse and grown without agro-chemicals as described in Chapter 3. They were used for olfactometer bioassays 3 weeks after sweet potato planting.

6.2.2.1.2 Butterflies: females butterflies used in the bioassay were reared as described in Chapter 3 (point 3.2.1.1)

6.2.2.1.3 Olfactometer

A four-armed olfactometer (Figure 6.1) originally described by Vet *et al.*(1983) was used for the pre-screening of potential repellent/disorienting plants to SPBs.

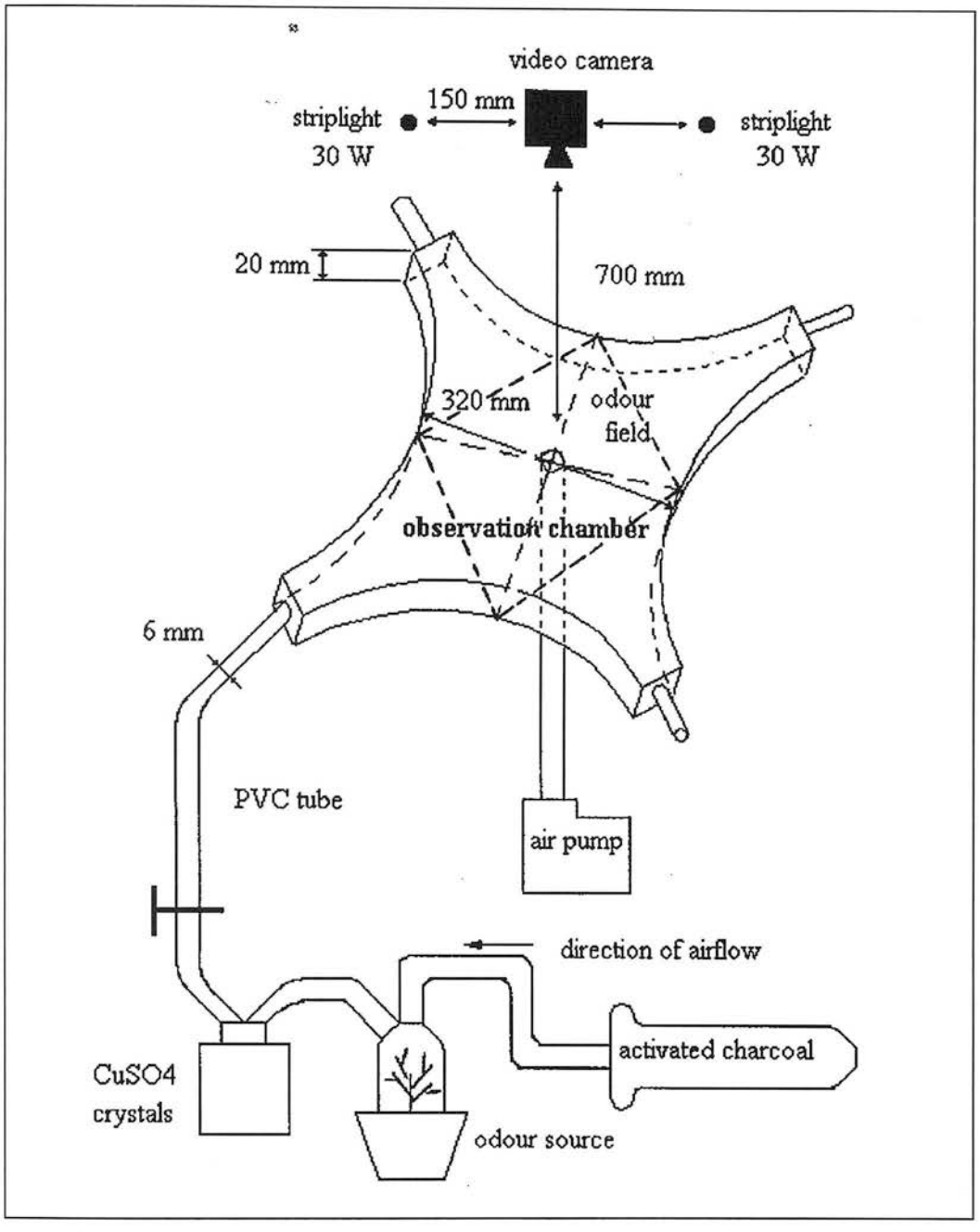


Figure 6.1 Diagrammatic representation of the olfactometer system (modified from Tréfás *et al.*, In press)

It consisted of :i) an exposure chamber in a four-pointed star shape made of four transparent perspex crescents on the top of a perspex sheet making the floor; bolts with wing nuts held the perspex lid of the chamber and rubber in O rings were

inserted under the nuts to assure the air-tightness of the system; the four-pointed star exits of the chamber terminated in 4 stainless steel tubes and; the chamber's internal dimensions were 20 mm in depth, 320 mm in the narrowest width, 380 mm in the widest width and 57 cm^2 in area; ii) volatile sources: 4 emplacements for volatile sources were provided (in our experiment different plants constituted volatile sources); iii) PVC tubes (6 mm inside diameter) which linked the exposure chamber to the volatile sources; iv) air humidity and odour filters: air was passed over activated charcoal to remove odours before entering the sources of volatiles chosen for the experiment; and the uniformity of volatiles' humidity was created by passing the flow of air over CuSO4 crystals; v) insect monitoring system made of a camera (Sony CCD-Iris) centrally positioned above the exposure chamber and connected to both a TV monitor (Panasonic WV-CM 1450) and a video cassette recorder (Panasonic time lapse video cassette recorder AG 6040); the system could record and display at the same time what was happening in the exposure chamber; vi) the lighting system was made of two 30 W strip light tubes fixed on either side of the camera at 70 cm above the olfactometer to provide uniform illumination of the observation chamber; light external to the olfactometer was blocked by a layer of black plastic sheeting on top of another layer of a black fabric covering the camera, strip lights and the exposure chamber and; vii) air pump (Edwards Vacuum Components EB3A, Crawley, UK) sucked air creating four distinct odour fields. The airflow was measured by a flowmeter (Platon Air Products, Basingstoke, UK) and adjusted using metal screw clamps over the PVC tubes. Four adjacent flow fields with minimum turbulence and mixing between them were obtained by visualising the flow fields using the 'smoke' produced by pouring hot water on to dry ice and adjusting the flow rate of each of the four arms to 0.35 l/min. Insects were introduced into the exposure chamber through a 2 cm diameter hole drilled on the top lid of the chamber. To seal the hole a small PVC sliding door was fixed on the top lid of the exposure chamber.

6.2.2.2 Methods

6.2.2.2.1 Bioassay

Four sets of bioassays were carried out in the olfactometer. For each set, twenty individual two-day old, mated and naive female butterflies were observed for a 30-minute period and their location, activities and time spent in each arm were recorded using The Observer software programme (Noldus Information Technology, 1993). To minimise the effect of diffusion and mixing of odours between adjacent odour fields, a butterfly was recorded as being located in one particular odour field after it had crossed one of the lines of the arbitrary 'first choice' square as shown in the observation chamber (Figure 6.1). After recording data for five individual butterflies, the positions of different treatments were rotated in the different arms to eliminate any positional bias. Butterflies did not appear to walk easily on the smooth perspex floor in the chamber. To rectify this, four rough tissue papers (Lotus professional: Dixcel professional plus) were placed on top of the perspex floor surface leaving free the hole through which air was drawn from different arms of the olfactometer. New papers were placed for each butterfly which kept the exposure chamber free from traces of the previous insect observed.

i) Test for random distribution of butterflies in the four flow fields of the olfactometer. This was done by offering butterflies 'clean ambient air' (a pot with peat only) in the four arms of the olfactometer.

ii) Test of the attractiveness of sweet potato plant volatiles in olfactometer. 20 butterflies were offered a choice between volatiles emanating from a sweet potato plant placed in one arm of the olfactometer and three fields of 'clean ambient air'. The sweet potato plant was regularly rotated to each arm of the olfactometer.

iii) Test of masking of sweet potato plant volatiles and/or repelling the SPB by mixing sweet potato + tomato, sweet potato + wild tomato and sweet potato + garlic. The treatments consisted of one plant mixture in each of three arms and 'clean ambient air' in the fourth arm as a control. As in the previous experiment, the

treatments were regularly rotated around the four arms. The null hypothesis to test was: sweet potato + masking/repellent plant volatiles are not attractive to SPBs in an olfactometer (i.e butterflies spent as much time in sweet potato + masking plant volatiles as in ambient air).

iv) Test of masking of sweet potato plant volatiles and/or repelling the SPB by mixing sweet potato +*Desmodium*, sweet potato + molass grass and sweet potato + onion. The bioassay was conducted in the same way as in iii) and the same hypothesis was tested.

6.2.2.2.2 Statistical analysis.

Three parameters were analysed: the time spent by butterflies in each arm of the olfactometer, the number of butterflies which made their first treatment choice and the number of butterflies which spent more than 25 % of the total observation time period in any of the four different arms. The analysis of variance was performed using Genstat for Windows for the time spent by butterflies in different arms of the olfactometer. The data were log-transformed [log10 (data + 1)]. The time spent by butterflies in each odour field was not independent to the time spent in the other three fields. This would be taken into account by conducting a randomisation test (as in Chapter 4) if the analysis of variance revealed statistically significant differences between treatments. The number of butterflies which spent more than 25% of their time in the odour field of each treatment as well as the number of butterflies which made their first choice for each treatment were analysed using a χ^2 test.

6.3 Results and Discussion

6.3.1 Results

When 'clean ambient air' was offered in the four arms of the olfactometer, butterflies spent a similar amount of time in the odour field from each arm ($P > 0.05$, Figure 6.2). There was no difference between the numbers of butterflies which made their first choice in odour fields from each arm neither between those which stayed more than 25% of the observation time in each odour field (Table 6.2).

Although butterflies seemed to spend relatively more time in the sweet potato plant odour field, there was no statistically significant difference between sweet potato plants as odour source and the 'clean ambient air' odour field ($P > 0.05$, Figure 6.3). The number of butterflies which crossed the line of first choice as well as the number of butterflies which spent more than 25% of the total time in each arm of the olfactometer did not differ statistically between the 'clean ambient air' and sweet potato plant odours (Table 6.3). These results are similar to those obtained with the mixtures of potential repelling/masking plants but butterflies were spending relatively less time in onion and tomato odour fields ($P > 0.05$, Figure 6.4a, Figure 6.4b, Table 6.4a, Table 6.4b).

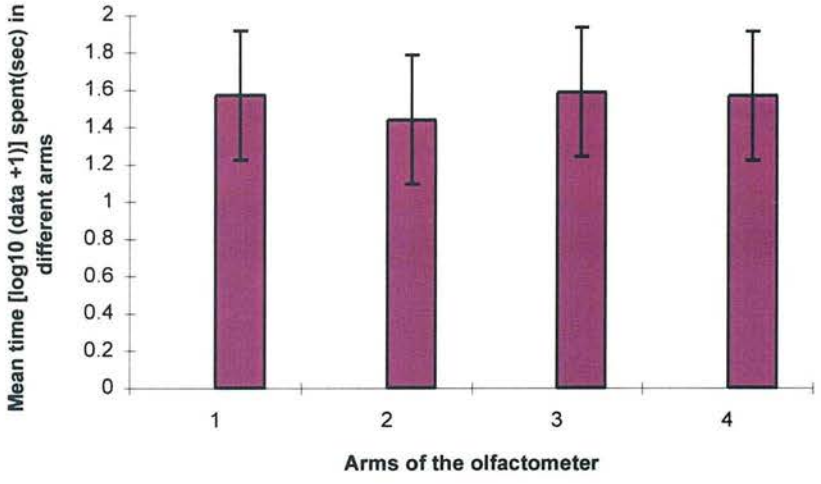


Figure 6.2 Mean time [log10 (data+1)] ± SED spent (sec) by female butterflies in different odour fields of the olfactometer when 'clean ambient air' was offered in each arm (n = 20)

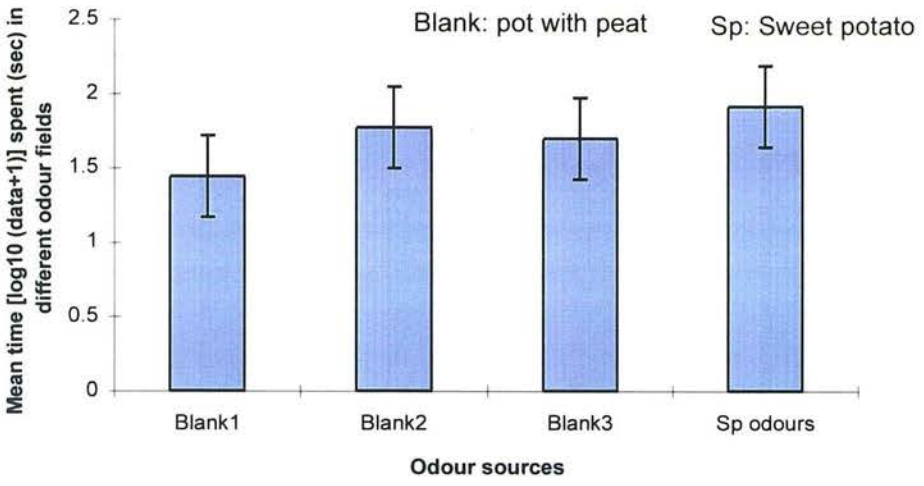


Figure 6.3 Mean time [log10 (data+1)] ± SED spent (sec) by female butterflies in different odour fields of the olfactometer when sweet potato plant was offered in one arm and 'clean ambient air'(or blank) in three other arms (n = 20)

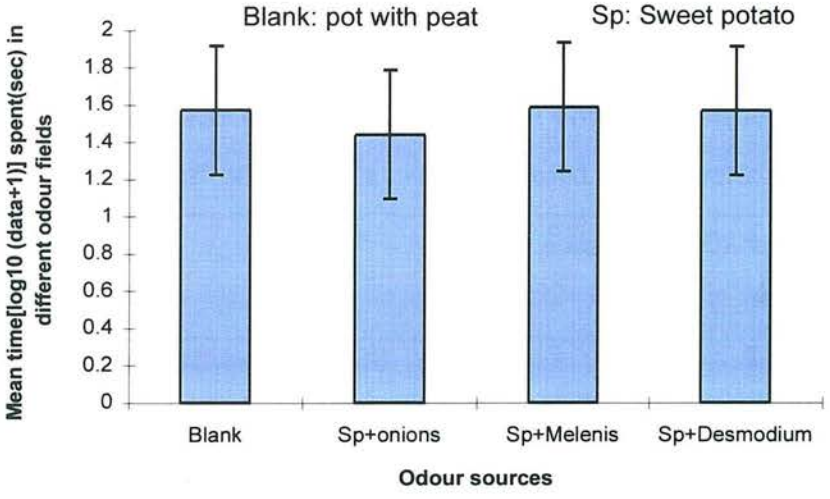


Figure 6.4a Mean time [log10 (data+1)] ± SED spent (sec) by female butterflies in different odour fields of the olfactometer when mixtures of sweet potato plant + potential masking plants were offered in three arms and 'clean ambient air' in one arm (n = 20)

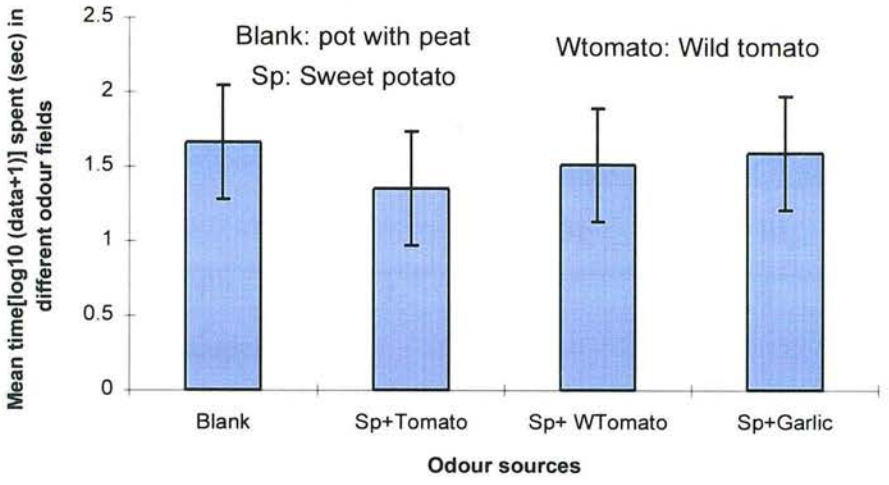


Figure 6.4b Mean time [log10 (data+1)] ± SED spent (sec) by female butterflies in different odour fields of the olfactometer when mixtures of sweet potato plant + potential masking plants were offered in three arms and 'clean ambient air' in one arm (n = 20)

Table 6.2 Response of mated female sweet potato butterflies to volatiles from four 'clean ambient air' odour fields in a four-armed olfactometer.

Response	n	Odour fields from the 4 arms				P
		1	2	3	4	
Number of butterflies which made 1st choices	20	6	1	6	7	0.118
Number of butterflies which spent > 25% of total observation time in any odour field	20	5	10	7	8	0.428

$\chi^2_{df=3}$ = 5.867 for the number of butterflies which selected either odour field for their first choice.

$\chi^2_{df=3}$ = 2.773 for the number of butterflies which spent more than 25 % of the total time spent in the four odour fields in one particular odour field.

Table 6.3 Response of mated female sweet potato butterflies to volatiles from sweet potato plant in one arm and 'clean ambient air' in each of the three other arms of the olfactometer.

Response	n	Odour fields from the 4 arms				P
		Sp	Bl_1	Bl_2	Bl_3	
Number of butterflies which made 1st choices	20	5	5	4	6	0.912
Number of butterflies which spent > 25% of total observation time in any odour field	20	9	5	9	9	0.475

Bl_1= Blank; Bl_2= Blank; Bl_3= Blank; Sp: Sweet potato

$\chi^2_{df=3}$ = 0.533 for the number of butterflies which selected either odour field for their first choice.

$\chi^2_{df=3}$ = 2.500 for the number of butterflies which spent more than 25 % of the total time spent in the four odour fields in one particular odour field.

Table 6.4a Response of mated female sweet potato butterflies to volatiles from three different mixtures of sweet potato plant + potential masking plants (one in each arm) and 'clean ambient air' (blank) in the fourth arm of the olfactometer.

Response	n	Odour fields from the four arms				P
		Sp+O	Sp+M	SP+D	Bl	
Number of butterflies which made 1st choices	20	5	6	4	4	0.859
Number of butterflies which spent > 25% of total observation time in any odour field	20	5	9	7	9	0.504

Sp+O: sweet potato + onion plants, Sp+M: sweet potato +*Melenis* plants

Sp+D: sweet potato + *Desmodium* plants, Bl: Blank

$\chi^2_{df=3}$ = 0.759 for the number of butterflies which selected either odour field for their first choice.

$\chi^2_{df=3}$ = 2.347 for the number of butterflies which spent more than 25 % of the total time spent in the four odour fields in one particular odour field.

Table 6.4b Response of mated female sweet potato butterflies to volatiles from three different mixtures of sweet potato plant + potential masking plants (one in each arm) and 'clean ambient air' (blank) in the fourth arm of the olfactometer.

Response	n	Odour fields from the four arms				P
		Sp+T	Sp+WT	SP+G	Bl	
Number of butterflies which made 1st choices	20	4	3	7	5	0.491
Number of butterflies which spent time > 25% of total observation time in any odour field	20	4	8	6	8	0.474

Sp+T: sweet potato + tomato plants, Sp+WT: sweet potato +wild tomato plants
Sp+G: sweet potato + garlic plants, Bl: Blank

$\chi^2_{df=3}$ = 2.507 for the number of butterflies which selected either odour field for their first choice.

$\chi^2_{df=3}$ = 2.416 for the number of butterflies which spent more than 25 % of the total time spent in the four odour fields in one particular odour field.

6.3.2 Discussion

The mean time spent by female SPBs in the four odour fields when 'clean ambient air' was offered in each arm of the olfactometer did not show statistically significant differences (Figure 6.2, P > 0.05). Moreover, the number of female SPBs which made their first choices in the odour field of different arms did not reveal any statistically significant difference between the four odour fields ($\chi^2_{df=3}$ = 5.867, P >

0.05, Table 6.2). In the same way, no statistically significant difference was found between the number of butterflies that spent more than 25 % of the total time spent in the four odour fields in one particular odour field ($\chi^2_{df=3}$ = 2.773, P > 0.05, Table 6.2). These results could well mean that the responses of the female SPBs in the four-armed olfactometer were randomly distributed, a very important condition which excludes any bias in the olfactometer's exposure chamber (Vet *et al.*, 1983). However, when the response of butterflies to odours emanating from sweet potato plants was compared to their response to 'ambient clean air', there was no statistically significant difference between them neither considering mean time spent in each odour field (Figure 6.3, P > 0.05), the number of female SPBs which made their first choices in a particular odour field ($\chi^2_{df=3}$ = 0.533, P > 0.05, Table 6.3) nor the number of butterflies that spent more than 25 % of the total time in one particular odour field ($\chi^2_{df=3}$ = 2.500, P > 0.05, Table 6.3). As previous wind tunnel experiments had shown the attractiveness of sweet potato plant volatiles to female SPBs (Chapter 4 and Chapter 5) it would appear that the olfactometer used in this bioassay could have hampered the responsiveness of the butterflies. In fact, the butterflies, which were normally expected to respond to host plant volatiles by walking and/or flying towards the volatile sources, might have been forced to respond by walking only in a very confined space without any possibility of flying. However, the cabbage seed weevil, *Ceutorhynchus assimilis* Payk., pollen beetles, *Meligethes aeneus* F. and the cabbage aphids, *Brevicoryne brassicae* L. which do fly towards their host plants in fields showed a positive response to attractive odours in a similar four-armed olfactometer (Evans, 1991). However Vet *et al.* (1983) warned that the 4-armed olfactometer, as initially built, was not appropriate for large insects which orient to odours only after initiation of flights. No study has yet shown whether or not the SPB is in that category of insects.

Another possible explanation might be that female SPBs spent more time in the sweet potato odour field but a proportion of that time was not taken into account because of the arbitrary decision to record as time spent by butterflies in a particular odour field only the time spent beyond the line of the first choice. In a similar

olfactometer bioassay, Evans (1991), working with pod midges, *D. brassicae*, argued that insects which made a significant choice for an odour field but did not move toward the source of odour might have been behaving as they would in the field where they stay in contact with host plant odours to maximise their chance of locating host plants or finding mates. This might apply particularly to the ovipositing female SPB which, in wind tunnel bioassays moved to host plants only when it was the right time to start laying eggs (Chapter 3).

Furthermore, the assumption, usually verified qualitatively using smoke, that there are sharp boundaries between odour fields in a 4-armed olfactometer was questioned by Giles, Heinz and Parrell (1996). They used ethylene as a gas tracer to quantitatively characterise the air flow and odour mixing in an olfactometer similar to that used by Vet *et al.* (1983). The measurement of the tracer gas at different locations of the exposure chamber provided evidence that flow boundaries between odour fields are rather gradual, mixed flow instead of being sharp, abrupt boundaries. It was found that boundaries between odour fields become less and less distinct near the centre of the chamber and only 35 to 70% of the tracer concentrations were found at the boundary locations. It was argued that this is not normally detected by the visualisation of odour fields using smoke (Giles *et al.*, 1996). If this had been the case with the bioassay testing the response of female SPBs to sweet potato plant volatiles where one attracting odour field was tested against three 'clean ambient air' odour fields, one might expect that the two 'clean ambient air' odour fields adjacent to the sweet potato plant odour field would have carried a certain percentage of the host plant volatiles making them attractive to the butterflies as well. The similar amount of time spent by butterflies in the 'clean ambient air' treatments called Blank2 and Blank3, and in the sweet potato plant odour field as well as the similar numbers of butterflies which spent more than 25% of the total observation time (Figure 6.2, Table 6.3) appeared to support the above argument. If this was what was happening in the four-armed olfactometer, it could be even more complicated in the case where two sources of identical odour are placed in two arms and tested against clean air in the other two arms as all four odour fields would carry a certain

percentage of the odour being tested due to diffusion and mixing from adjacent odour fields. However, previous experimental studies showed the predominance of the attractive odour field against the control (Vet *et al.*, 1983, Phamdelegue *et al.*, 1991, Evans, 1991, Kirkland, 1999). But such mixing between odour fields should not be excluded from consideration as most experiments which fail to demonstrate attraction of a particular odour to a certain insect when it was expected are not generally reported.

The experimental set up of the bioassays for the pre-screening of plants which could mask the attractiveness of sweet potato plant volatiles to SPBs or repel them was based on the assumption that sweet potato plant volatiles would be found attractive to the butterfly in the olfactometer. But the results discussed above revealed that there was no statistically significant difference between sweet potato plant volatiles and 'clean ambient air'. Nevertheless, as the sweet potato plant volatiles seemed relatively more attractive than the control and as the olfactometer bio-assays were used only as a screen for potential repellent/masking plants to include in wind tunnel screening bioassays, it was decided to continue the screening using the 4-armed olfactometer.

The investigation of the repelling/masking effects of onions, *M. minutiflora*, *D. uncinatum*, tomato, wild tomato and garlic in binary mixtures with sweet potato plants did not reveal any statistically significant difference between the mixtures and 'clean ambient air' (Figure 6.4a, Figure 6.4b, Table 6.4a, Table 6.4b, $P > 0.05$). This would have meant that all the plants tested were masking the attractiveness of sweet potato plant volatiles to female SPBs. Such a conclusion would have been safe if the olfactometer bioassay had shown that sweet potato plant volatiles were more attractive to SPBs than the control. Although this was not the case, there were some indications of a repelling/masking effect (to be confirmed by further experiments) of the onions and *D. uncinatum* for the first group of mixtures and tomato and wild tomato for the second group of mixtures. Female SPBs spent relatively less time in odour fields from the mixtures of sweet potato + onions and sweet potato + tomato

(Figure 6.4a , Figure 6.4b). Furthermore the number of butterflies which spent more than 25% of the total time spent in all odour fields was lowest for these mixtures (Table 6.4a, Table 6.4b). The female SPBs showed a similar trend in their response to the mixture of sweet potato + *D. uncinatum* and sweet potato + wild tomato. Conversely, the butterflies spent relatively more time in the odours from the mixtures sweet potato + *M. minutiflora* and sweet potato + garlic (Figure 6.4a, Figure 6.4b, Table 6.4a, Table 6.4b). In particular, one butterfly laid eggs in the narrow end of the odour field of the mixture sweet potato + *M. minutiflora* suggesting that the butterfly responded as if it was in the presence of sweet potato plant volatiles alone. Hence, *M. minutiflora* and garlic were considered as not being capable of repelling/masking sweet potato plant volatiles.

6.4 Conclusion

Although there was no convincing significant response of SPBs to sweet potato plant volatiles in the olfactometer compared to the control, there were some indications which suggested that onions, tomato, wild tomato and *D. uncinatum* might repel or mask sweet potato volatiles from female SPBs. The suspected repelling/masking effect of these plants would have to be confirmed in a more appropriate experimental setting.

Chapter 7

Screening of odour masking and/or repellent plants to *Acraea acerata* in a wind tunnel

Chapter 7 Screening of odour masking and/or repellent plants to *Acraea acerata* in a wind tunnel

7.1 Introduction

An initial screening of potential odour masking/repellent plants had been done using olfactometer bioassays. Though the results of those bioassays were not conclusive, they provided some indications which suggested that the volatiles from the mixtures of sweet potato + onion, sweet potato + *Desmodium* and sweet potato + tomato plants might be masking volatiles from sweet potato plants or repelling to the SPB with the effect of disorienting the host plant seeking female SPB. Since wind tunnel assays simulate reality better than olfactometers, it was decided to carry on the screening for repellency (to SPBs) or masking (of sweet potato volatiles) of the mixtures of sweet potato with the three plants: tomato, onions, and *D. uncinatum*. Moreover, as the results which suggested the attractiveness of sweet potato plant volatiles to female SPBs were obtained in wind tunnel bioassays (Chapter 4, Chapter 5), equally the disruption of that attractiveness, if any, would be well established using wind tunnel bioassays.

Unlike the olfactometer used in the previous chapter, the wind tunnel, as described in Chapter 3 (Figure 3.1), offered a relatively large space which allowed butterflies to respond (or not) to volatile stimuli without much hindrance. Although the wind tunnel bioassays could not investigate the repellency of the volatiles tested because the experimental setting did not allow butterflies to move away from the odour due especially to the presence of the wind at a relatively constant speed which carried plant volatiles uniformly (at least theoretically) downwind (Dogan and Rossignol, 1999), it was, however, appropriate to study and compare the responses of female SPBs to the volatiles from the mixtures of selected plants + sweet potato plants. The aim of the bioassays was to study the responses of the mated female SPB to the volatiles from the mixtures of sweet potato + onion plants, sweet potato + *Desmodium* plants and sweet potato + tomato plants in two phases. Phase 1: use of volatiles emanating from whole plants, and phase 2: use of volatiles collected using

headspace entrainment. Only plants which had shown some effects on the response of the SPB to its host plant volatiles in phase 1 were considered for the second phase.

7.2 Materials and Methods

7.2.1 Materials

Sweet potato, tomato, onion and *Desmodium* plants were grown in the glasshouse as described in point 6.2.2.1 but they were not mixed in the same pot with sweet potato plants. Tomato, onion and *Desmodium* plants were four to six weeks old after transplanting when they were used in the bioassays whereas sweet potato plants were four to six weeks old after planting. Butterflies were reared as described in Chapter 3 (point 3.2.1.1). The wind tunnel was described in Chapter 3 (point 3.2.1.3). Volatiles were collected using the same equipment described in Chapter 5 (point 5.2.1).

7.2.2 Methods

7.2.2.1 Bioassays using whole plants as sources of volatiles

Two day old, mated and naive butterflies were offered four treatments: ambient air (6 pots with peat only), sweet potato plants (6 pots of plants), potential repellent/masking plants (6 pots of tomato, *Desmodium* or onion plants) and mixture sweet potato + potential odour masking/repellent plants (3 pots of sweet potato plants + 3 pots of potential odour masking/repellent plants). The plants were arranged in a grid of 2 × 3 behind the muslin screen. In the case of mixtures, sweet potato plants were alternated with potential odour masking/repellent plants in 1:1 ratio. Each of the twenty individual female SPBs (per treatment) was observed for a period of 30 minutes. The releasing of SPBs was done as described in Chapter 3 (point 3.2.2). As in previous wind tunnel bioassays, average distance moved, time allocated by butterflies to different activities as well as landing on treatments were recorded. The observations were done between 10h30 min and 17h30 min in June 1999. During the bioassays, the average temperature was 28.1 ± 0.1 °C and the average wind speed was 27.97 ± 0.19 cm/s. The bioassays were planned and executed as a 2 × 2 factorial experiment and the data collected were analysed using Genstat 5, Release 3.2 (PC/Windows/Win32s) for average distances moved by butterflies and the time they

spent moving (as in Chapter 4, point 4.2.2). The normality of the data was checked using Genstat command: DAPLOT fitted, normal, halfnormal, histogram. The proportion as well as the frequency of landings of butterflies were analysed using a Chi-square test performed by MINITAB for Windows (Release 11.1).

7.2.2.2 Bioassays using plant volatiles collected by headspace entrainment

Volatiles from sweet potato, onion and *Desmodium* plants were collected as described in Chapter 5 (point 5.2.2.1). The bioassay was run in the same way as detailed in Chapter 5, and only the differences are given here. For onion and *Desmodium* plant volatile collection, whole plants of onion and *Desmodium* were unearthed, the roots washed in tap water and used as whole plants for volatile collection. The aim of the bioassay was to compare the response of female SPBs to the interactions of volatiles from sweet potato + onion plants, sweet potato + *Desmodium* plants and sweet potato plant volatiles alone. As the main effects were not a concern, the experimental set-up was simplified and only four treatments were offered to the butterflies: ambient clean air, sweet potato plant volatiles alone, sweet potato + *Desmodium* plant volatiles (1:1) and sweet potato + onion plant volatiles (1:1). 16 butterflies were observed for each treatment. Analysis of variance (ANOVA) on average distances moved by butterflies was performed using Genstat 5, Release 3.2 (PC/Windows/Win32s). The normality of the data was checked using Genstat command: DAPLOT fitted, normal, halfnormal, histogram. When the ANOVA showed statistically significant differences between treatments, Fisher's Least Significant Difference (LSD) comparison (P = 0.05) (in Genstat) was used to reveal means which were different. The data on the time butterflies allocated to different activities, and the proportion as well as the frequency of landings were analysed using a Chi-square test performed by MINITAB for Windows (Release 11.1).

7.3 Results and Discussion

7.3.1 Results

7.3.1.1 Bioassays using whole plants as sources of volatiles

For the mixture sweet potato + onion plants, there were no statistically significant effects of the main factors ($P > 0.05$) on the mean average distances moved by butterflies but the interaction of the two factors (i.e. sweet potato plants and onion plants) had a statistically significant effect on the distance moved by SPBs for the observation periods 1-10 minutes (Table 7.1a, $P < 0.05$), 11-20 minutes (Table 7.1b, $P < 0.05$) and 21-30 minutes (Table 7.1c, $P < 0.05$).

Although the percentage and the frequency of landings, and the time SPBs spent moving in the wind tunnel were not statistically different between the two factors (sweet potato plants and onion plants) or their interaction, they were consistently the lowest when SPBs were in the presence of volatiles emanating from onion plants (alone or in mixture with sweet potato plants) (Table 7.1d, Table 7.1e, $P > 0.05$).

Considering the mixture sweet potato + *Desmodium* plants, neither the main effects nor the effect of interaction were statistically significant for all the parameters analysed (average distances moved towards treatments, time butterflies spent moving, landing and frequency of landing) (Table 7.2a, Table 7.2b, Table 7.2c, Table 7.2d, Table 7.2e, $P > 0.05$). However, it was observed that, in most cases, the presence of *Desmodium* plants was having a positive effect on the movement of female SPBs towards the sources of volatiles (Table 7.2f).

The response of female SPBs to the volatiles emanating from the mixtures of sweet potato + tomato plants did not show any statistically significant effect of the main factors (sweet potato plants and tomato plants) nor their interaction for all the parameters analysed (Table 7.3a, Table 7.3b, Table 7.3c, Table 7.3d, Table 7.3e, $P > 0.05$). Unlike the presence of *Desmodium* plants, tomato plants, when offered

together with sweet potato plants, negatively affected the movement of SPBs towards the sources of sweet potato plant volatiles (Table 7.3f).

Table 7.1a Repelling/odour masking effects of volatiles given off by sweet potato + onion plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 1-10 minutes.

Sweet potato plants	Onion plants		Sweet potato means
	No	Yes	
No	59.6	56.1	57.8
Yes	87.0	38.5	62.7
Onion means	73.3	47.3	
Effects (± SE)			
Sweet potato plant effect	4.9 ± 10.4 (n=40)		
Onion plant effect	-26.0 ± 10.4 (n=40)		
Sweet potato × onion effect	-45 ± 20.8 (n=20)		

Table 7.1b Repelling/odour masking effects of volatiles given off by sweet potato + onion plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 11-20 minutes.

Sweet potato plants	Onion plants		Sweet potato means
	No	Yes	
No	61.9	73.0	67.4
Yes	96.9	46.1	71.5
Onion plant means	79.4	59.5	
Effects (± SE)			
Sweet potato plant effect	4.1 ± 12.4 (n=40)		
Onion plant effect	-19.9 ± 12.4 (n=40)		
Sweet potato × onion effect	61.9 ± 24.8 (n=20)		

Table 7.1c Repelling/odour masking effects of volatiles given off by sweet potato + onion plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 21-30 minutes.

Sweet potato plants	Onion plants		Sweet potato means
	No	Yes	
No	69.2	82.0	75.6
Yes	94.0	52.3	73.1
Onion plant means	81.6	67.1	
Effects (± SE)			
Sweet potato plant effect	-2.5 ± 12.8 (n=40)		
Onion plant effect	-14.5 ± 12.8 (n=40)		
Sweet potato × onion effect	-54.5 ± 25.7 (n=20)		

Table 7.1d Repelling/odour masking effects of volatiles given off by sweet potato + onion plants on the mean time (min) female *Acraea acerata* spent moving (walking + flying) in a wind tunnel.

Sweet potato plants	Onion plants		Sweet potato plant means
	No	Yes	
No	4.40	4.15	4.28
Yes	6.55	5.40	5.97
Onion plant means	5.47	4.78	
Effects (± SE)			
Sweet potato plant effect	1.68 ± 1.01 (n=40)		
Onion plant effect	-0.69 ± 1.01 (n=40)		
Sweet potato × Onion effect	-0.9 ± 2.02 (n=20)		

Table 7.1e Landing and frequency of landing of butterflies in the presence of volatiles given off by sweet potato plants, onion plants and their mixture in a wind tunnel.

Source of volatiles	Landing of butterflies (%) (n =20)	Butterflies (%) which landed more than once (n = 20)
1. Clean ambient air	50	30
2. Sweet potato plants	55	40
3. Onion plants	45	25
4. Sweet potato + onion plants	30	30
All treatments	NS	NS

NS: Non statistically significant differences ($\chi^2_{df=3}$ =2.83, P > 0.05 for landing, $\chi^2_{df=3}$ =3.26, P > 0.05 for frequency of landing)

Table 7.2a Repelling/odour masking effects of volatiles given off by sweet potato + *Desmodium* plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 1-10 minutes.

Sweet potato plants	*Desmodium* plants		Sweet potato plant mean
	No	Yes	
No	66.3	55.2	60.7
Yes	56.1	55.2	55.8
Desmodium plant mean	61.3	55.2	SED for comparing the combination of means = 15.5 (n = 20); SED for comparing main effect means = 10.9 (n = 40)

Table 7.2b Repelling/odour masking effects of volatiles given off by sweet potato + *Desmodium* plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 11-20 minutes.

Sweet potato plants	*Desmodium* plants		Sweet potato plant mean
	No	Yes	
No	84.7	96.1	90.2
Yes	90.2	92.3	91.1
Desmodium plant mean	87.6	94.0	SED for comparing the combination of means = 19.5 (n = 20); SED for comparing main effect means = 13.8 (n = 40)

Table 7.2c Repelling/odour masking effects of volatiles given off by sweet potato + *Desmodium* plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 21-30 minutes.

Sweet potato plants	*Desmodium* plants		Sweet potato plant mean
	No	Yes	
No	103.6	97.8	100.7
Yes	85.5	109.5	97.8
Desmodium plant mean	94.6	103.6	SED for comparing the combination of means = 18.6 (n = 20); SED for comparing main effect means = 13.1 (n = 40)

Table 7.2d Repelling/odour masking effects of volatiles given off by sweet potato + *Desmodium* plants on the mean time (min) female *Acraea acerata* spent moving (walking + flying) in a wind tunnel.

Sweet potato plants	*Desmodium* plants		Sweet potato plant mean
	No	Yes	
No	3.95	4.45	4.20
Yes	5.75	5.05	5.40
Desmodium plant mean	4.85	4.75	SED for comparing the combination of means =1.291 (n =20); SED for comparing main effect means = 0.913 (n = 40)

Table 7.2e Landing and frequency of landing of butterflies in the presence of volatiles given off by sweet potato plants, *Desmodium* plants and their mixture in a wind tunnel.

Source of volatiles	Landing of butterflies (%) (n =20)	Butterflies (%) which landed more than once (n = 20)
1. Clean ambient air	75	35
2. Sweet potato plants	65	40
3. *Desmodium* plants	65	35
4. Sweet potato + *Desmodium* plants	45	40
All treatments	NS	NS

NS: Non statistically significant differences ($\chi^2_{df=3} = 4.05$, $P > 0.05$ for landing, $\chi^2_{df=3} = 0.213$, $P > 0.05$ for frequency of landing)

Table 7.2f Trends of the effects of volatiles given off by the mixture of sweet potato + *Desmodium* plants on the mean average distances moved by female *Acraea acerata* in a wind tunnel.

Observation period	Source of plant volatiles	Effect on mean distance (cm) ± SE	Trends of the effects
1-10 minutes	Sweet potato	−5.2 ± 11 (n = 40)	−
	Desmodium	− 6.1 ± 11 (n = 40)	−
	Sweet potato + *Desmodium*	10.2 ± 22 (n = 20)	+
11-20 minutes	Sweet potato	0.9 ± 14 (n = 40)	+
	Desmodium	6.7 ± 14 (n = 40)	+
	Sweet potato + *Desmodium*	−9.6 ± 27.6 (n = 20)	−
21-30 minutes	Sweet potato	−3.2 ± 13.1 (n = 40)	−
	Desmodium	9 ± 13.1 (n = 40)	+
	Sweet potato + *Desmodium*	29.5 ± 26.2 (n = 20)	+

Table 7.3a Repelling/odour masking effects of volatiles given off by sweet potato + tomato plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 1-10 minutes.

Sweet potato plants	Tomato plants		Sweet potato plant mean
	No	Yes	
No	37.1	52.5	44.9
Yes	50.5	33.3	41.7
Tomato plant mean	43.8	42.9	SED for comparing the combination of means = 13.5 (n = 20); SED for comparing main effect means = 9.6 (n = 40)

Table 7.3b Repelling/odour masking effects of volatiles given off by sweet potato + tomato plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 11-20 minutes.

Sweet potato plants	Tomato plants		Sweet potato plant mean
	No	Yes	
No	50.2	85.0	67.5
Yes	71.8	74.2	73
Tomato plant mean	61.0	79.4	SED for comparing the combination of means = 20.1 (n = 20); SED for comparing main effect means = 14.2 (n = 40)

Table 7.3c Repelling/odour masking effects of volatiles given off by sweet potato + tomato plants on the mean average distances (cm) moved by female *Acraea acerata* in a wind tunnel for the observation period 21-30 minutes.

Sweet potato plants	Tomato plants		Sweet potato plant mean
	No	Yes	
No	65.1	91.9	78.5
Yes	79.7	78.5	79.1
Tomato plant mean	72.4	85.3	SED for comparing the combination of means = 20.8 (n = 20); SED for comparing main effect means = 14.7 (n = 40)

Table 7.3d Repelling/odour masking effects of volatiles given off by sweet potato + tomato plants on the mean time (min) female *Acraea acerata* spent moving (walking + flying) in a wind tunnel.

Sweet potato plants	Tomato plants		Sweet potato plant mean
	No	Yes	
No	3.90	4.55	4.22
Yes	6.85	4.20	5.53
Tomato plant mean	5.37	4.37	SED for comparing the combination of means =1.593 (n = 20); SED for comparing main effect means = 1.126 (n = 40)

Table 7.3e Landing and frequency of landing of butterflies in the presence of volatiles given off by sweet potato plants, tomato plants and their mixture in a wind tunnel.

Source of volatiles	Landing of butterflies (%) (n =20)	Butterflies (%) which landed more than once (n = 20)
1. Clean ambient air	40	20
2. Sweet potato plants	55	50
3. Tomato plants	50	35
4. Sweet potato + tomato plants	40	30
All treatments	NS	NS

NS: Non statistically significant differences ($\chi^2_{df=3} = 1.36$, $P > 0.05$ for landing, $\chi^2_{df=3} = 4.19$, $P > 0.05$ for frequency of landing)

Table 7.3f Trends of the effects of volatiles given off by the mixture of sweet potato + tomato plants on the mean average distances moved by female *Acraea acerata* in a wind tunnel.

Observation period	Source of plant volatiles	Effect on mean distance (cm) ± SE	Trends of the effects
1-10 minutes	Sweet potato	–2.9 ± 9.6 (n = 40)	–
	Tomato	– 0.87 ± 9.6 (n = 40)	–
	Sweet potato + tomato	–32.7 ± 19.1 (n = 20)	–
11-20 minutes	Sweet potato	5.2 ± 14.2 (n = 40)	+
	Tomato	18.4 ± 14.2 (n = 40)	+
	Sweet potato + tomato	–32.7 ± 28.5 (n = 20)	–
21-30 minutes	Sweet potato	0.87 ± 14.7 (n = 40)	+
	Tomato	12.8 ± 14.7 (n = 40)	+
	Sweet potato + tomato	–28.0 ± 29.4 (n = 20)	–

7.3.1.2 Bioassays using plant volatiles collected by headspace entrainment

The screening of odour masking/repellent plants using whole plants as source of volatiles pointed to onion plants as having an odour masking/repellent effect on female SPBs when onions were mixed with sweet potato plants. By contrast, it appeared that the volatiles emanating from the mixture of sweet potato + *Desmodium* plants were having a more attracting effect on female SPBs than the volatiles from sweet potato plants alone. Therefore volatiles from sweet potato, onion and *Desmodium* plants were collected and the results presented below show the responses of female SPBs to the presence of the different volatiles in a wind tunnel.

Statistically significant differences were found between mean average distances moved by female SPBs in the presence of volatiles collected from sweet potato plants and the mixtures of volatiles collected from sweet potato + onion plants and sweet potato + *Desmodium* plants for the observation periods 1-10 minutes ($P < 0.001$, df = 4, Figure 7.1), 11-20 minutes ($P < 0.001$, df = 4, Figure 7.2) and 21-30

minutes ($P < 0.01$, df = 4, Figure 7.3). During the whole period of observation, mean average distances moved by SPBs in the presence of the mixtures of volatiles collected from sweet potato + *Desmodium* plants were very high. Conversely, mean average distances moved by SPBs in the presence of the mixture of volatiles from sweet potato + onion plants were very low (Figure 7.1, Figure 7.2, Figure 7.3).

There were very highly significant differences between the proportion of time butterflies spent moving in the presence of different volatiles ($\chi^2_{df=4} = 44.922$, $P < 0.001$) and SPBs spent more time in the mixture of volatiles from sweet potato + *Desmodium* plants and less time in the mixture of volatiles from sweet potato + onion plants (Table 7.4). Statistically significant differences were also detected in butterfly landings ($\chi^2_{df=4} = 9.94$, $P < 0.05$) with more landings on the mixture of volatiles from sweet potato + *Desmodium* plants and less landings on the mixture of volatiles from sweet potato + onion plants (Table 7.4). There was a similar trend with the frequency of landings.

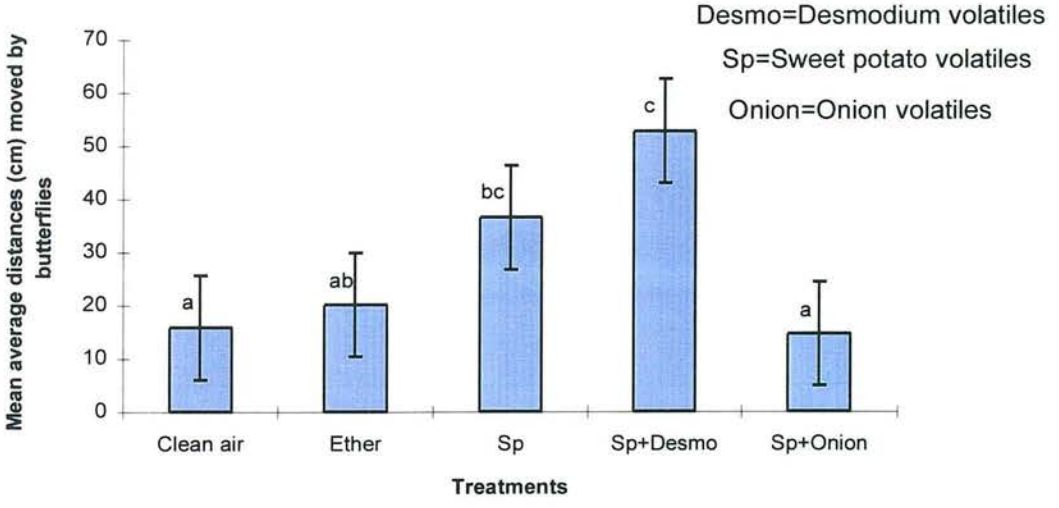


Figure 7.1 Mean average distances (cm) (± SED) moved by female *Acraea acerata* in a wind tunnel in the presence of volatiles from sweet potato plants and the mixtures with onion and *Desmodium* plant volatiles (ether as solvent) during the period 1-10 minute of the observation time (n = 16). Means with different letters are statistically different ($P < 0.05$, LSD test).

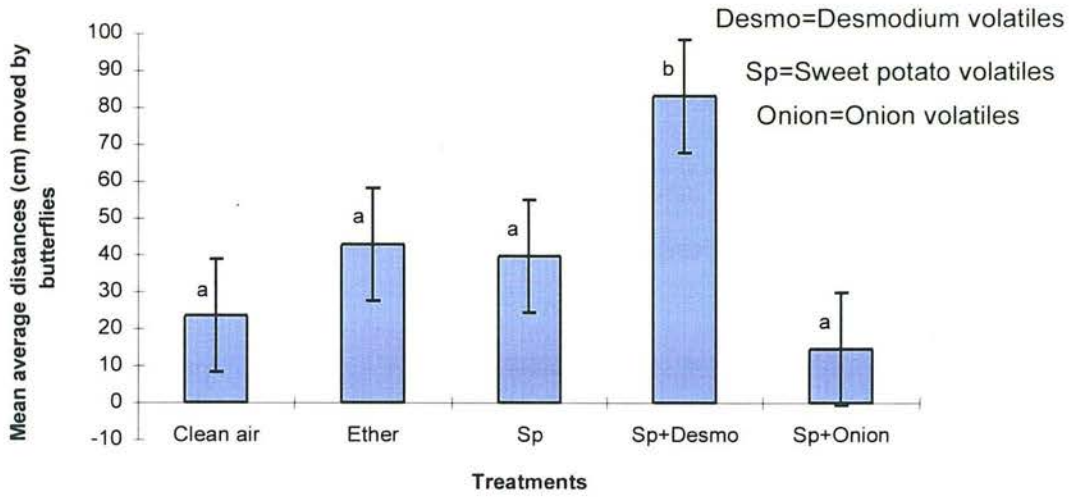


Figure 7.2 Mean average distances (cm) (± SED) moved by female *Acraea acerata* in a wind tunnel in the presence of volatiles from sweet potato plants and the mixtures with onion and *Desmodium uncinatum* plant volatiles (ether as solvent) during the period 11-21 minutes of the observation time (n = 16). Means with different letters are statistically different ($P < 0.05$, LSD test).

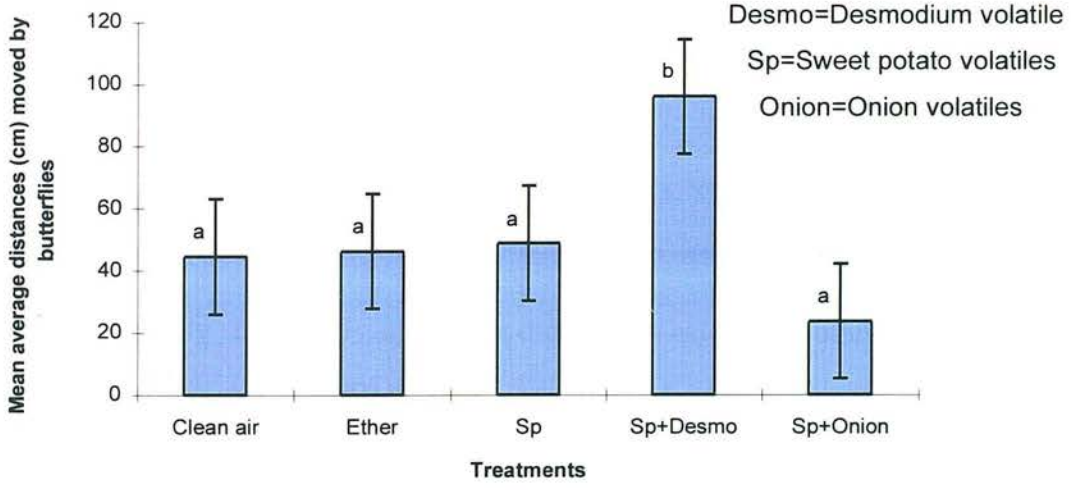


Figure 7.3. Mean average distances (cm) (± SED) moved by female *Acraea acerata* in a wind tunnel in the presence of volatiles from sweet potato plants and their mixtures with onion and *Desmodium uncinatum* plant volatiles (ether as solvent) during the period 21-30 minutes of the observation time (n = 16). Means with different letters are statistically different ($P < 0.05$, LSD test).

Table 7.4 Response of female *Acraea acerata* to the presence of sweet potato plant volatiles, the mixture of sweet potato + *Desmodium* plant volatiles and sweet potato + onion plant volatiles in a wind tunnel.

Source of volatiles	Time (%) spent by butterflies moving (total time = 480 minutes)	Landing of butterflies (%) (n = 16)	Butterflies (%) which landed more than once (n = 16)
1.Clean ambient air	18.5	12.5	0
2.Ether	21	12.5	6
3.Sweet potato plants	26	31	25
4.Sweet potato + *Desmodium* plants	31	50	44
5.Sweet potato + onion plants	15	12.5	6
All treatments	***	*	**
1 vs 2	NS	NS	NS
2 vs 3	*	NS	NS
3 vs 4	NS	NS	NS
3 vs 5	***	NS	NS
4 vs 5	***	*	*

*** :Very highly statistically significant differences between sources of volatiles ($\chi^2_{df=4}$ or $\chi^2_{df=1}$, $P < 0.001$)

** :Highly statistically significant differences between sources of volatiles ($\chi^2_{df=4}$, $P < 0.01$)

* :Statistically significant differences between sources of volatiles ($\chi^2_{df=4}$ or $\chi^2_{df=1}$, $P < 0.05$)

NS: Non statistically significant differences ($\chi^2_{df=1}$, $P > 0.05$)

7.3.2 Discussion

The possibility of masking host plant volatiles which attract phytophagous insects by mixing host plants with non host plants has been reported as one of the mechanisms involved in the reduction of pest damage in intercropping (Perrin and Phillips, 1978, Hill, 1983, Nottingham, 1988, Altieri, 1994, Finch, 1996). The results of the previous chapter suggested that volatiles given off by onions, *Desmodium* and tomato plants had an odour masking effect on sweet potato plant volatiles or a repellent effect on SPBs when a mixture of one of these plants with sweet potato plants was offered to ovipositing female SPBs in a four-armed olfactometer. The results of the wind tunnel bioassays using whole plants as sources of volatiles did not support that suggestion with the exception of onion plants.

The mean average distances moved by SPBs towards the source of sweet potato plant volatiles was considerably reduced when onion plants were mixed with sweet potato plants ($P < 0.05$, Table 7.1a) for the first 10 minutes of the observation period. When the SPB stayed longer in the presence of the mixture of sweet potato + onion plant volatiles, the relative negative effect of the mixture sweet potato + onion plant volatiles on the average distances moved by butterflies increased ($P < 0.05$, Table 7.1b for the period 11-20 minutes and Table 7.1c for the period 21-30 minutes). It could be argued that as the SPBs were spending more time exposed to the mixture of sweet potato + onion plant volatiles, they responded increasingly negatively to it. The negative effect of onion plant volatiles alone was almost half that of the mixture for the first ten minutes ($P < 0.05$, Table 7.1a) whereas for the second and third ten-minute observation periods, it was almost less than a third of that of the mixture of volatiles from sweet potato + onion plants ($P > 0.05$ for the second and third ten minutes). Plant odours are known to be made of complex blends of many chemical compounds. The mixtures of some of these compounds were found to act synergistically to attract herbivorous insects (Visser, 1986). For instance *Delia antiqua* and *Hylemya platura* (Diptera) were attracted to traps by the synergistic effect of the mixture of 2-phenylethanol and pentanoic acid (Ishakawa, Matsumoto, Tsutsumi and Mitui, 1984). Interestingly, there seemed to be a negative synergistic

effect of the onion + sweet potato plant volatiles as the effect of the interaction of onion + sweet potato plant volatiles was greater (in absolute value) than either that of sweet potato plant volatiles or onion plant volatiles. These results suggested that the mixture of volatiles from sweet potato + onion plants was having an important negative effect on the ability of female SPBs to respond positively to its host plant volatiles.

As there was no statistically significant difference between the mean times the butterflies spent moving in the presence of sweet potato plant volatiles alone and the mixture of volatiles from sweet potato + onion plants, it appeared that butterflies spent relatively similar amounts of time moving (walking + flying) in the presence of different plant volatiles ($P > 0.05$, df =2). However, unlike the case of butterflies in the presence of sweet potato plant volatiles alone, the movement of butterflies in the presence of the mixture of volatiles from sweet potato + onion plants did not result in bringing them closer to the source of the mixture of volatiles (Table 7.1a, Table 7.1b, Table 7.1c). Furthermore, the proportions of butterfly landings on the muslin screen in the presence of sweet potato plant volatiles, onion plant volatiles and their mixture were not statistically different ($\chi^2_{df=3} = 2.828$, $P > 0.05$). The muslin screen (on which butterflies landed) was the nearest point to the volatile sources the SPBs could reach. With similar proportions of landings and similar amounts of time spent moving by SPBs, similar mean average distances moved by SPBs in the presence of the different odour sources were expected. But as discussed above, mean average distances moved by SPBs towards the source of the mixture of volatiles from sweet potato + onion plants were statistically different from the other volatiles ($P < 0.05$). Therefore it could be argued that female SPBs that moved and landed (on the muslin screen) in the presence of the mixture of volatiles from sweet potato + onion plants were probably attempting to avoid the mixture of volatiles from sweet potato + onion plants.

It is generally accepted that phytophagous insects use non host plant volatile stimuli to avoid non host plants (Renwick and Radke, 1988, Renwick, 1989). If female SPBs

were trying to avoid the odour plume from the mixture of volatiles emanating from sweet potato + onion plants, this may indicate that, in ideal conditions (field for instance), the butterflies would have left it and consequently given up following the odour and reaching its source as it would be expected if it was made of their host plant volatiles alone (Murlis *et al.*, 1992). Unfortunately, the wind tunnel set up used could not allow such an observation to be made (Dogan and Rossignol, 1999).

Further evidence of the negative effect of the mixture of volatiles from sweet potato + onion plants on the response of female SPBs to their host plant volatiles was obtained by using a mixture of volatiles collected from sweet potato and onion plant headspaces. The investigation was carried out in a wind tunnel bioassay which sought to compare the responses of female SPBs to headspace collected volatiles from sweet potato plants, the combination of sweet potato + onion plants and sweet potato + *Desmodium* plants.

The mixture of sweet potato + *Desmodium* plant volatiles were included in the comparison because of the unexpected positive effect volatiles from *Desmodium* plants and/or mixture of sweet potato + *Desmodium* plants had on the movement of female SPBs towards volatile sources in wind tunnel bioassays which used whole plants. Though there was no statistically significant difference between the response of female SPBs to the mixture of volatiles given off by sweet potato + *Desmodium* plants and sweet potato plants alone, the effect of the mixture of volatiles from both plants and/or the effect of *Desmodium* plant volatiles alone on the mean average distances moved by butterflies was higher than any other source of volatiles (Table 7.2f). It was therefore decided to use the volatiles collected from sweet potato and *Desmodium* plants to investigate if the response of female SPBs to the mixture of these volatiles followed the same trend as observed with volatiles from whole plants.

Like *Desmodium* plants, tomato plants did not interfere significantly with the response of female SPBs to sweet potato plant volatiles either for the mean average distances moved towards volatile sources (though the presence of tomato plant

volatiles seemed to affect negatively the movement of SPBs towards the source of its host plant volatiles (Table7.3f)), the time spent moving ($P > 0.05$) or for landing of SPBs ($\chi^2_{df=3} = 1.36$, $P > 0.05$ for the proportion of butterfly landing and $\chi^2_{df=3} = 4.193$, $P > 0.05$ for the frequency of landings). Although, tomato plant volatiles had been reported as having a repellent or odour masking effect on a number of phytophagous insects including some Lepidoptera like *P. xylostella* (Perrin and Phillips, 1978), *P. operculla* (CIP, 1983) and *P. rapae* (Wu *et al.*, 1999), they did not seem to show an important effect on the response of female SPBs to sweet potato plant volatiles. Therefore tomato plants were abandoned and were not included in the bioassay which used headspace collected volatiles.

The analysis of variance of average distances moved by female SPBs in the presence of ambient clean air, ether (solvent), sweet potato plant volatiles, sweet potato + onion plant volatiles and sweet potato + *Desmodium* plant volatiles revealed very highly significant differences between the responses of SPBs to these different volatiles for the observation periods 1-10 and 11-20 minutes ($P < 0.001$, $df = 3$) and highly significant differences for the observation period 21-30 minutes ($P < 0.01$, $df = 3$).

For the observation period 1-10 minutes, the differences were mainly due to the relatively large distances moved by female SPBs towards sweet potato plant volatiles and sweet potato + *Desmodium* plant volatiles (Figure 7.1, $P < 0.05$). The mixture of sweet potato + *Desmodium* plant volatiles provoked the highest response from SPBs. Apart from sweet potato, the response of SPBs to the mixture of sweet potato + *Desmodium* plant volatiles was statistically different from all of the other volatiles. The same positive high response to the mixture of sweet potato + *Desmodium* plant volatile was observed for the periods 11-20 and 21-30 minutes when statistically significant differences were detected even between mean average distances moved by female SPBs towards the mixture of sweet potato + *Desmodium* plants and sweet potato plant volatiles alone ($P < 0.05$, Figure 7.2, Figure 7.3). Female SPBs spent more time, landed in larger proportion and more often in the presence of the mixture

of sweet potato + *Desmodium* plant volatiles than any other source of volatiles (Table 7.4). Although there was no statistically significant difference between the time (%) SPBs spent moving, the percentage and the frequency of butterfly landings in the presence of the mixture of volatiles from sweet potato + *Desmodium* plants and sweet potato plant volatiles alone, SPBs seemed to spend more time moving, landed more frequently and in larger number when sweet potato volatiles were offered with *Desmodium* plant volatiles than when they were offered alone. The results supported the previous supposition that the mixture of volatiles from sweet potato + *Desmodium* plants might be more attractive to female SPBs than sweet potato plant volatiles alone. This was a startling finding because *Desmodium* species had never been reported as SPB host plants (Lefèvre, 1948, Smit *et al.*, 1997, Azerfegne, 1999) and they are not part of the Convolvulaceae family to which belong all recorded host plants of the SPB.

Compared to the time SPBs spent moving in the presence of ether (solvent), there was a statistically significant difference between the time SPBs spent walking and flying in the presence of sweet potato plant volatiles ($\chi^2_{df=1} = 4.232$, $P < 0.05$, Table 7.4). Considering the average distances moved, SPBs moved longer distances towards the sweet potato plant volatiles than they did in the presence of the solvent for the observation period 1-10 minutes although no statistically significant differences were detected ($P > 0.05$, Figure 7.1). Likewise, there was no statistically significant difference between landings and frequency of landings in the presence of the solvent and sweet potato plant volatiles even though the response of SPBs to sweet potato plant volatiles was consistently higher (Table 7.4). However, there are clear indications that sweet potato plant volatiles are attractive to female SPBs as discussed in Chapters 3 and 4.

7.4 Conclusion

The presence of onion plant volatiles (from whole plants or headspace collected) in a wind tunnel affected negatively the movement of female SPBs towards the sources of their host plant volatiles and decreased their level of activities (walking, flying,

landing). Conversely, the presence of *Desmodium* plant volatiles (from whole plants or headspace collected) affected positively the movement of female SPBs towards the sources of their host plant volatiles and increased their level of activities. The results revealed two very interesting mixtures with opposite effects on the behaviour of female SPBs towards their host plant: the mixture of volatiles from sweet potato + *Desmodium* plants which appeared to be more attractive than sweet potato volatiles alone and the mixture of volatiles from sweet potato + onion plants which appeared to be repelling to female SPBs or masking odours from sweet potato plants.

Chapter 8

Effects of intercropping sweet potato with onion or *Desmodium* plants on *Acraea acerata*

Chapter 8 Effects of intercropping sweet potato with onion or *Desmodium* plants on *Acraea acerata*[4]

8.1 Introduction

The attractiveness of female SPBs to sweet potato plant volatiles in wind tunnel bioassays suggested that it might be possible to reach some level of control of the SPB by interfering with the response of SPBs to sweet potato plant volatiles using intercropping sweet potato with repellent and/or odour masking non host plants. The screening of some reported repellent plants in the olfactometer and the wind tunnel bioassays suggested that the volatiles from the mixtures of sweet potato + onion plants and sweet potato + *Desmodium* plants might affect the response of the host-seeking female SPB to its host plants in an intercropping system.

One of the potential advantages of intercropping is a reduction in herbivorous insect attacks. Many researchers have been trying to understand the mechanisms explaining the reduction of pest damage in diverse habitats. Diagram 8.1 gives a condensed picture of existing hypotheses and the relationships between them which would support the reduction of insect pests by intercropping. Reviewing various explanatory hypotheses, Vandermeer (1989) summarised them as: the disruptive crop, the trap crop and the enemies hypotheses. Andow (1991) reviewed the responses of arthropod herbivores to mixtures of different plant species (polycultures). He discussed proposed hypotheses explaining the responses of arthropods to polycultures in order to elaborate a common theory which would account for all the different arthropod responses to polycultures. He found that many cases of herbivore responses to plant mixtures were mostly explained by the resource concentration hypothesis (Diagram 8.1). He underlined, however, that the response of polyphagous insects and the effects of natural enemies are still unpredictable. He suggested that there might be more than one ecological explanatory theory. Altieri (1994) listed the factors involved in the reduction of insect pest incidence in mixed crops: increase of parasitoid and predator populations, higher availability of alternate

[4] A paper based on the introduction of this chapter has been published. See Published paper 3

food for natural enemies, decrease in pest colonisation and reproduction, chemical repellency, masking and/or feeding inhibition due to non host plants, physical barriers to pest movement and/or emigration, and optimum synchrony between pests and natural enemies.

Discussing the mechanisms described by Altieri (1994), Finch (1996) agreed with the mechanism of physical interference but underlined the incompleteness of available experimental data. He pointed out the need for scientific experimental proofs for the other mechanisms apart from the alteration of the host plant odour profiles which is currently being studied. In opposition to the previous mechanisms, he presented another mechanism termed 'appropriate/inappropriate landings' to describe why host plants grown in bare soil become populated with more insect pests than when host plants are undersown with another plant. All landings on host plants growing on bare soil will be 'appropriate' as insects were said to land solely on host plants, being the only green objects available. Conversely, 'inappropriate' landings occurred when host plants were undersown because 'pest species do not discriminate between host and non-host plants when both are green'(Finch, 1996). It was suggested that this mechanism might apply to phytophagous insects on the whole. However, landing by a phytophagous insect on a host plant is only one event in an involved behavioural repertoire associated with host plant location and not all pest species do not discriminate between host and non-host plants when both are green. Lepidoptera, for instance, are able to discriminate between green host and non-host plants (Bernays and Chapman, 1994).

Discovering a unique explanatory mechanism that accounts for all different reactions of all insect pests to intercropping seems unlikely at the moment and, as a consequence, consideration should be given to uncovering mechanisms with more specific types of insects. In this context, the recent successful control of maize borers (*C. partellus and B. fusca*) in Kenya by intercropping maize with *M. minutiflora* (one row of maize alternating with one row of *M. minutiflora*) (Khan *et al.*,1997b), a repellent plant, is an excellent example of the success of such an approach.

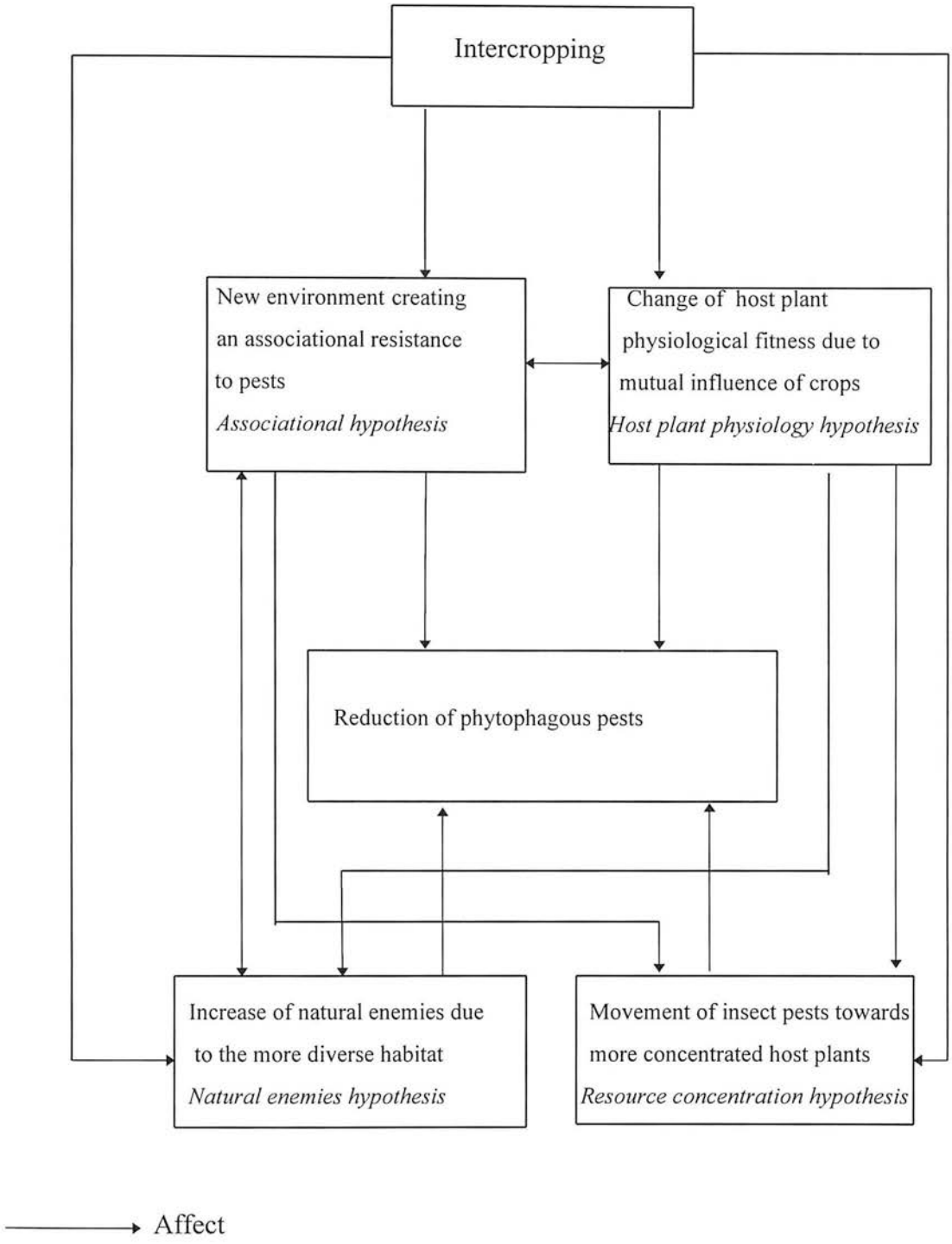


Diagram 8.1 The effects of intercropping on phytophagous pests

The aim of this preliminary field experiment was to study the effects of the intercrops sweet potato + onion plants and sweet potato + *Desmodium* plants on the attraction of

the ovipositing female SPBs to sweet potato plants. Particularly, the main interest was to test whether or not volatiles given off by sweet potato + *Desmodium* plants or sweet potato + onion plants had the effect of attracting more or less ovipositing female SPBs, as suggested by the results of laboratory experiments (Chapters 6 and 7).

8.2 Materials and Methods

8.2.1 The experimental site

The site of the field experiment was located in Uganda at Namulonge Agricultural and Animal Production Research Institute (NAARI), at the latitude of 0° 32' N and longitude 0° 32' 35'' E , 1128 m above sea level and 27 km north of Kampala (Plate 8.1). Namulonge area belongs to 'the banana-coffee system' one of the seven Ugandan agroecological zones characterised by evenly distributed bimodal rainfall with annual average of 1270 mm, mean maximum temperature around 27 °C and mean minimum temperature around 15 °C, deep and well drained tropical red clay loamy soils of medium to high productivity. The long rainy season starts in March and finishes in July whereas the short one starts in October and runs to January. In addition to banana and coffee, perennial major crops, farmers grow maize and sweet potato as secondary seasonal crops (Mwebaze, 2000).

Sweet potato is a staple crop for some regions of Uganda, the largest producer of sweet potato in Africa (Table 1.1). Though the SPB was earlier reported in few agroecological zones of Uganda, the list of infested zones is now quickly expanding and includes the 'montane system' (which includes the highlands of the south west), the 'banana coffee system' which borders the shores of Lake Victoria and the 'Teso system' which includes Soroti district (Mwebaze, 2000, Odongo, *personal communication*). The SPB is becoming a major concern for farmers in many areas of Uganda.

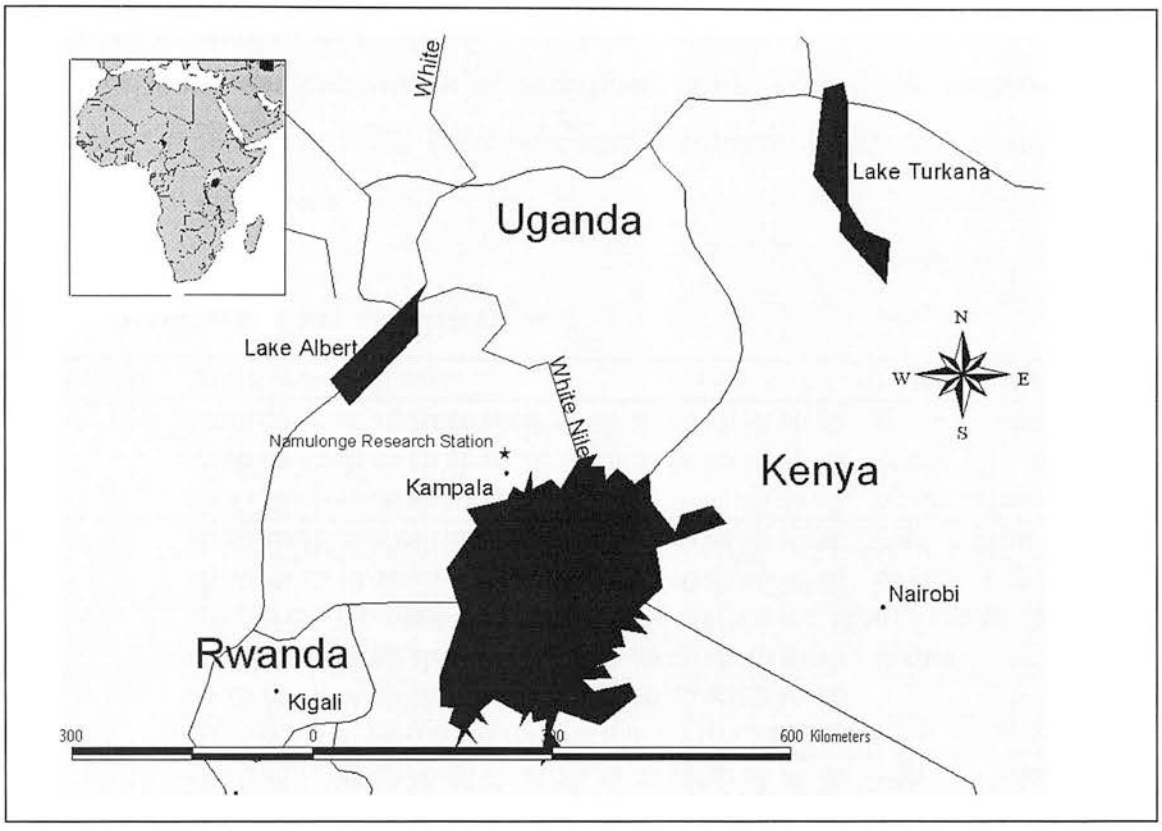


Plate 8.1 Location of Namulonge research station in Uganda

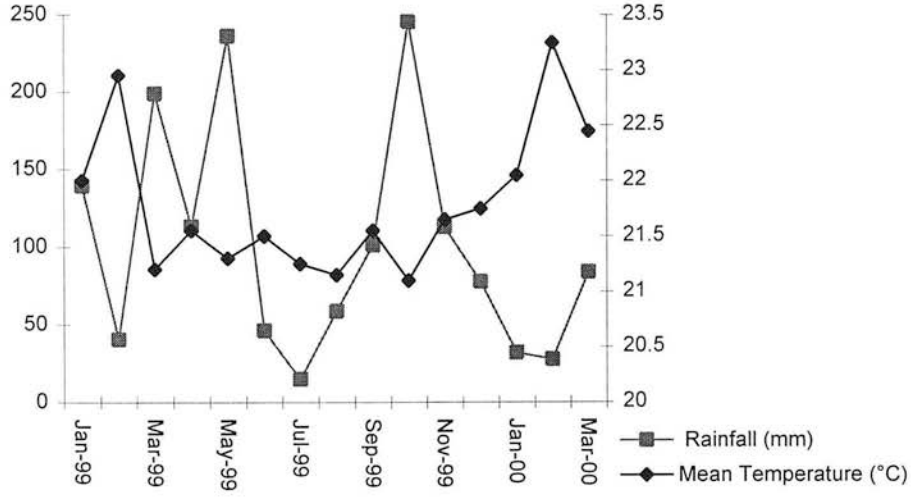


Figure 8.1 Monthly rainfall and mean monthly average temperature at Namulonge research station (Uganda).

8.2.2 Experimental design

The experiment was designed as a randomised block. Each block constituted a replicate (4 replicates in total). There were seven treatments (Table 8.1) arranged in 28 plots of 20 × 6 m each.

Table 8.1 Treatments and intercrop ratios

Treatment	Arrangement of rows	Intercrop ratio
T0	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp	**0** :no intercrop sweet potato plants alone
T1	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o	**1/2**: 1row of onions + 2 rows of sweet potato plants
T2	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o	**1/1**: 1 row of onions + 1 row of sweet potato
T3	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o o	**2/1**: 2 rows of onions + 1 row of sweet potato
T4	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d	**1/2**: 1 row of *Desmodium* + 2 rows of sweet potato
T5	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d	**1/1**: 1 row of *Desmodium* + 1 row of sweet potato
T6	sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp sp d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d d	**2/1**: 2 rows of *Desmodium* + 1 row of sweet potato

sp: sweet potato plants; o: onion plants; d : *Desmodium* plants

8.2.3 Field preparation, planting and weeding

The field was located at a place called Kilimantungo at Namulonge research station. The field had not been cultivated for a long time and couch grass (*Elymus repens*) was the main weed growing at the site. The field was slashed in the first week of October 1999 and the herbicide, glyphosate (Roundup (Monsanto)) was applied (at manufacturers recommended rate) to control the couch grass. The first ploughing was done on 15 October 1999 followed by a second ploughing 3 weeks later. Ridges one meter wide were made by a tractor-mounted mechanical rigger. Vine tips (0.25-0.30 m long) of sweet potato variety New Kawogo were planted at 0.3 m between the plants on the ridge tops during the second week of November 1999. Stolons of *Desmodium uncinatum* were locally obtained from the fields of Namulonge research institute and cuttings of 0.25-0.30 m planted at 0.3 m between plants at the same time as the planting of sweet potato. Onion bulbs bought in the local market were sprouted before being planted on the ridge tops at 0.15 m between plants in the fourth week of November 1999. Treatment plots were separated by 0.5 m. Weeding was done twice manually using a hoe on 14 December 1999 and at the end of February 2000. Apart from the herbicide applied before ploughing, no other agrochemical was used in the experiment.

8.2.4 Data collection and analysis

The data were collected each week starting from the last week of January 2000 until the onions were harvested in the last week of February 2000. Each time, 20 plants from every plot were randomly selected and the main information recorded was the number of egg batches laid by SPBs, the number of SPB damaged leaves per sweet potato plant, the number of damaged plants per plot, the number of SPB larvae per plant, and the score of damage caused by the SPB larvae to sweet potato plants (Appendix 8.1 for the data recording sheet). The experiment was designed to attract more or less ovipositing female SPBs depending on the treatment it contained (Table 8.1). The best indication of the number of female SPBs attracted to a plot was the number of SPB egg batches laid on sweet potato plants.

The analysis of variance of the numbers [$\log_{10}$(data +1)] of SPB larvae, damaged leaves, damaged plants, and score of damage on sweet potato plants was performed using Genstat for Windows using the commands"General Analysis of Variance, BLOCK: Plot, TREATMENTS : Control/(Intercrop*Ratio of Intercrop); COVARIATE "No Covariate" ANOVA [PRINT = aovtable, information, mean; FACT=3; FPROB=yes; PSE = diff, lsd, means] Number of larvae (or other factors). The normality of the data was checked using Genstat command: DAPLOT fitted, normal, halfnormal, histogram.

8.3 Results and Discussion

8.3.1 Some general observations

The attack of the SPB in the experimental field was low to moderate. However, in another field experiment on sweet potato plant varieties, about 1 km away, the attack of SPBs was so severe that insecticide had to be applied to control the butterfly. Moreover in farmer's fields around Namulonge station, serious attacks of SPBs were also reported.

Due to a severe drought (Figure 8.1), *Desmodium* plants were lost in early February 2000. Onions were harvested earlier than planned because of some attempts to steal them. Neither the yield of onions, nor that of sweet potato was recorded.

8.3.2 Results

The analysis of variance of the numbers [$\log_{10}$(data +1)] of SPB larvae, damaged leaves, damaged plants, and the score of damage on sweet potato plants did not show any statistically significant difference between the seven treatments ($P > 0.05$, $df = 6$) either for the average data accumulated on four consecutive weekly recordings or for each weekly data recording. However, to check for any consistent trend in the data, the following factors were considered: i) sweet potato cropping systems (sweet potato alone and sweet potato intercrops); ii) types of sweet potato intercrops (sweet potato + onion plants and sweet potato + *Desmodium* plants); iii) sweet potato

intercrop ratios and; iv) the interactions between sweet potato intercrops and intercrop ratios.

The trends of the effects of different treatments are shown in Figures 8.2 to 8.5) and the overall trend is shown in Table 8.2 for the average data (from four weekly data recordings). With sweet potato intercrops, the number of SPB larvae tended to increase which consequently increased the damage of sweet potato plants (Figure 8.2, Figure 8.3, Figure 8.4, Figure 8.5, Table 8.2). However, the two types of sweet potato intercrops had opposite effects: compared to sweet potato plants alone, sweet potato + onion plants decreased the number of SPB larvae which resulted in a decrease of damage caused to sweet potato plants whereas the intercrop sweet potato + *Desmodium* plants had an opposite effect (Figure 8.2a, Figure 8.3a, Figure 8.4a, Figure 8.5a, Table 8.2).

Considering the intercrop ratios; the ratio 1/2 had an effect of increasing the number of SPB larvae whereas the intercrop 2/1 had an opposite effect. The trend of the effects of the intercrop ratio 1/1 was variable (Figure 8.2b, Figure 8.3b, Figure 8.4b, Figure 8.5b, Table 8.2). It was variable whether sweet potato plants were intercropped with onion or *Desmodium* plants. The trend of the effects of the intercrop ratio 1/2 changed depending on the crop which was intercropped with sweet potato plants. With onion plants, the effect of the intercrop ratio 1/2 showed a trend of increasing the number of SPB larvae and their damage but opposite trend was observed when sweet potato plants were intercropped with *Desmodium* plants. The same situation occurred with the ratio 2/1: intercropping with onions tended to decrease the number of SPB larvae whereas the opposite trend was observed when sweet potato was intercropped with *Desmodium* plants (Figure 8.2c, Figure 8.3c, Figure 8.4c, Figure 8.5c, Table 8.2).

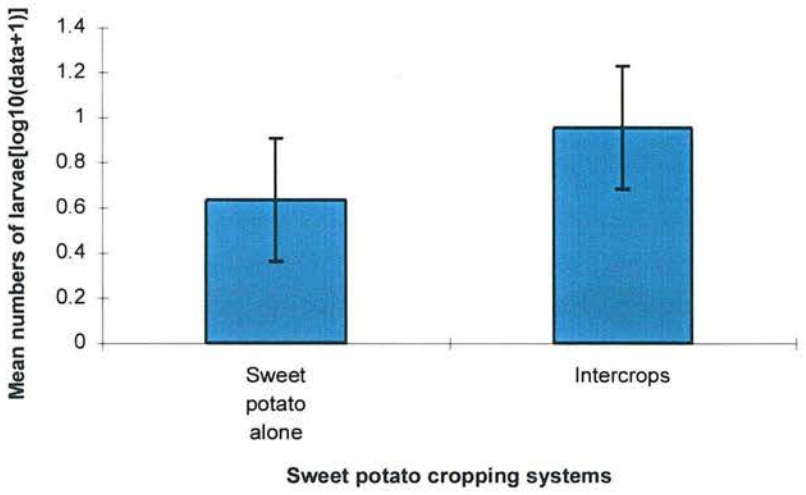


Figure 8.2 Comparison of mean numbers (± SED) of *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Average of 4 weekly data recordings).

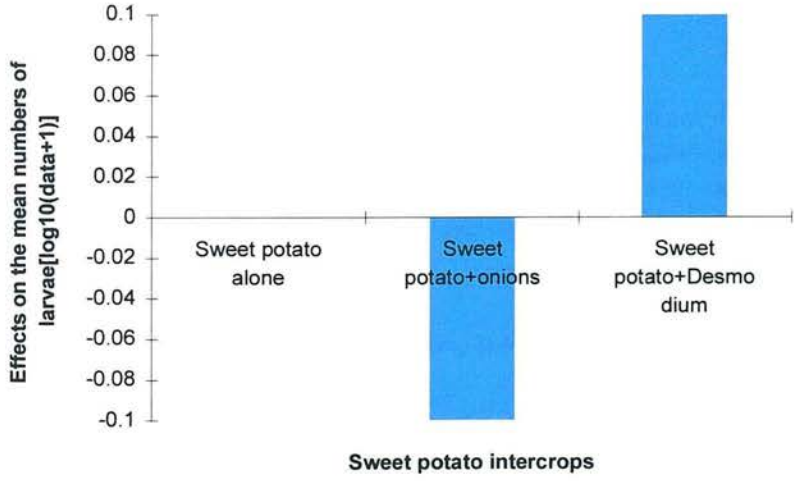


Figure 8.2a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* larvae. (Average of 4 weekly data recordings).

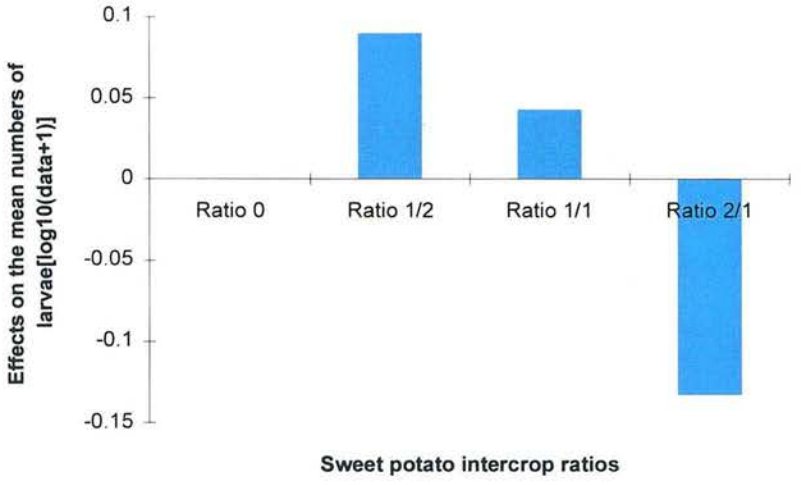


Figure 8.2b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Average of 4 weekly data recordings).

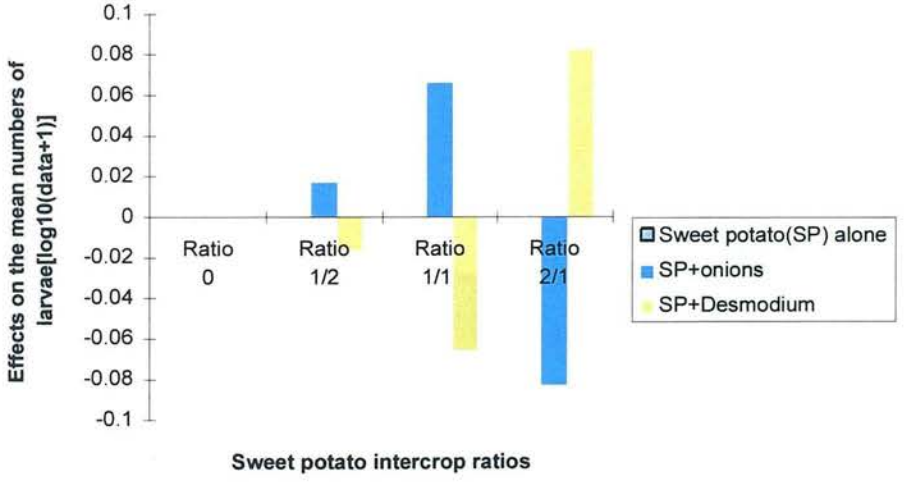


Figure 8.2c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Average of 4 weekly data recordings).

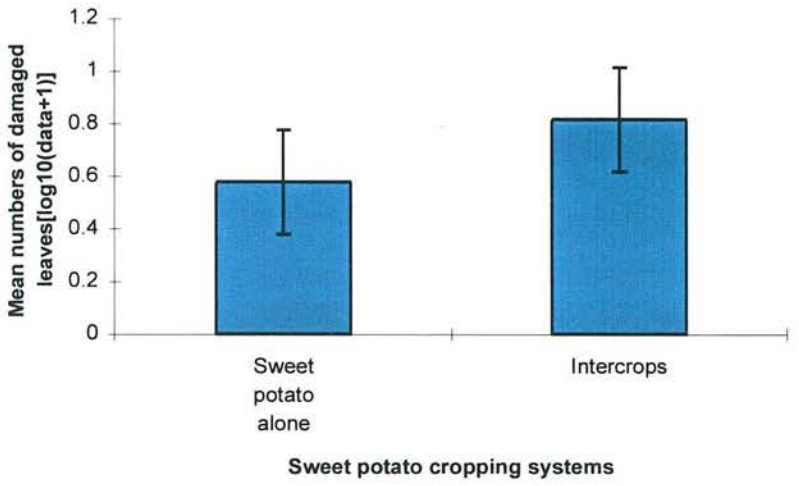


Figure 8.3 Comparison of mean numbers (± SED) of sweet potato plant leaves damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Average of 4 weekly data recordings).

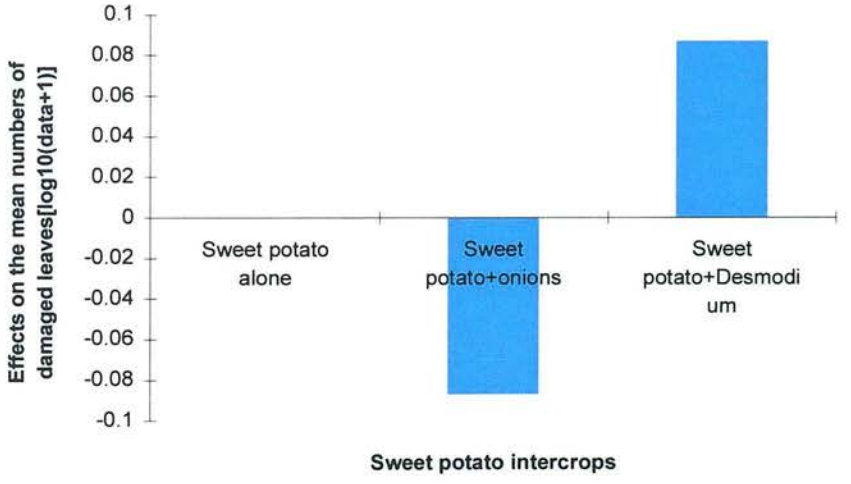


Figure 8.3a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

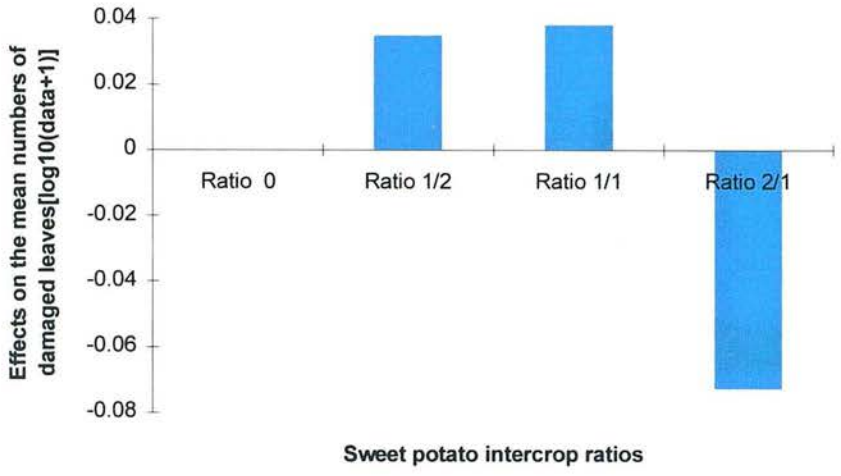


Figure 8.3b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

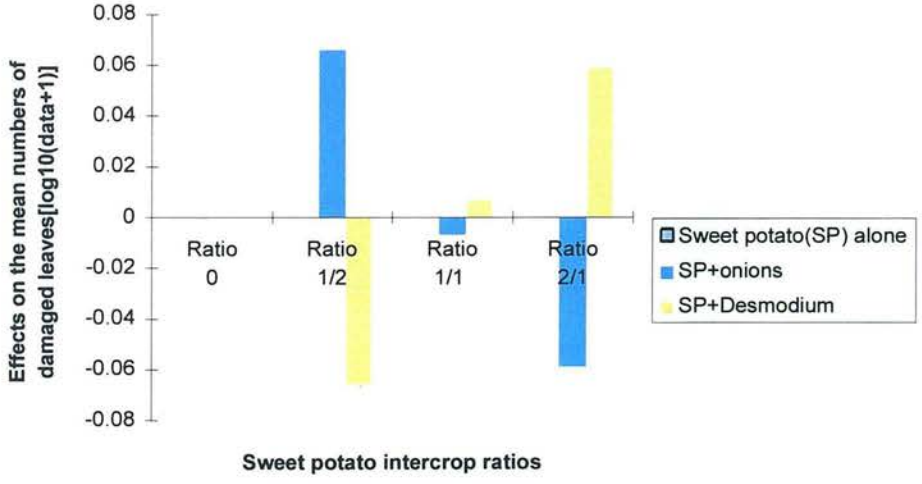


Figure 8.3c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

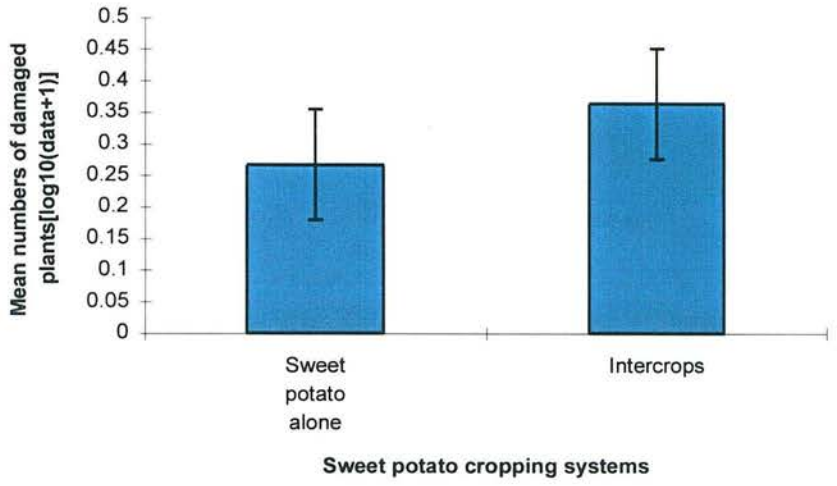


Figure 8.4 Comparison of mean numbers (± SED) of sweet potato plants damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Average of 4 weekly data recordings).

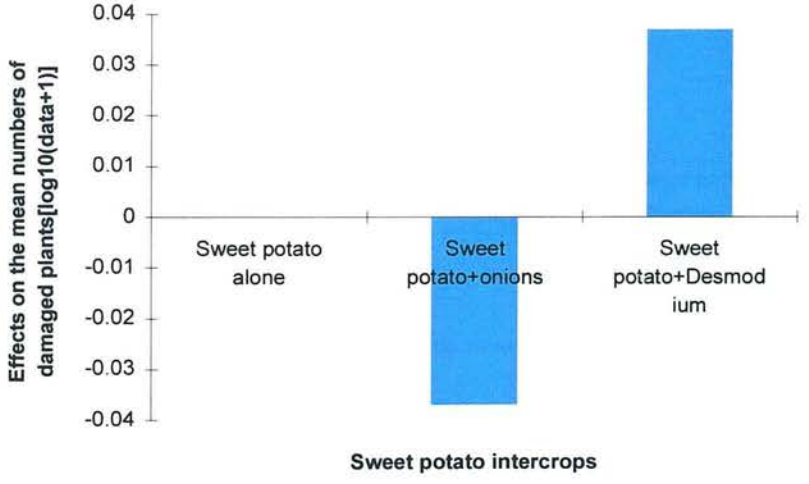


Figure 8.4a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

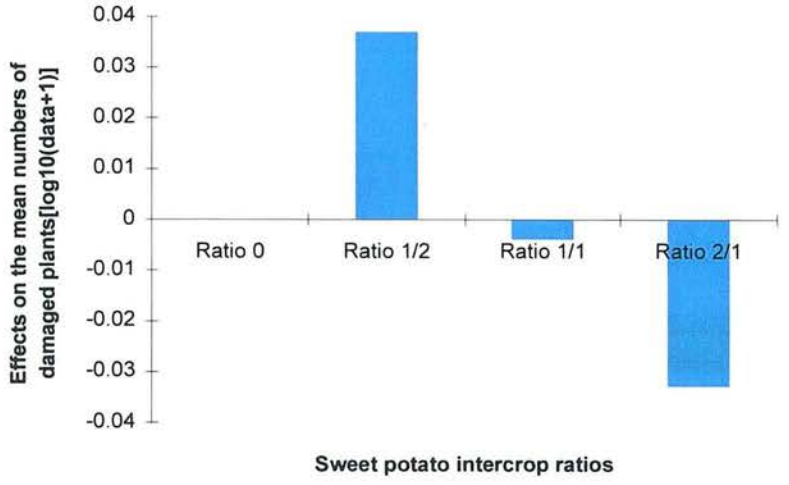


Figure 8.4b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

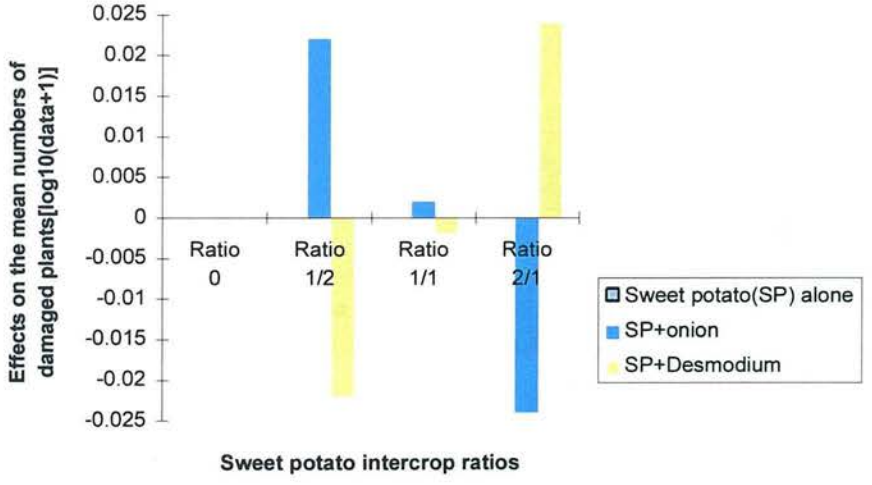


Figure 8.4c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Average of 4 weekly data recordings).

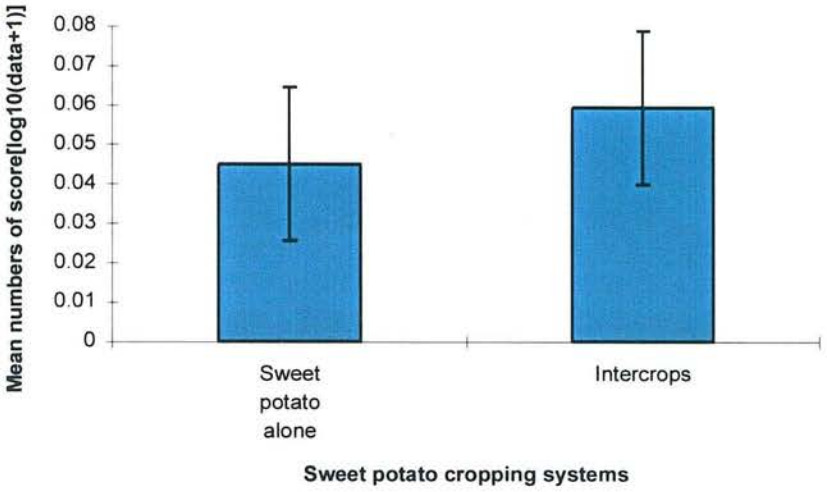


Figure 8.5 Comparison of mean numbers (± SED) of *Acraea acerata* damage score in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Average of 4 weekly data recordings).

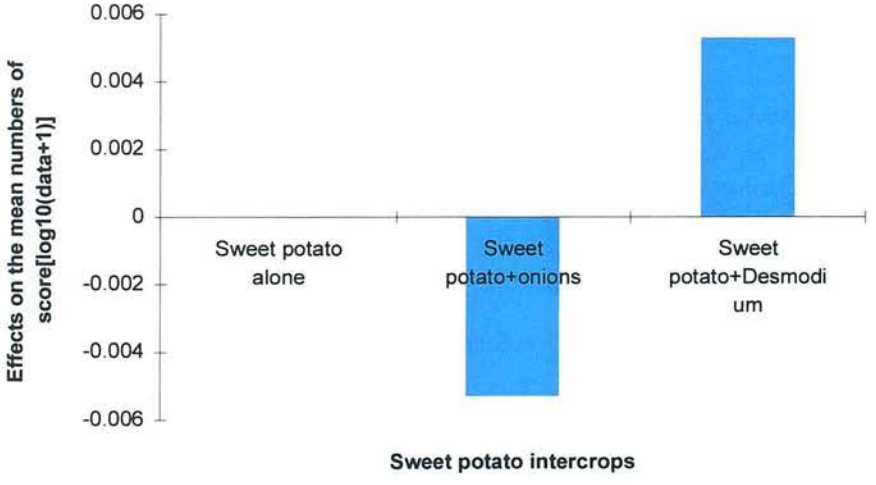


Figure 8.5a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* damage score. (Average of 4 weekly data recordings).

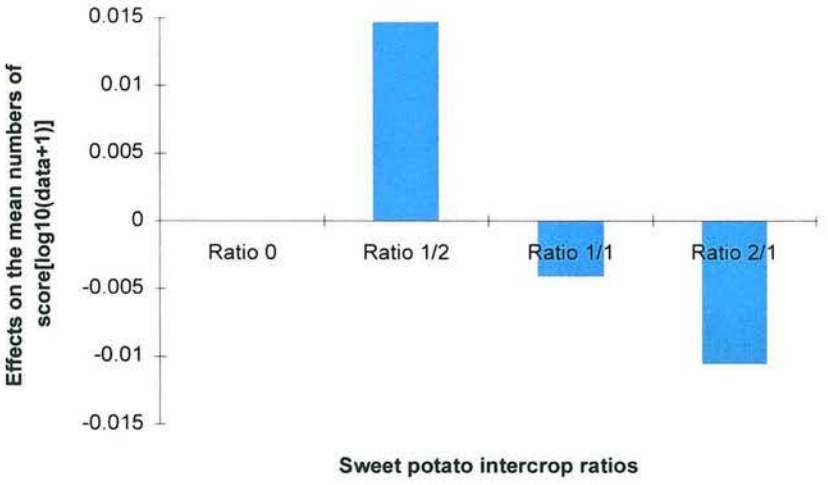


Figure 8.5b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Average of 4 weekly data recordings).

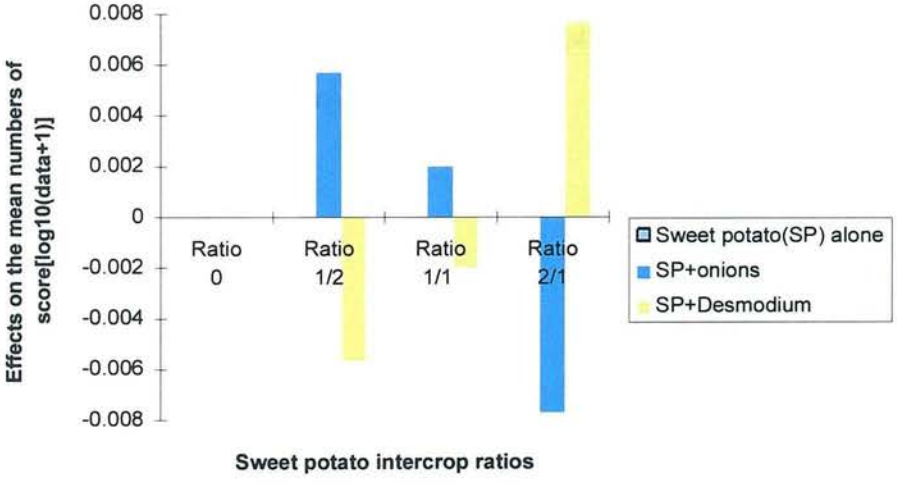


Figure 8.5c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* damage score.(Average of 4 weekly data recordings).

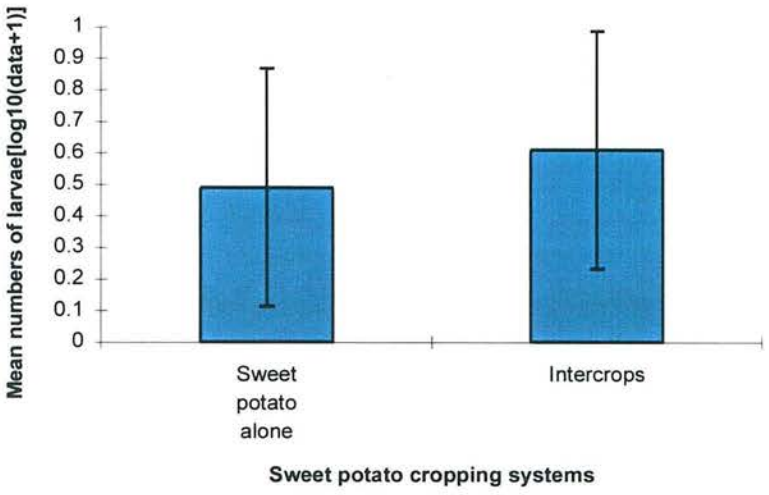


Figure 8.6 Comparison of mean numbers (± SED) of *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 27-28/01/2000: first week of data collection).

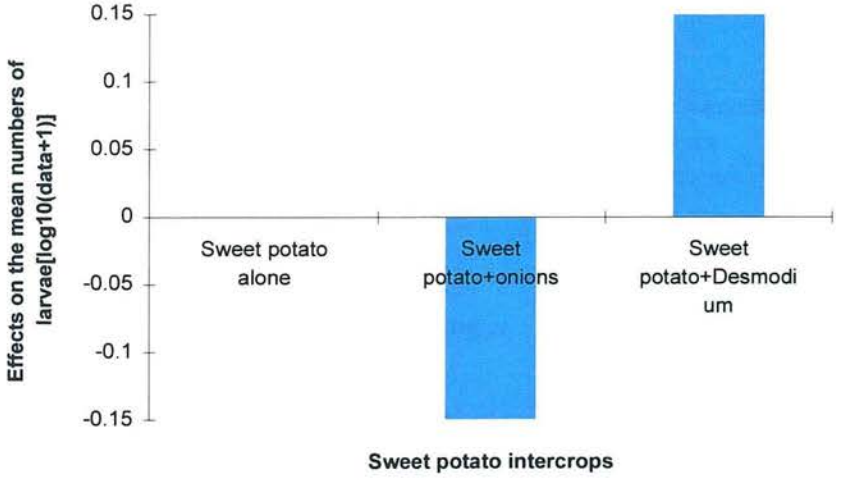


Figure 8.6a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

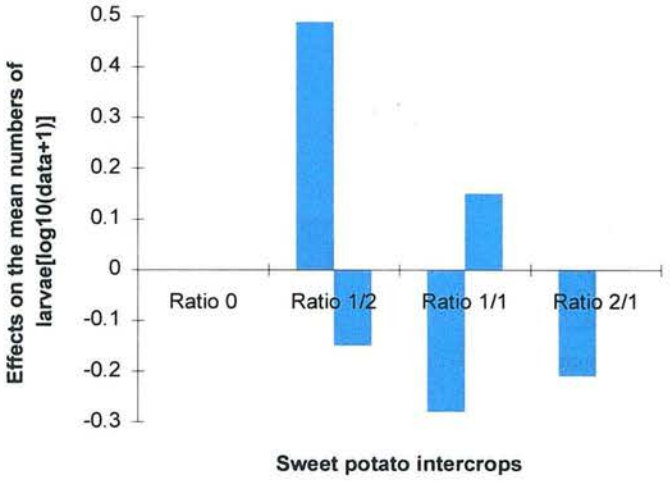


Figure 8.6b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

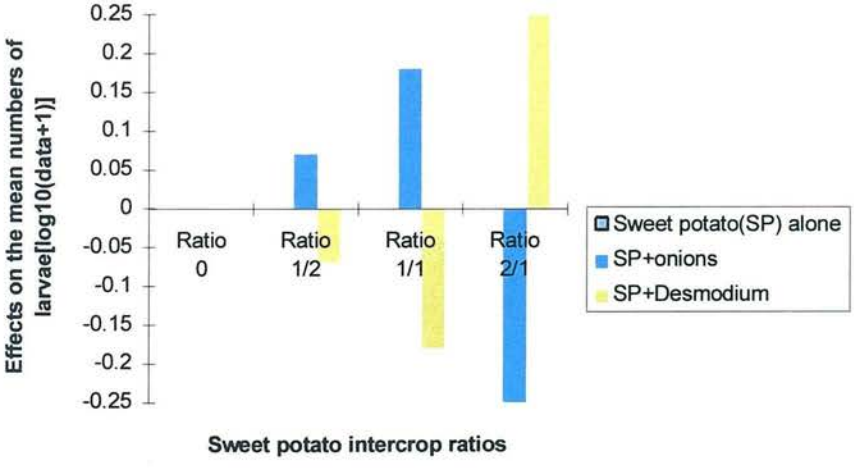


Figure 8.6c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

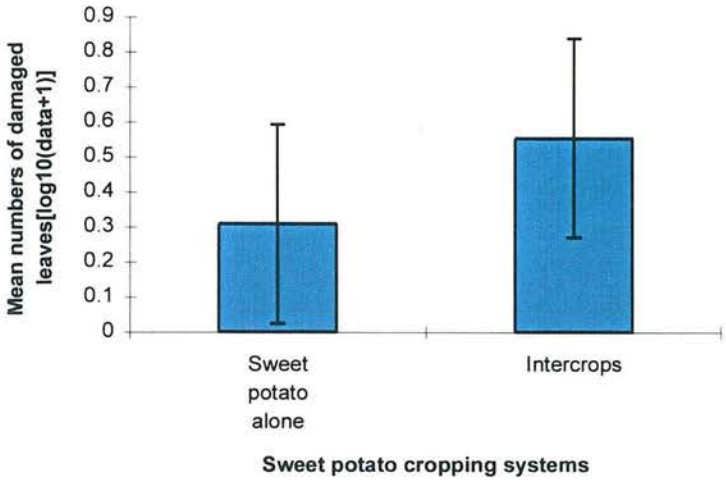


Figure 8.7 Comparison of mean numbers (± SED) of sweet potato plant leaves damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 27-28/01/2000: first week of data collection).

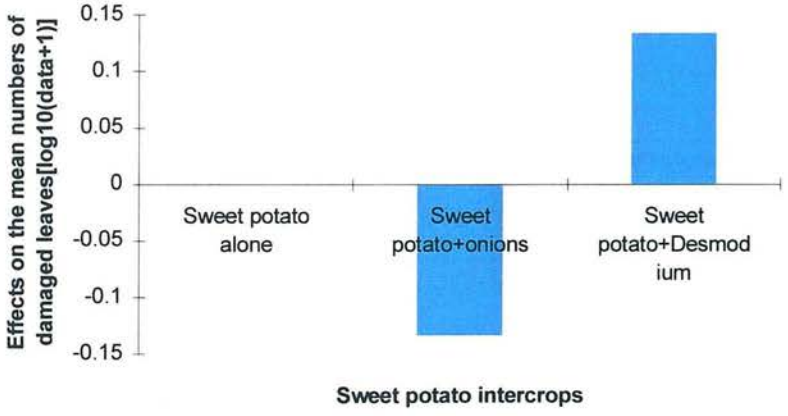


Figure 8.7a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

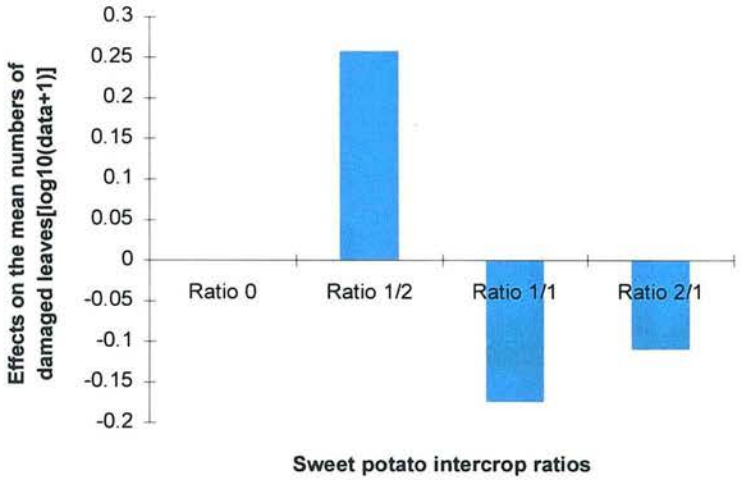


Figure 8.7b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

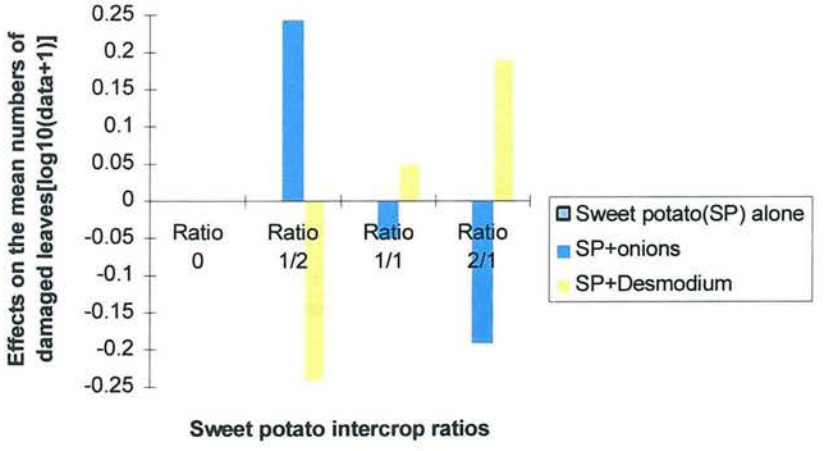


Figure 8.7c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

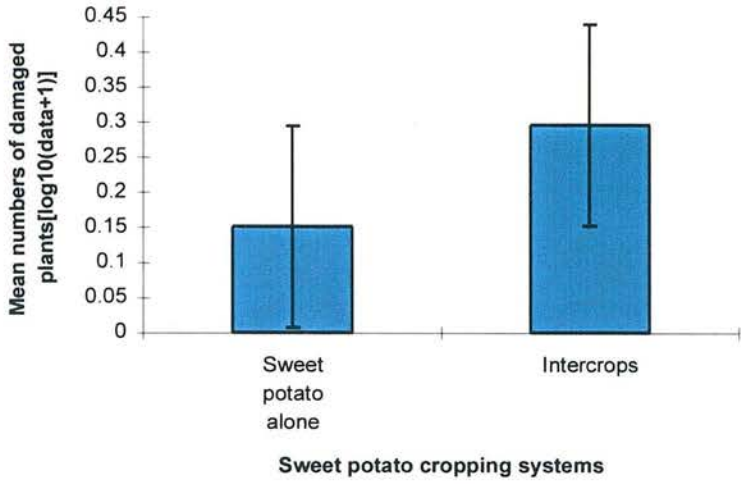


Figure 8.8 Comparison of mean numbers (± SED) of sweet potato plants damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 27-28/01/2000: first week of data collection).

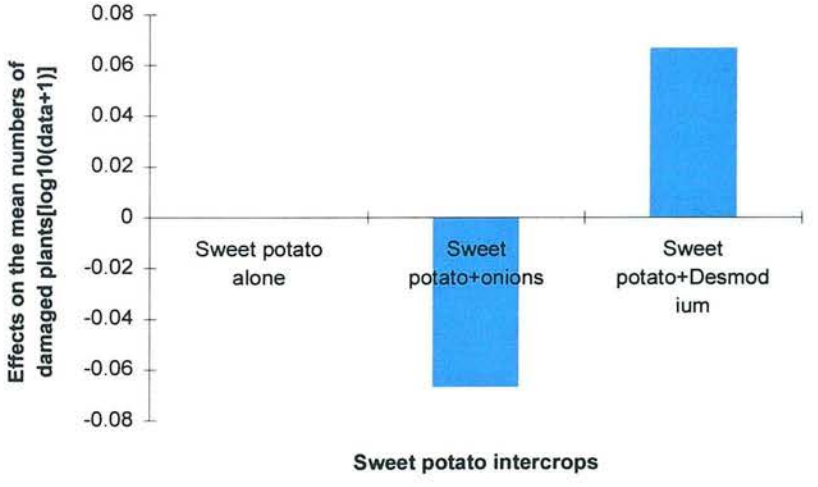


Figure 8.8a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

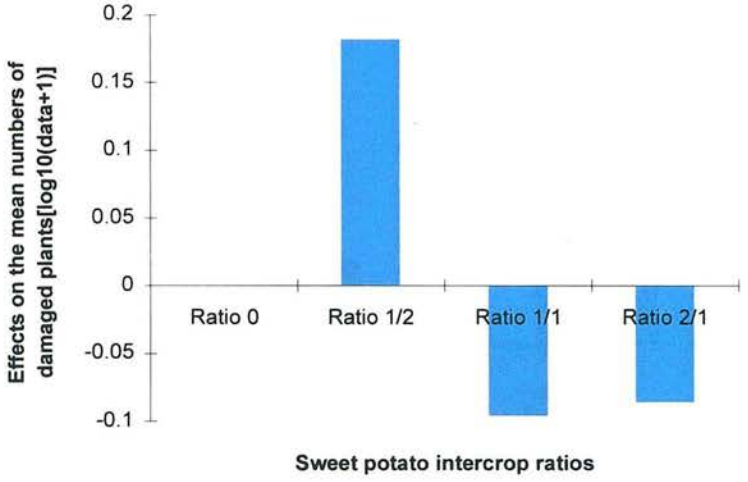


Figure 8.8b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

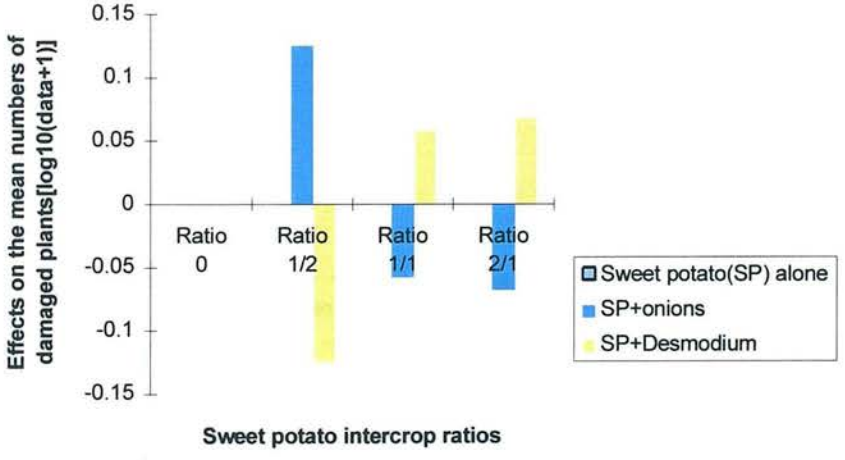


Figure 8.8c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 27-28/01/2000: first week of data collection).

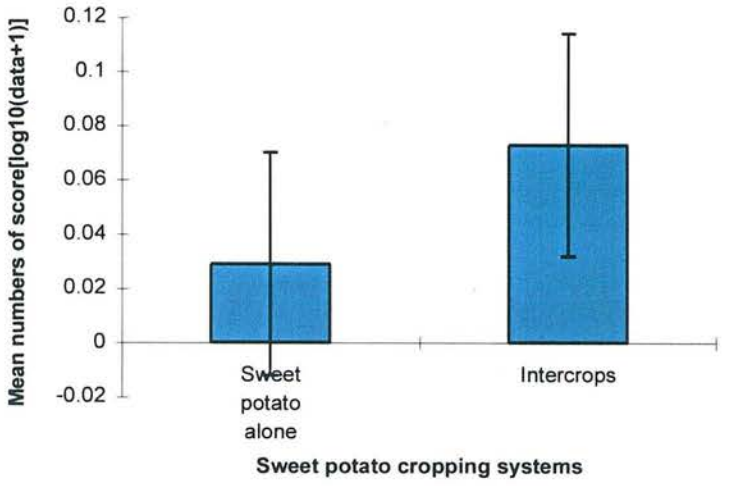


Figure 8.9 Comparison of mean numbers (± SED) of *Acraea acerata* damage score in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 27-28/01/2000: first week of data collection).

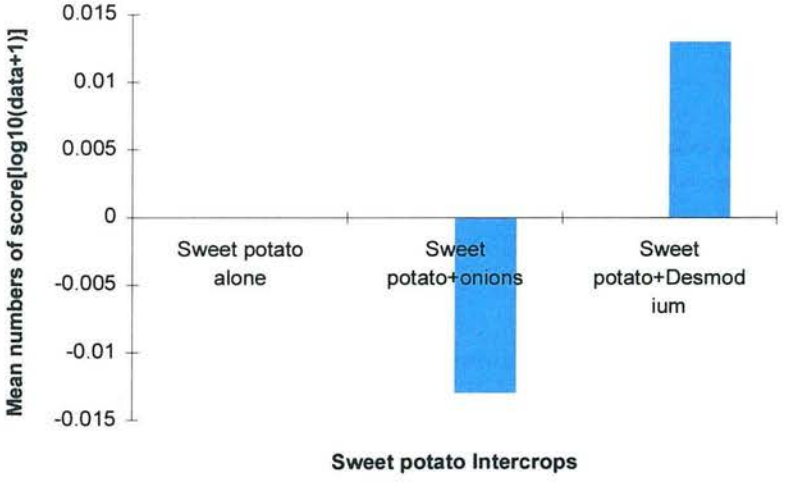


Figure 8.9a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* damage score. (Data collected on 27-28/01/2000: first week of data collection).

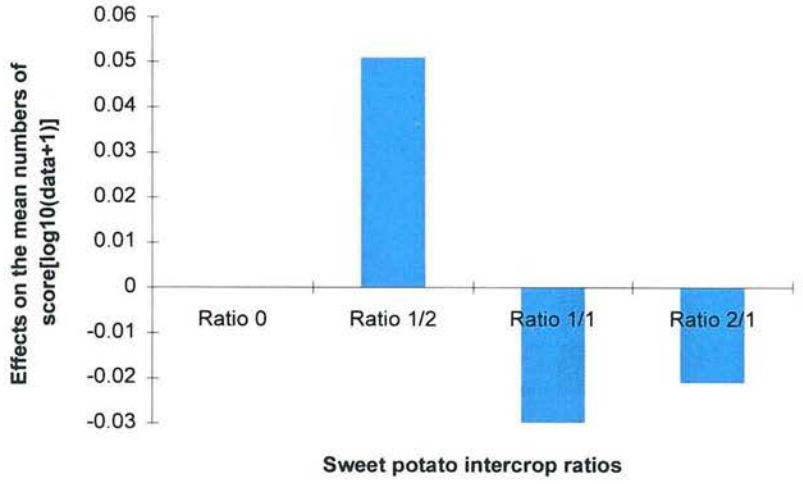


Figure 8.9b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 27-28/01/2000: first week of data collection).

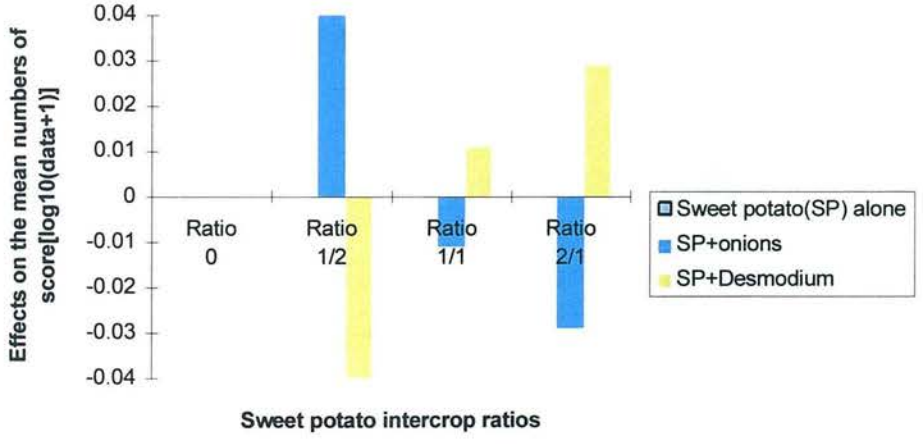


Figure 8.9c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 27-28/01/2000: first week of data collection).

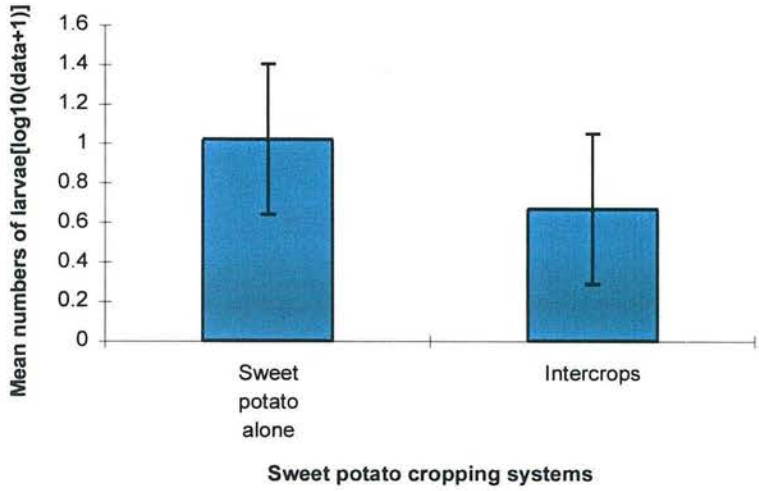


Figure 8.10 Comparison of mean numbers (± SED) of *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 2-3/02/2000: second week of data collection).

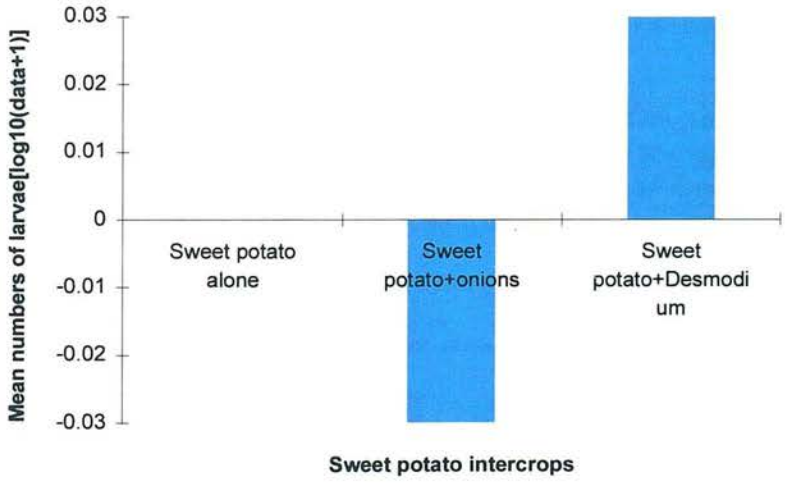


Figure 8.10a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

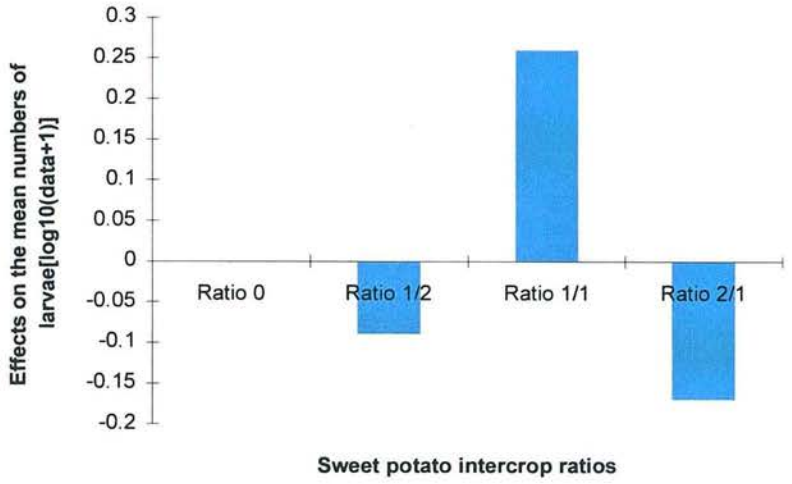


Figure 8.10b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

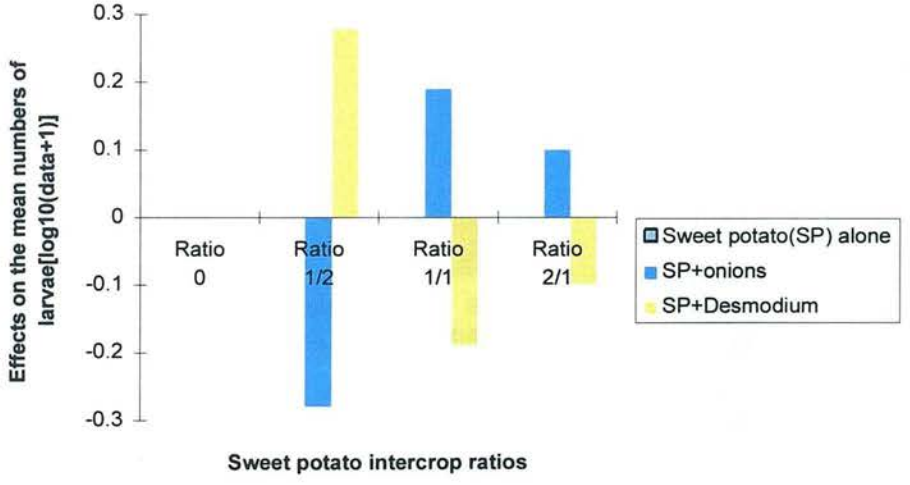


Figure 8.10c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

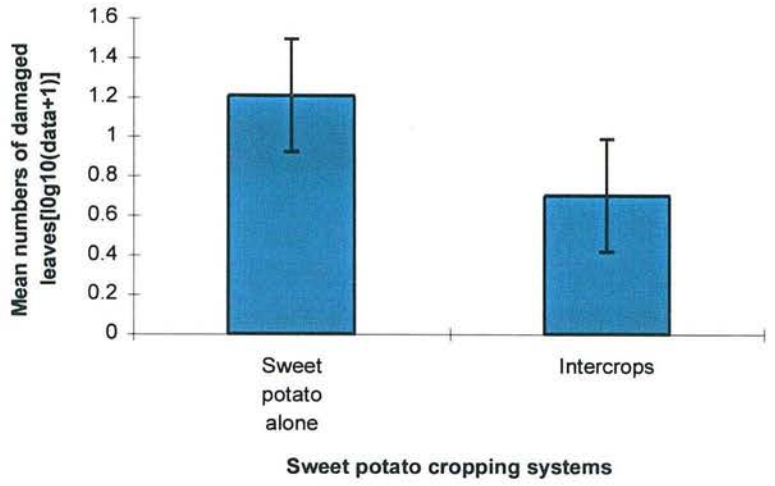


Figure 8.11 Comparison of mean numbers (± SED) of sweet potato plant leaves damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 2-3/02/2000: second week of data collection).

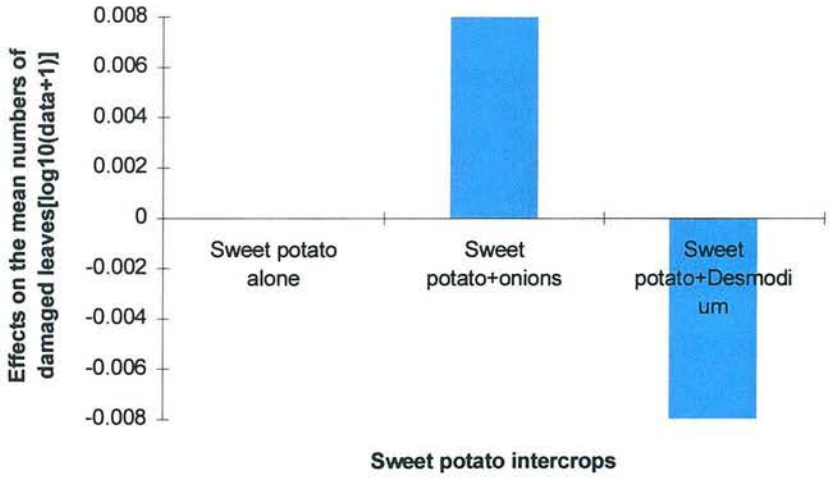


Figure 8.11a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

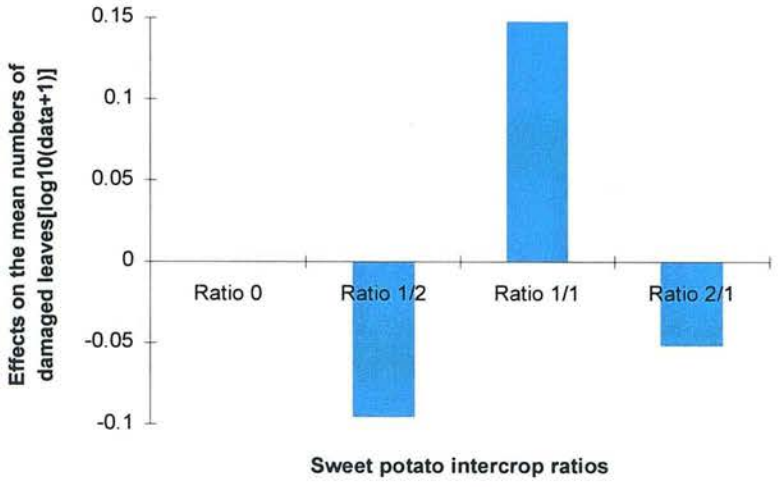


Figure 8.11b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

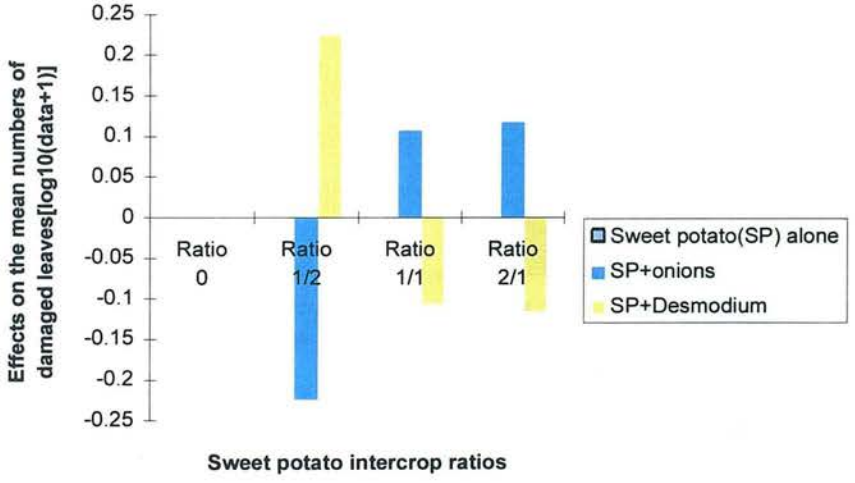


Figure 8.11c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

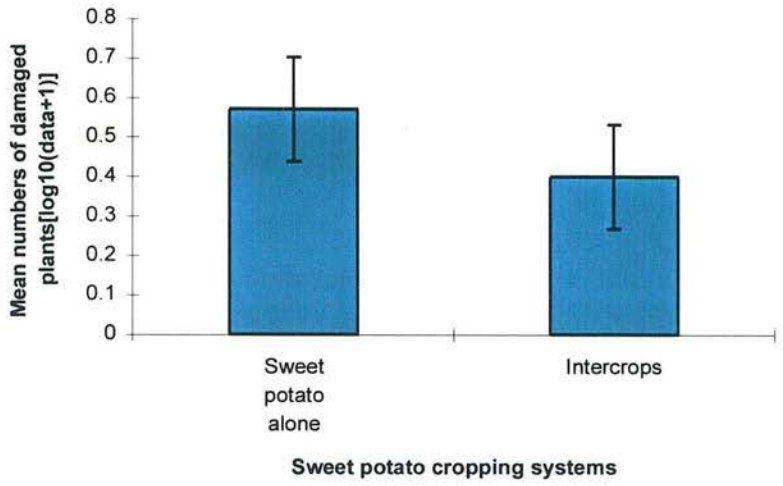


Figure 8.12 Comparison of mean numbers (± SED) of sweet potato plants damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 2-3/02/2000: second week of data collection).

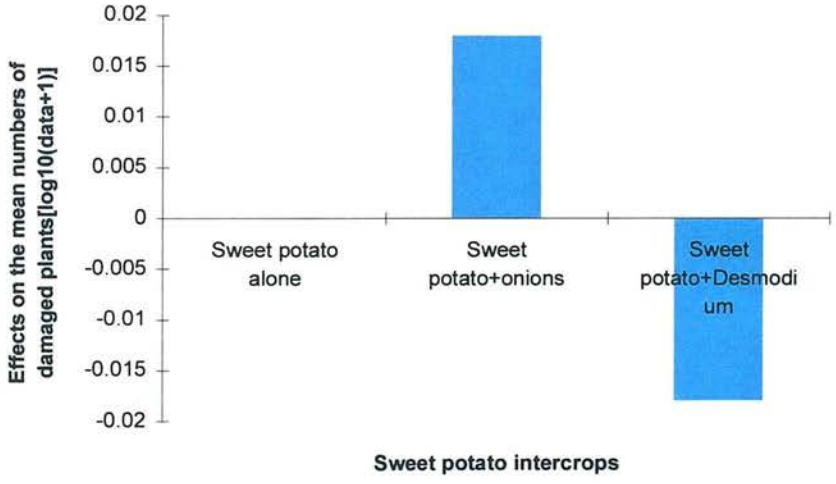


Figure 8.12a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

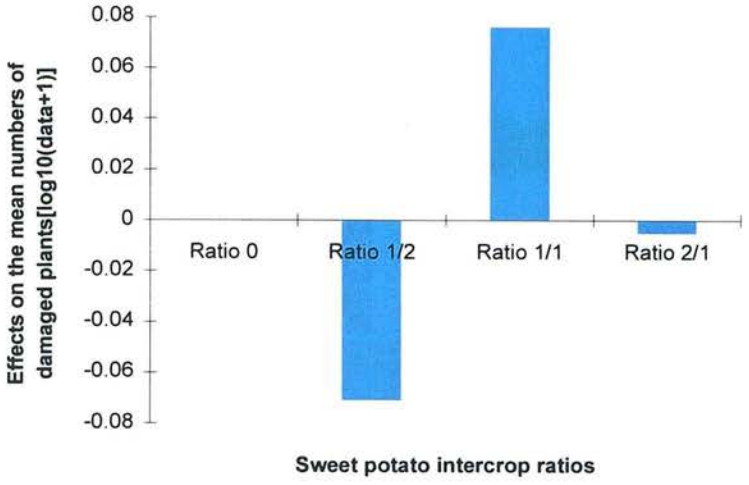


Figure 8.12b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

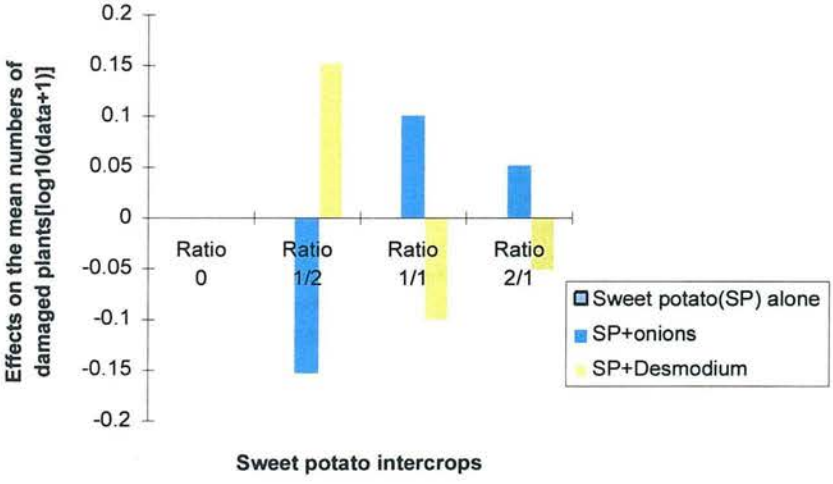


Figure 8.12c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 2-3/02/2000: second week of data collection).

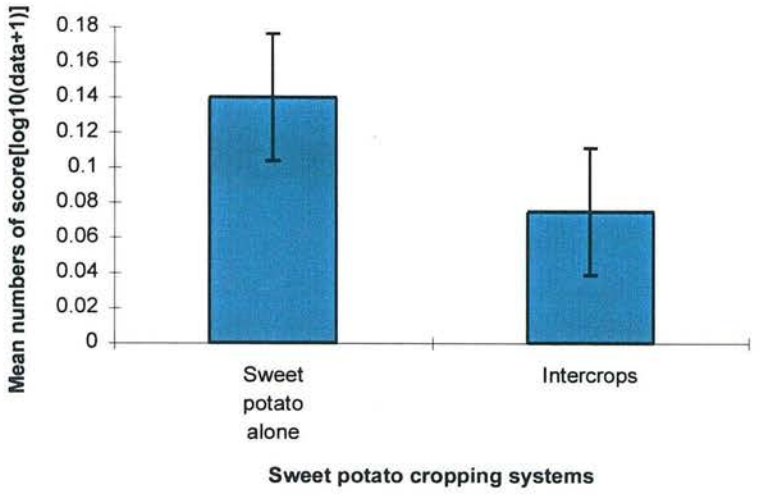


Figure 8.13 Comparison of mean numbers (± SED) of *Acraea acerata* damage score in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 2-3/02/2000: second week of data collection).

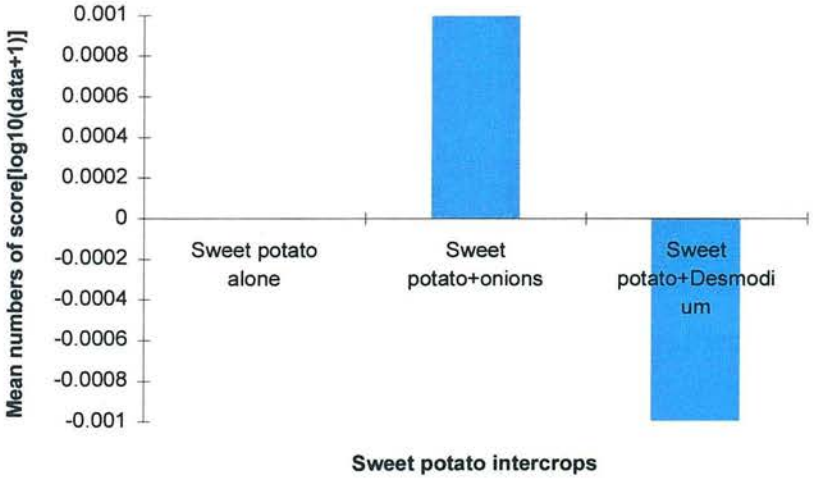


Figure 8.13a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* damage score. (Data collected on 2-3/02/2000: second week of data collection).

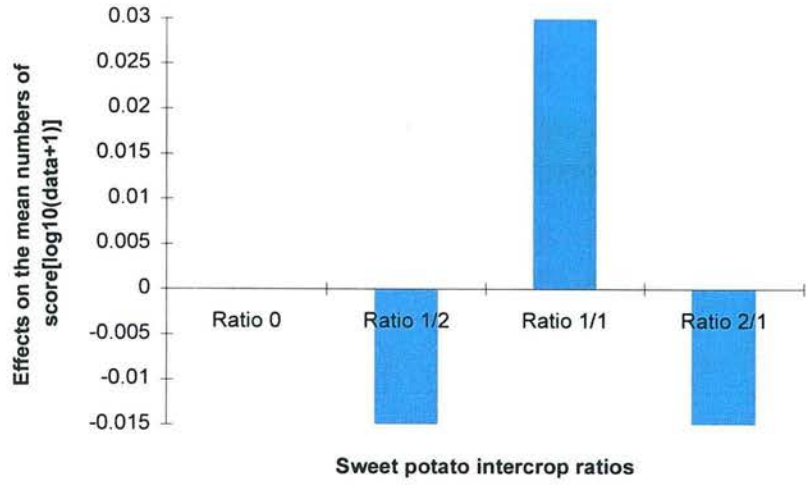


Figure 8.13b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 2-3/02/2000: second week of data collection).

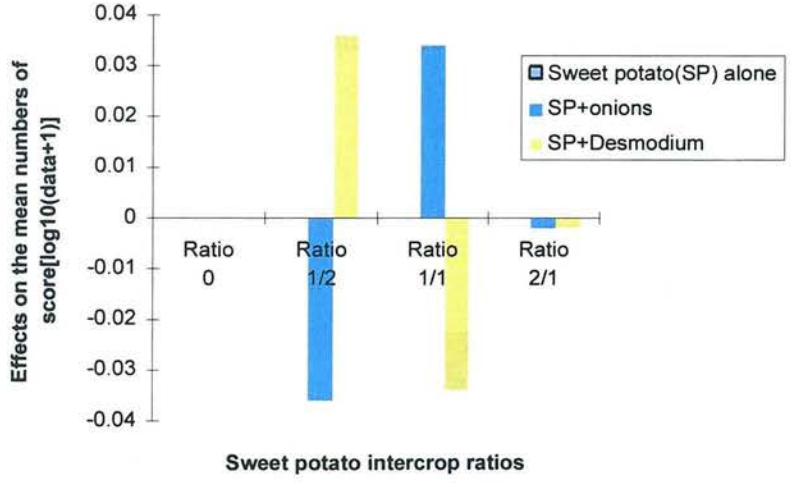


Figure 8.13c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 2-3/02/2000: second week of data collection).

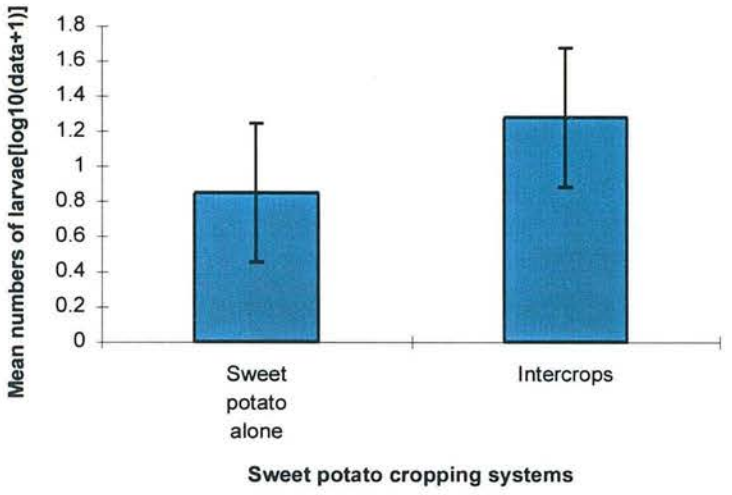


Figure 8.14 Comparison of mean numbers (± SED) of *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 9-10/02/2000: third week of data collection).

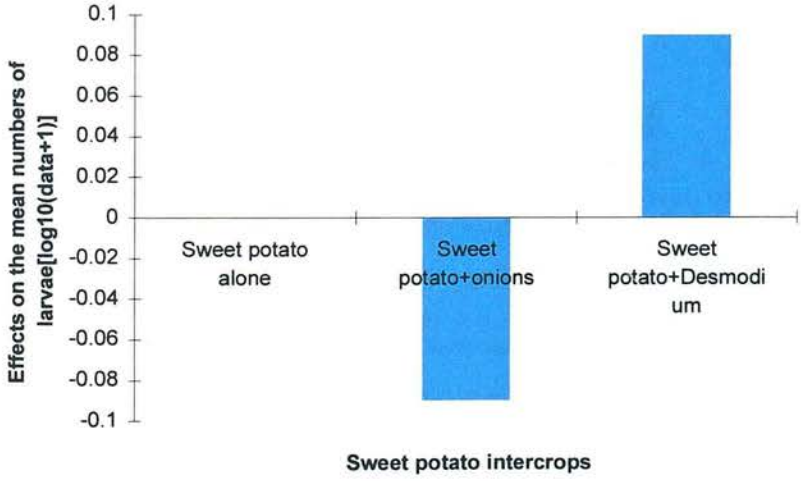


Figure 8.14a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

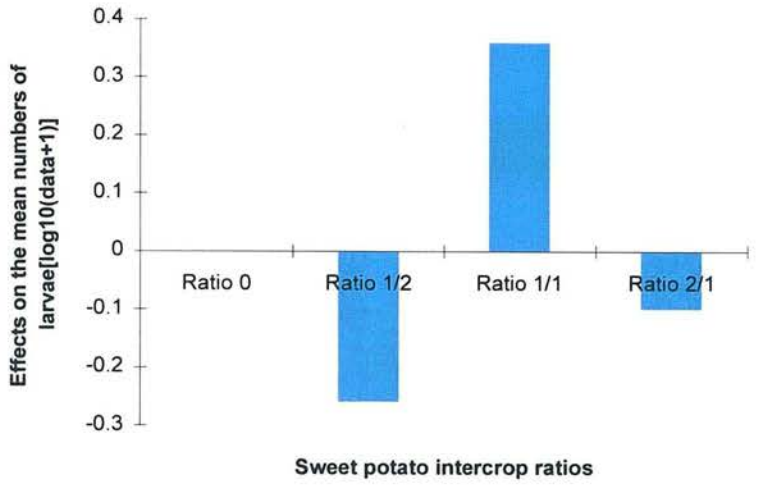


Figure 8.14b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

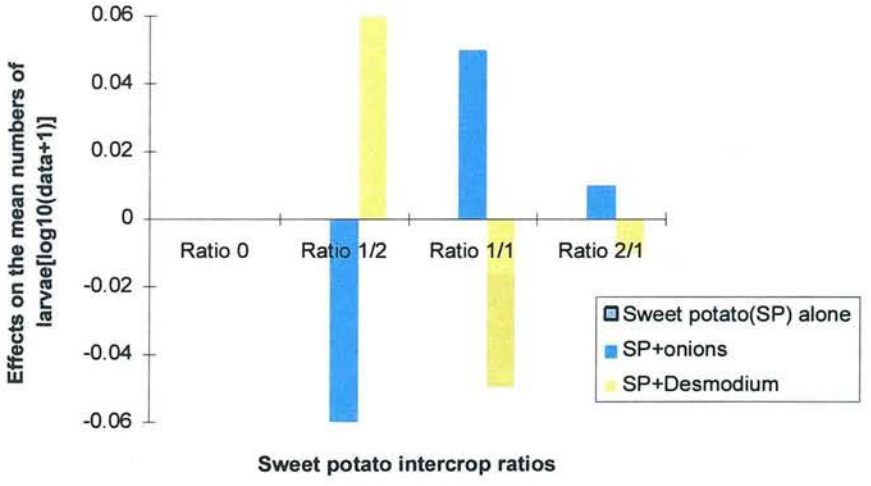


Figure 8.14c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

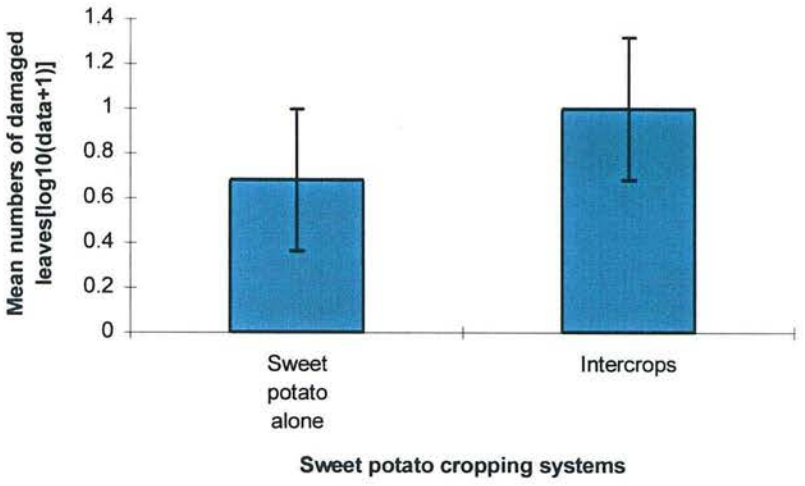


Figure 8.15 Comparison of mean numbers (± SED) of sweet potato plant leaves damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 9-10/02/2000: third week of data collection).

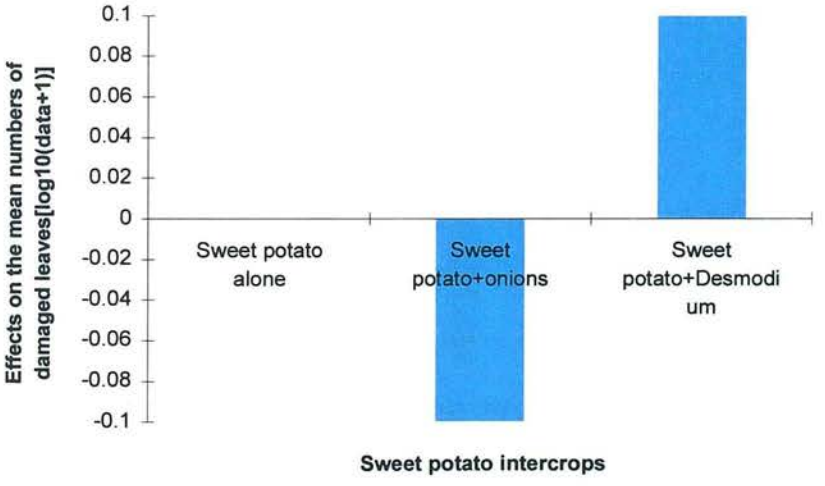


Figure 8.15a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

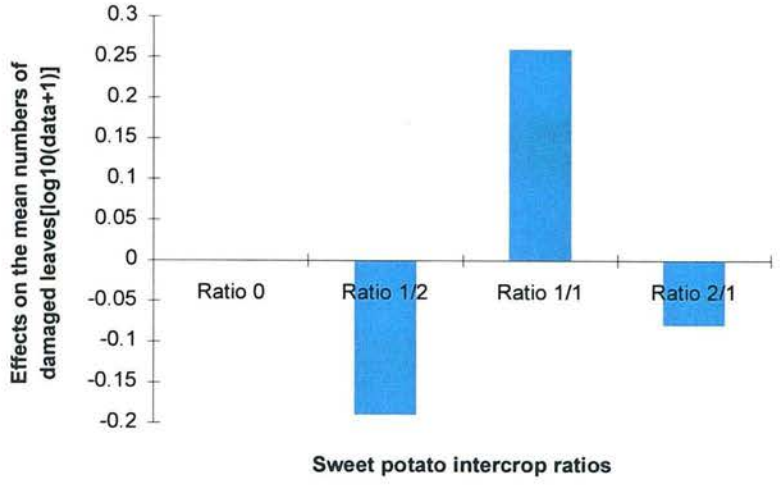


Figure 8.15b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

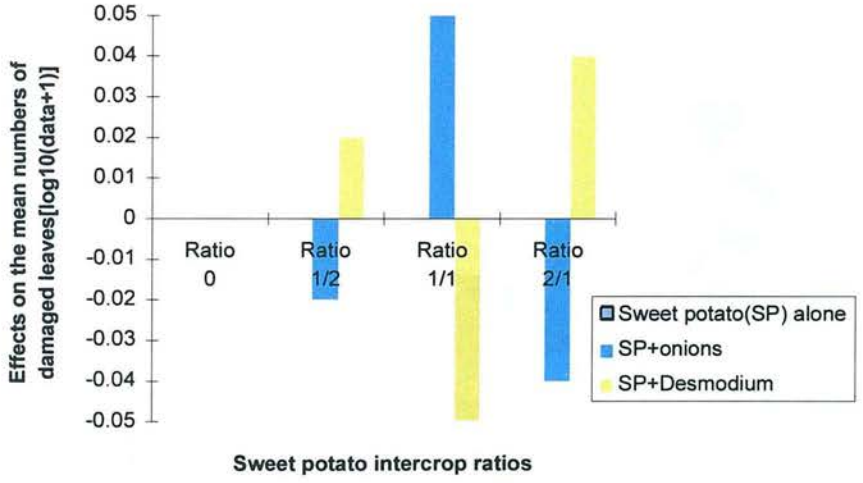


Figure 8.15c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

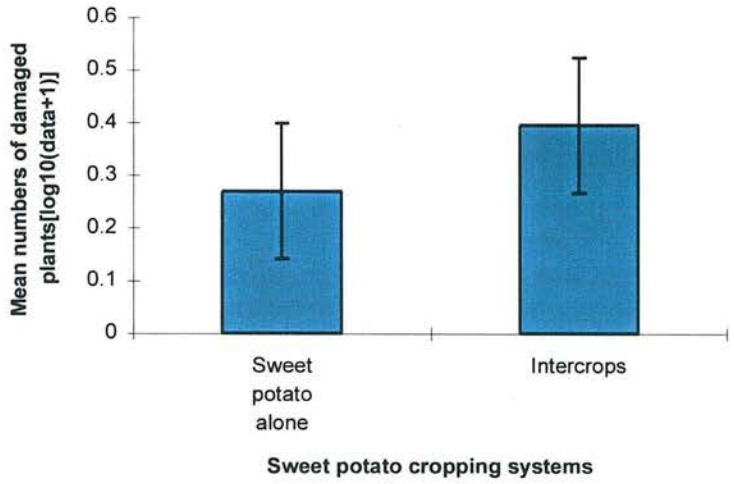


Figure 8.16 Comparison of mean numbers (± SED) of sweet potato plants damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 9-10/02/2000: third week of data collection).

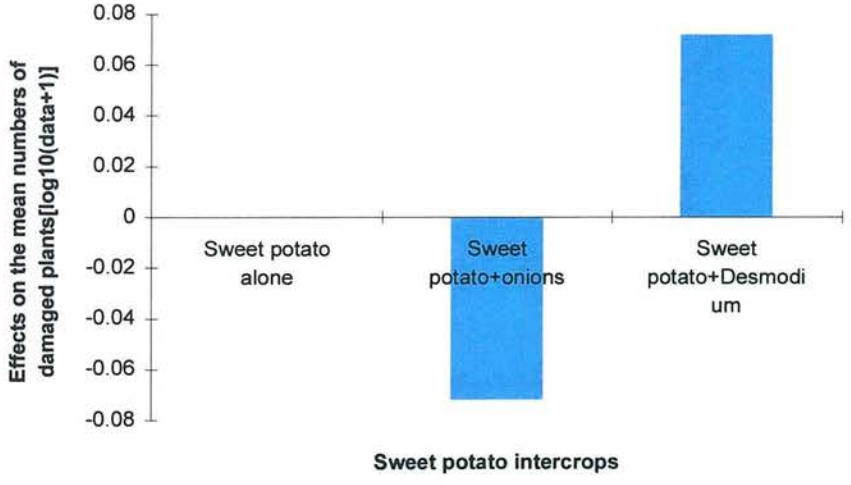


Figure 8.16a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

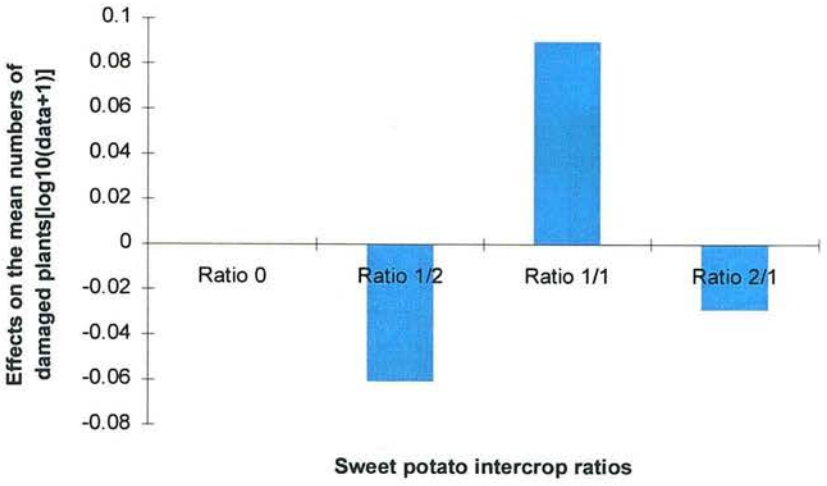


Figure 8.16b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

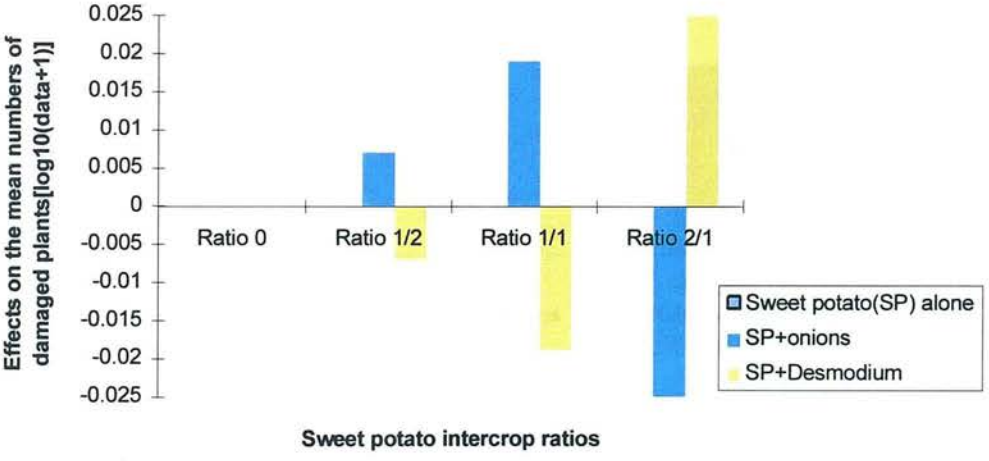


Figure 8.16c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 9-10/02/2000: third week of data collection).

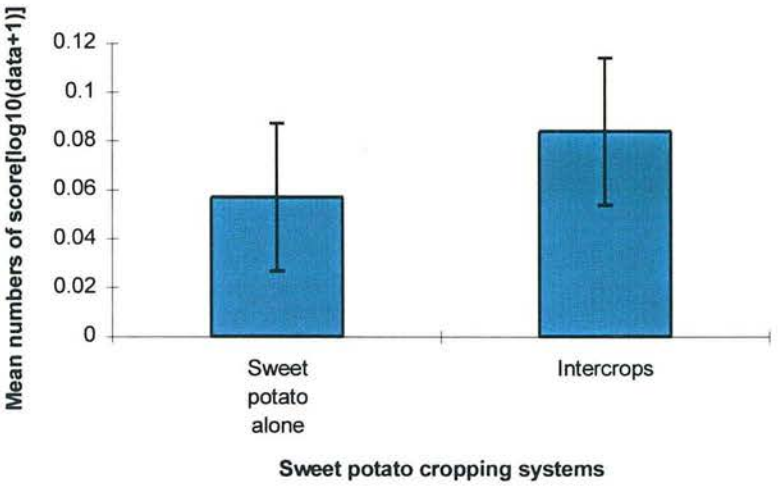


Figure 8.17 Comparison of mean numbers (± SED) of *Acraea acerata* damage score in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (Data collected on 9-10/02/2000: third week of data collection).

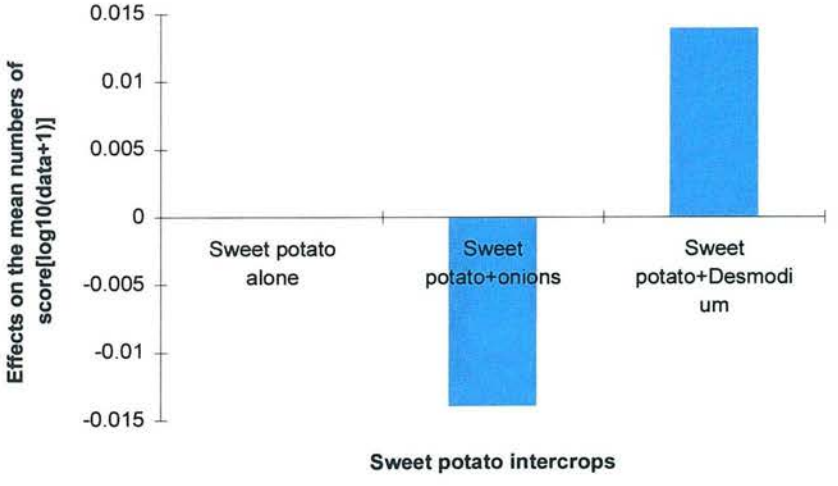


Figure 8.17a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* damage score. (Data collected on 9-10/02/2000: third week of data collection).

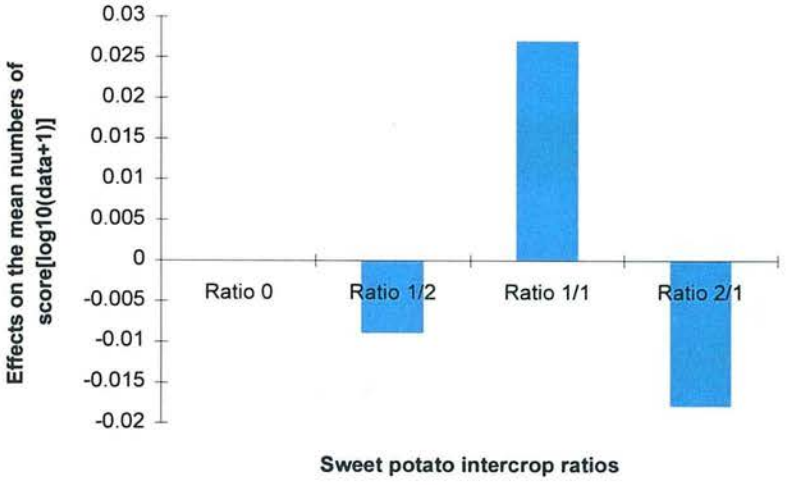


Figure 8.17b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 9-10/02/2000: third week of data collection).

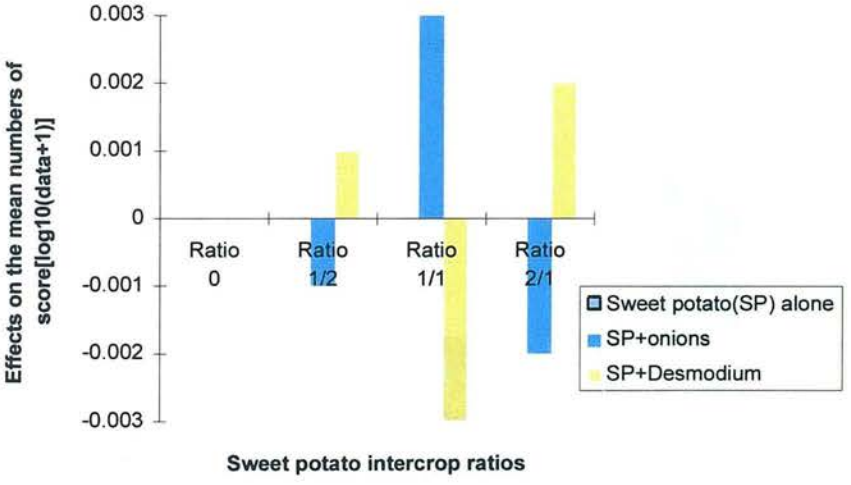


Figure 8.17c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 9-10/02/2000: third week of data collection).

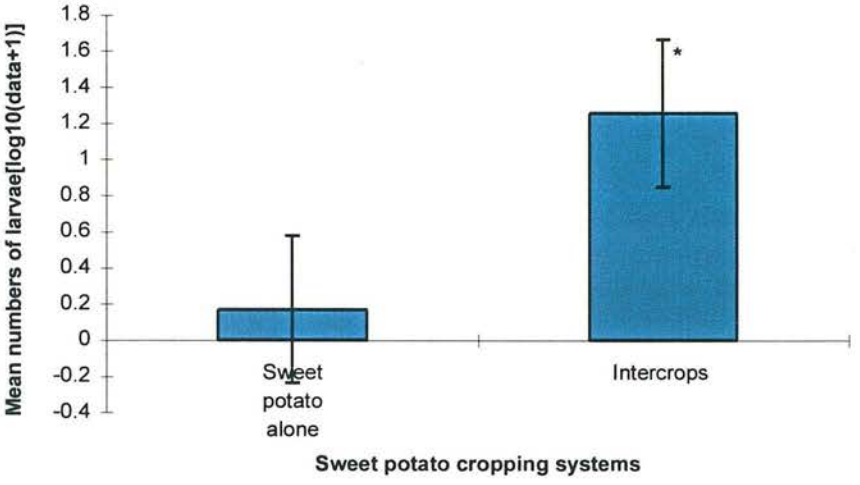


Figure 8.18 Comparison of mean numbers (± SED) of *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (* $P < 0.05$) (Data collected on 16-17/02/2000: fourth week of data collection).

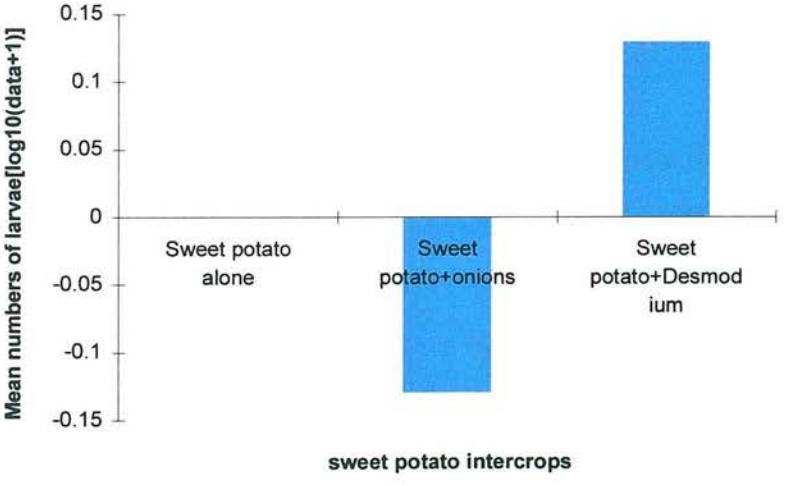


Figure 8.18a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

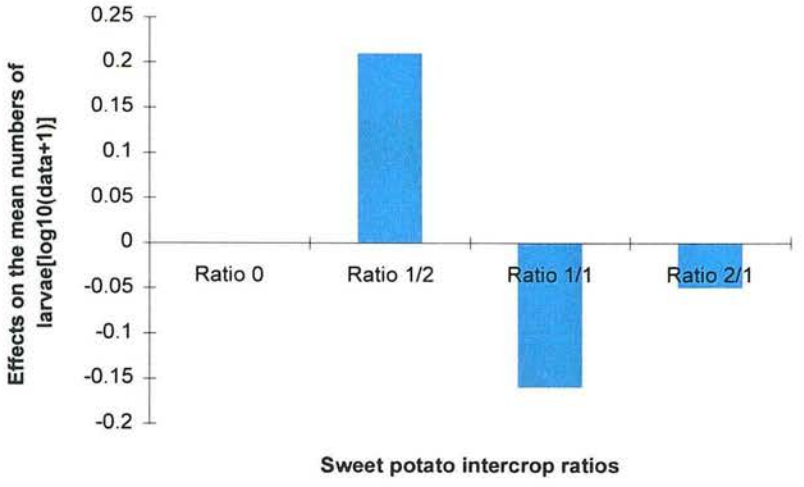


Figure 8.18b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

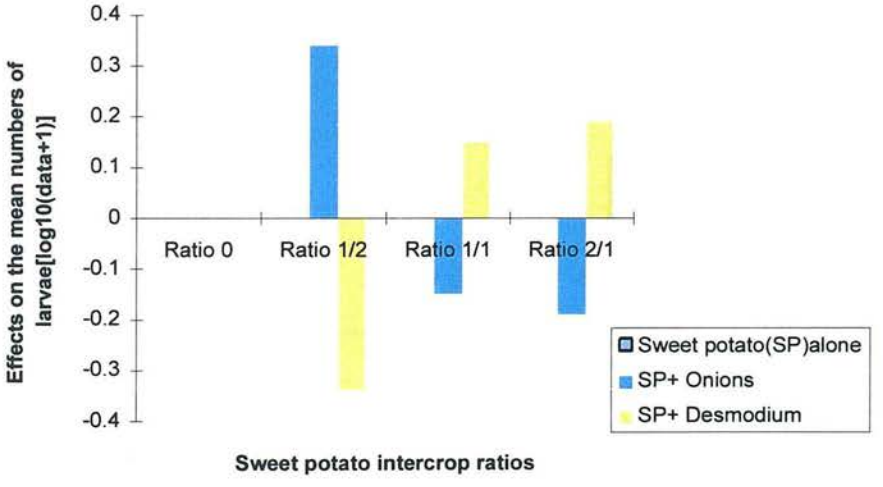


Figure 8.18c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

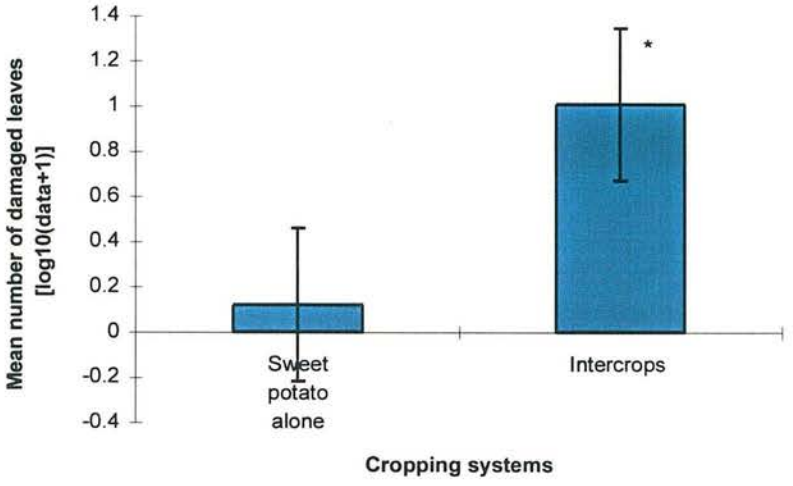


Figure 8.19 Comparison of mean numbers (± SED) of sweet potato plant leaves damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (* $P < 0.05$) (Data collected on 16-17/02/2000: fourth week of data collection).

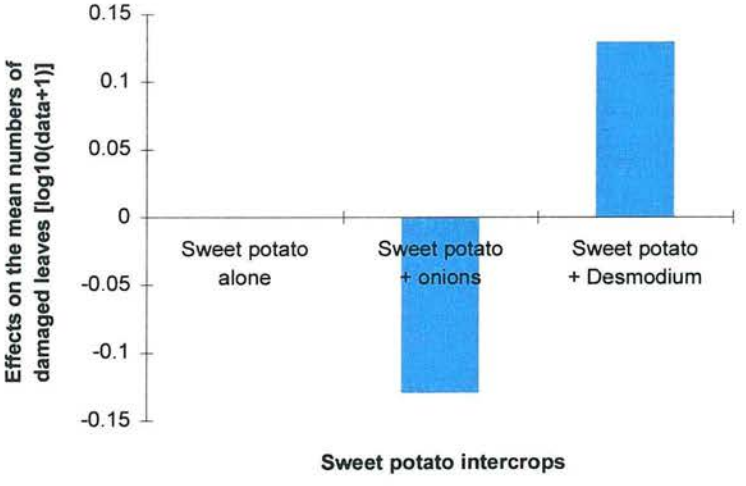


Figure 8.19a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

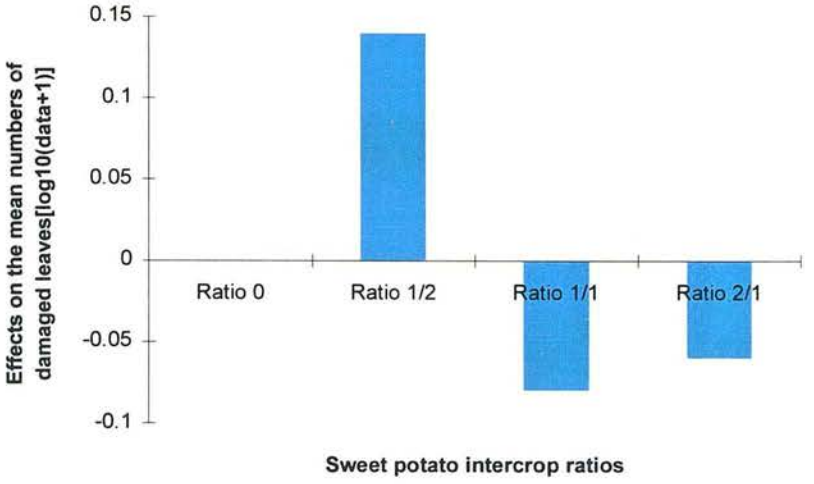


Figure 8.19b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

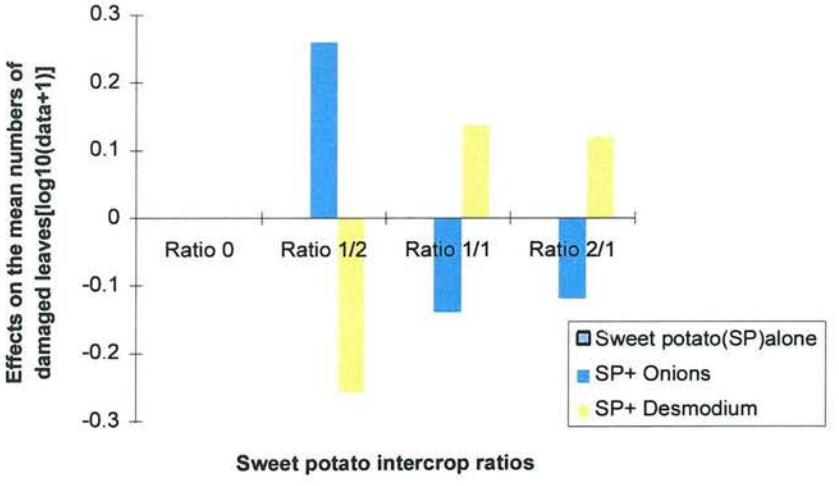


Figure 8.19c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plant leaves damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

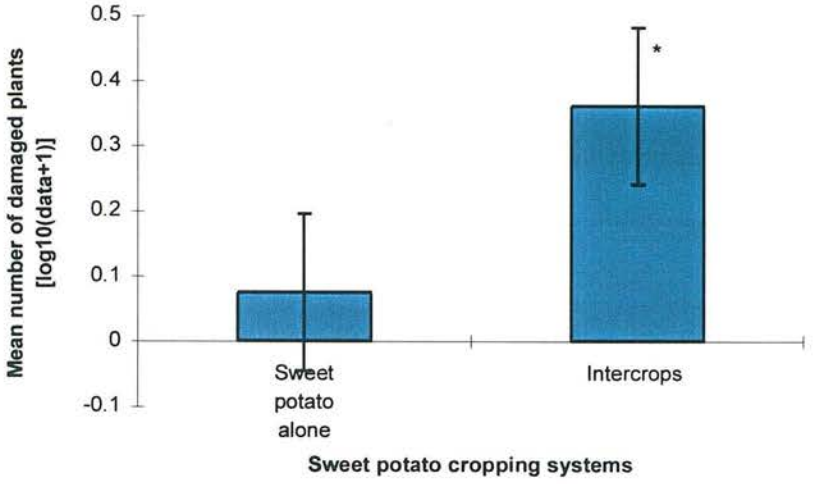


Figure 8.20 Comparison of mean numbers (± SED) of sweet potato plants damaged by *Acraea acerata* larvae in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (* $P < 0.05$) (Data collected on 16-17/02/2000: fourth week of data collection).

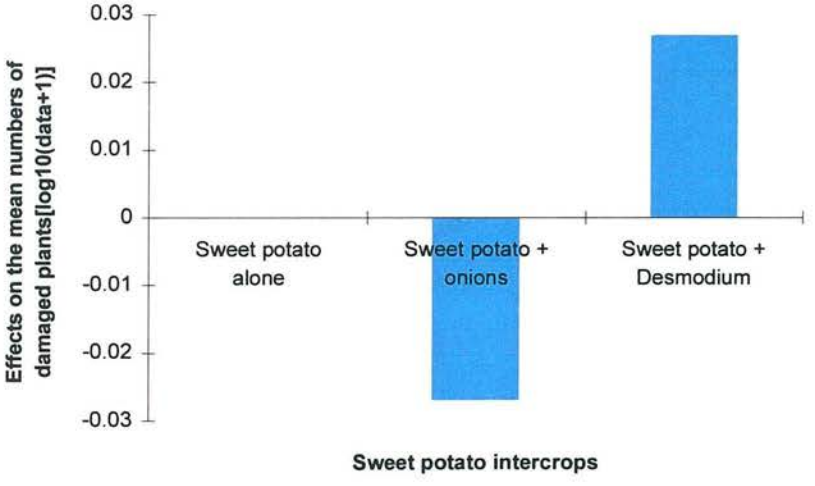


Figure 8.20a Comparison of the effects of different sweet potato intercrops on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

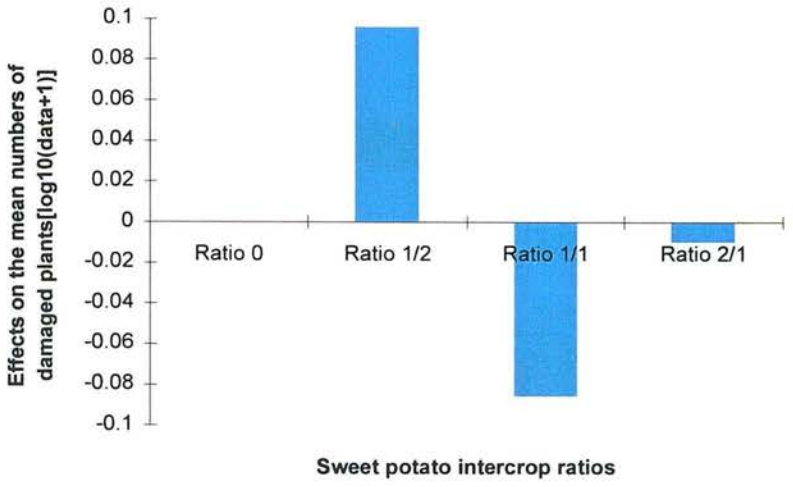


Figure 8.20b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

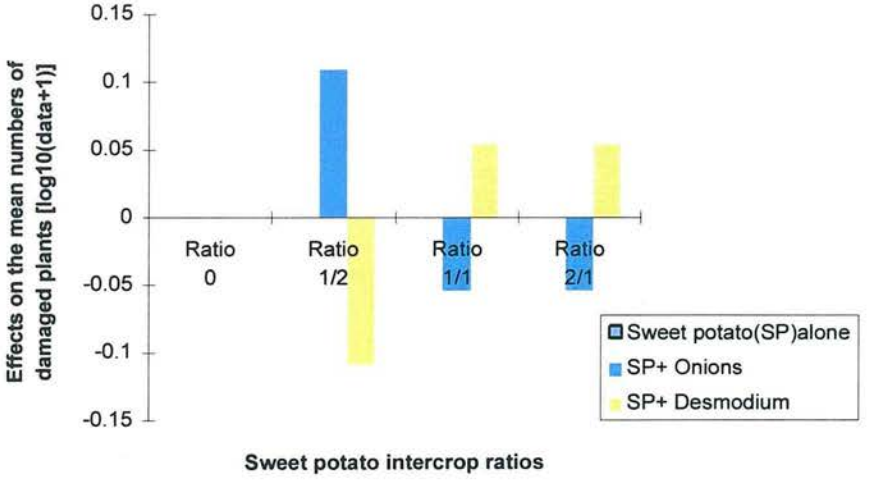


Figure 8.20c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of sweet potato plants damaged by *Acraea acerata* larvae. (Data collected on 16-17/02/2000: fourth week of data collection).

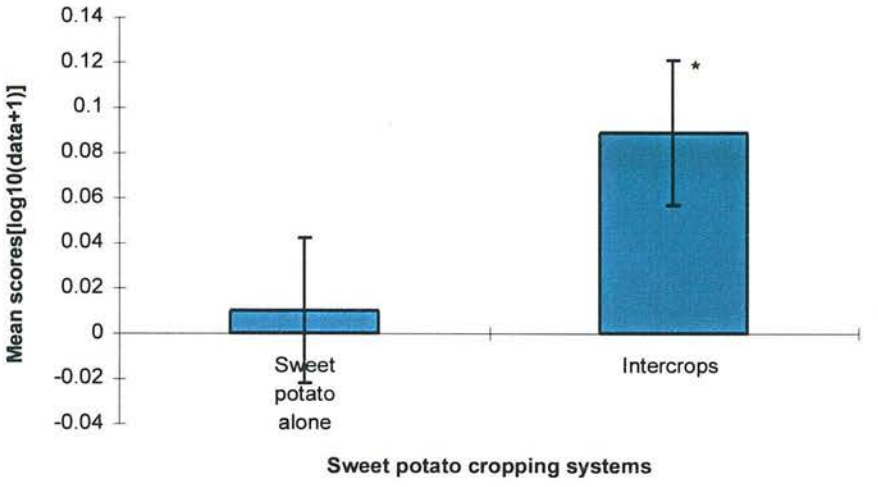


Figure 8.21 Comparison of mean numbers (± SED) of *Acraea acerata* damage score in plots with sweet potato plants alone (n = 4) and the intercrops of sweet potato + onions and sweet potato + *Desmodium* plants (n = 24). (* $P < 0.05$) (Data collected on 16-17/02/2000: fourth week of data collection).

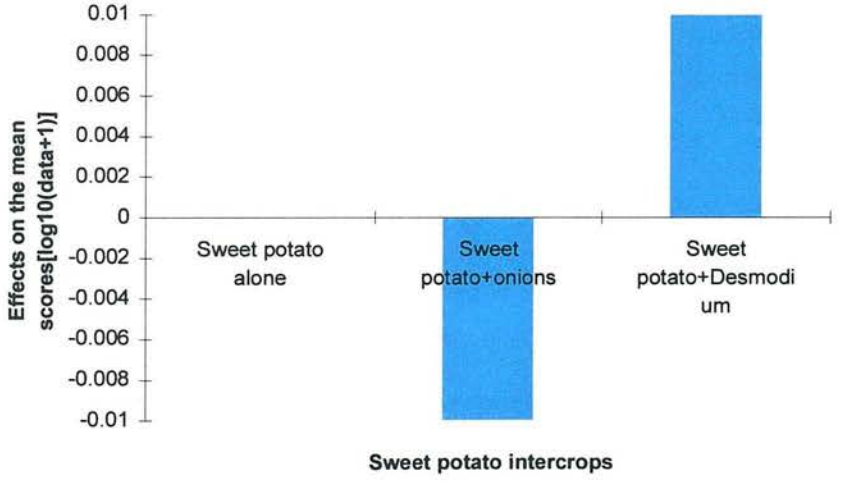


Figure 8.21a Comparison of the effects of different sweet potato intercrops on the mean numbers of *Acraea acerata* damage score. (Data collected on 16-17/02/2000: fourth week of data collection).

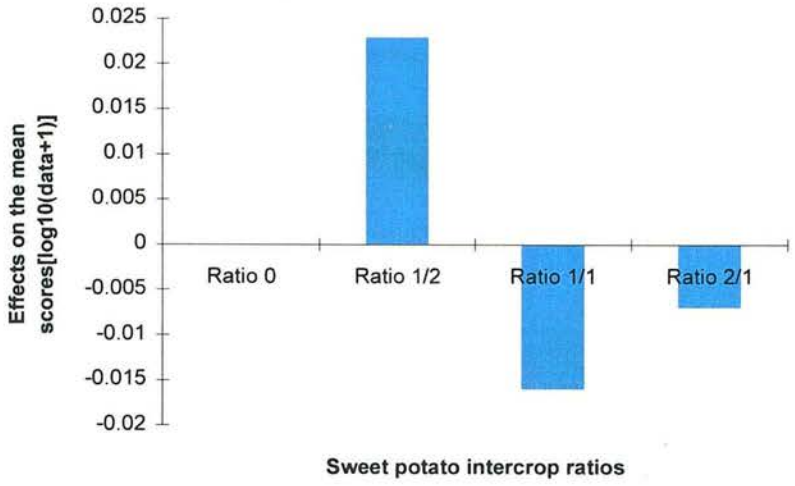


Figure 8.21b Comparison of the effects of sweet potato intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 16-17/02/2000: fourth week of data collection).

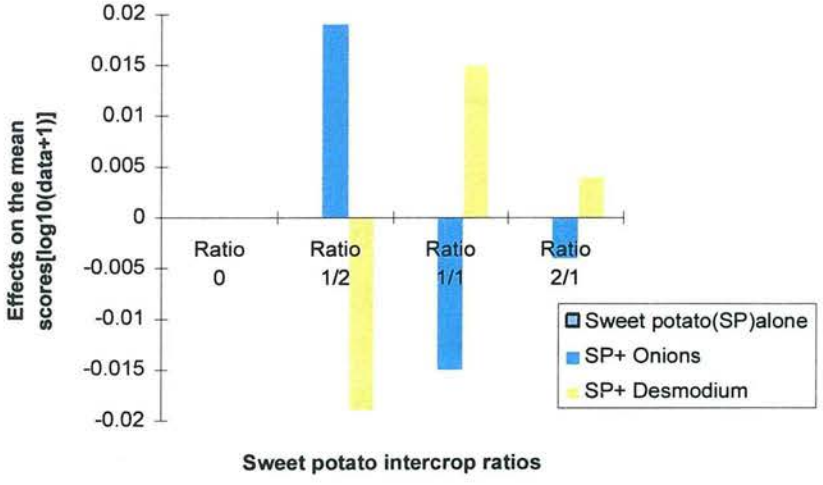


Figure 8.21c Comparison of the effects of the interactions between sweet potato intercrops and their intercrop ratios on the mean numbers of *Acraea acerata* damage score. (Data collected on 16-17/02/2000: fourth week of data collection).

Table 8.2 Trends of the effects of the intercrops sweet potato + onion plants and sweet potato + *Desmodium* plants on the number of *Acraea acerata* larvae and their damage to sweet potato plants.

Factors	Treatments	Number of larvae	Leaves damaged	Plants damaged	Score	**Overall trend**
Cropping systems	Sp alone	0	0	0	0	0
	Sp intercrops	+	+	+	+	+
Intercrops of Sp	Sp alone	0	0	0	0	0
	Sp + O	–	–	–	–	–
	Sp + D	+	+	+	+	+
Ratios of intercrops of Sp	Ratio 0	0	0	0	0	0
	Ratio 1/2	+	+	+	+	+
	Ratio 1/1	+	+	–	–	+/–
	Ratio 2/1	–	–	–	–	–
Interactions of Sp intercrops with intercrop ratios	Sp + O: ratio 0	0	0	0	0	0
	Sp + O: ratio 1/2	+	+	+	+	+
	Sp + O: ratio 1/1	+	–	+	+	+/–
	Sp + O: ratio 2/1	–	–	–	–	–
	Sp + D: ratio 0	0	0	0	0	0
	Sp + D: ratio 1/2	–	–	–	–	–
	Sp + D: ratio 1/1	–	+	–	–	+/–
	Sp + D: ratio 2/1	+	+	+	+	+

Sp: Sweet potato; O: Onions; D: *Desmodium*

0: no effect

+: increasing effect on the number of SPB larvae and their damage

–: decreasing effect on the number of SPB larvae and their damage

The analysis of the effects of the different treatments on the SPB and its damage on sweet potato plants using data collected weekly revealed the same trend as the average data (Figure 8.6 to Figure 8.9c, Figure 8.14 to Figure 8.21c) except for the data collected on the second week of data collection (02-03/02/2000) which showed totally opposite trends (Figure 8.10 to Figure 8.13c). The data collected on the fourth week (16-17/2/2000) presented the same trend as the average data but, unlike the data collected in previous weeks, there were statistically significant differences ($P < 0.05$, $df = 1$) between the number of SPB larvae and their damage on sweet potato plants in plots with intercropped sweet potato compared to sweet potato alone (Figure 8.18, Figure 8.19, Figure 8.20, Figure 8.21).

8.3.3 Discussion

The damage of the SPB to sweet potato plants in all the plots of the field experiment was qualitatively assessed as low to moderate. This was confirmed by the very low mean average score of sweet potato plant damage which was less than 1 (Figure 8.5, Figure 8.9, Figure 8.13, Figure 8.17, Figure 8.21, using anti-log means). This low level of infestation by the SPB might partly explain why no statistically significant differences were found between the effects of different treatments ($P > 0.05$, $df = 6$) on the mean number of SPB larvae and their damage to sweet potato plants. Therefore, one might wonder if the overall low infestation of sweet potato plants by the SPB might not have been due to the overall effect of intercropping sweet potato plants with onions and *Desmodium* plants. The reported serious attacks of the SPB in other sweet potato fields in the same research station and in its vicinity lend support to this suggestion (see 8.3.1). All the factors (Diagram 8.1) which may lead to pest reduction were potentially present: different plants mixed in the same field which could have created an associational resistance, the possible change of physiological fitness due to mutual influence of the three crops, the increase of natural enemies of the SPB by the more diverse habitat and the movement of the SPBs towards more concentrated host plants. Considering the important role of host plant volatiles in the process of host plant finding by female SPBs, fields of monocropped sweet potato plants (concentrated host plants) in the vicinity of the sweet potato intercrop field

experiment might have attracted more ovipositing female SPBs. This seemed to be supported only by the data collected on the second week of data recording which showed that sweet potato plant intercrops tended to decrease the number of SPB larvae and their damage (Figure 8.10, Figure 8.11, Figure 8.12, Figure 8.13). All other data collected on the other sampling dates as well as the average data suggested that the sweet potato intercrops tended to increase the number of SPB larvae and their damage to sweet potato plants (Figure 8.2, Figure 8.3, Figure 8.4, Figure 8.5, Table 9.2). However, as in the process of host plant finding where phytophagous insects first locate their host plant habitat then select patches of host plants in which individual plant resources are chosen (Miller and Strickler, 1984), the whole intercrop field experiment (not the different treatment plots) might have been seen by ovipositing female SPBs as a patch of host plants. As such, intercropped sweet potato plants might have been a less attractive patch to SPBs compared to neighbouring patches in the same habitat which might have been monocrops of sweet potato plants. This might explain why the experimental field, as a 'patch' of host plants mixed with other plants was infested with a low incidence of SPBs. Within the 'patch' (= experimental field), the plots of the intercrop sweet potato + *Desmodium* plants tended to host more SPB larvae and suffer more damage. Compared to sweet potato plant volatiles alone, the mixture of sweet potato + *Desmodium* plant volatiles seemed to attract more female SPBs in laboratory experiments (Chapter 7). In the field, the volatiles emanating from the plots of sweet potato + *Desmodium* plants may have had a similar effect (on ovipositing female SPBs) resulting in more SPB eggs being laid on sweet potato plants. This might explain the trend of higher incidence of SPB larvae in sweet potato/*Desmodium* intercrop plots than sweet potato monocrop plots.

Although, the level of infestation of the SPB was low in the field experiment and the differences between treatments were not statistically significant, the results of the experiment revealed some interesting trends:

i) Sweet potato + *Desmodium* intercrops seemed to have generally an increasing effect on the numbers of SPB larvae and their damage on sweet potato plants whereas intercropping sweet potato with onion plants seemed to have an opposite effect (Figure 8.2a, Figure 8.3a, Figure 8.4a, Figure 8.5a, Table 8.2). The trends of these results could be taken as a confirmation of the early laboratory findings that the mixture of volatiles from sweet potato + *Desmodium* plants attracted more ovipositing female sweet potato butterflies than sweet potato plant volatiles alone whereas the mixture of volatiles from sweet potato + onion plants had an opposite effect (Chapter 8). Onion intercrop has been widely seen as capable of repelling or disorienting host-seeking phytophagous insects (Perrin and Phillips, 1978, Altieri, 1994) and the trend of the above results seemed to support this view. However, cases of intercropping with unrelated non host plant species which resulted in the attraction of more phytophagous insects as the trend of the effects of sweet potato + *Desmodium* plants on the SPB appeared to suggest, have not yet been reported. Unfortunately the full extent of the effect of *Desmodium* plants on the SPB could not be assessed as *Desmodium* plants did not survive an unusual drought in January 2000.

ii) Different sweet potato intercrop ratios appeared to have different effects on the SPB and its damage to sweet potato plants. At low intercrop ratio 1/2, the intercrop sweet potato + *Desmodium* which generally seemed to increase the number of SPB larvae and their damage had an opposite trend and the trend of the effect of the intercrop sweet potato + onions was also reversed (Figure 8.2c, Figure 8.3c, Figure 8.4c, Figure 8.5c, Table 8.2). This might have resulted from the imbalance of the attractants and repellents contained in each of these mixtures of volatiles from sweet potato + onions and sweet potato + *Desmodium* plants due to the difference of biomass between sweet potato and onion plants or sweet potato and *Desmodium* plants in the ratio 1/2 i.e. assuming that host plant volatiles played a role in attracting SPBs to the intercrop sweet potato + onions or intercrop sweet potato + *Desmodium* plants, the volume of volatiles emanating from one row of onion or *Desmodium* plants might have been very low compared to the volume of volatiles emanating

from two rows of sweet potato plants resulting in an imbalance of the volatile blend from sweet potato + *Desmodium* plants (which appeared to attract female SPBs) or the volatile blend from sweet potato + onion plants (which appeared to repel female SPBs or to mask sweet potato plant volatiles) with opposite effects. In fact, the wind tunnel bioassays which suggested that onion plant volatiles might be repelling to the SPB or masking sweet potato plant volatiles and that the mixture of volatiles from sweet potato + *Desmodium* plants had an opposite effect, used sweet potato, onion and *Desmodium* plants of similar biomasses (for whole plant bioassay) and equal volumes and the same concentration for sweet potato, onion and *Desmodium* plant volatiles (for the bioassay which used headspace collected volatiles). Maybe with the intercrop ratio 1/2 and some plots of the intercrop ratio 1/1 (where the effect had been variable), the balance of volatile chemicals in the field experiment was different to the balance reached in the laboratory. It has been suggested that herbivore responses to plant volatiles are based on a delicate balance between attractants and repellents and some insect repellents at low concentrations might act as attractants (Visser, 1986, Foster and Harris, 1997, Theunissen, 1997). Likewise, the opposite effect of attractant acting as repellent at low concentration cannot be excluded. This might have been the case with low concentrations of onion volatiles acting synergistically with sweet potato plant volatiles to affect positively the response to sweet potato plant volatiles and the opposite effect with low concentrations of volatiles from *Desmodium* plants.

The effects of the intercrop ratio 2/1 was conform to the trends of the effects of the two sweet potato intercrops i.e. the ratio 2/1 of the intercrop sweet potato + onion plants tended to decrease the number of SPB larvae and their damage to sweet potato plants, and the ratio 2/1 of the intercrop sweet potato + *Desmodium* plants tended to increase the number of SPB larvae and their damage to sweet potato plants. It seemed that the optimum intercrop ratio to promote the trends of the effects of sweet potato + onion plants and sweet potato + *Desmodium* plants on the SPB as observed in laboratory and field experiments might be situated between ratios 1/1 and 2/1.

iii) With time, the number of SPB larvae increased with more larvae in the last two weeks of data collection (Figure 8.6, Figure 8.10, Figure 8.14, Figure 8.18). Interestingly, data collected on the fourth week revealed statistically significant differences ($P < 0.05$, $df = 1$) between the mean numbers of SPB larvae and their damage in the monocropped sweet potato plants compared to intercropped sweet potato plants (Figure 8.18, Figure 8.19, Figure 8.20. Figure 8.21). This suggested that, not only the hatching of more eggs which might have been laid by butterflies at different times was increasing the number of larvae which fed on sweet potato plants but also SPB larvae were growing which resulted in more damage to sweet potato plants.

The SPB larvae which were recorded at the first sampling dates (27-28/01/2000) indicated that female SPBs had laid eggs at least 5 days (minimum observed period between egg laying and hatching) earlier. It might have been better therefore to start the sampling as early as the first week or second week of January 2000 i.e. before or at the time SPBs laid the first egg batches. It was assumed that depending on the level of attractiveness of the volatiles given off by the plants of a (treatment) plot, a number of ovipositing female SPBs would reach that plot and lay eggs on sweet potato plants. The number of egg batches which were not recorded (assumed to be equal to the number of damaged leaves), could have been the best indicator of how many female SPBs were attracted to different plots (treatments). However, it is reasonable to assume that the number of SPB larvae was proportional to the number of SPBs that laid eggs on sweet potato plants in each plot. Thus, plots which had more SPB larvae attracted more ovipositing female SPBs. Conversely plots which had less SPB larvae attracted less ovipositing female SPBs.

8.4 Conclusion

The endeavour to confirm laboratory findings on the effects of the intercrops sweet potato + onions and sweet potato + *Desmodium* on the response of the SPB on its host plants in a field experiment was an important step. The trends of these intercrops showed that all cases of intercropping do not result in pest reduction. For a given

pest, there are some types and ratios of intercrops which should be avoided. It was interesting to observe that intercropping with unrelated plant species tended to increase the attractiveness of the host plants to the SPB.

The trends of the effects of the intercrops confirmed earlier laboratory findings that the response of the SPB to sweet potato plant volatiles was negatively affected by the mixture of volatiles from sweet potato + onion plants but positively affected by the mixture of volatiles from sweet potato + *Desmodium* plants. Both intercrops could be combined to build up a 'push-pull' management strategy for the SPB (see Chapter 9, section 9.4). Furthermore the trends of the results showed that the components as well as the ratio of an intercrop have to be carefully selected depending on the aim of the intercrop. Further field experiments will be needed to establish firmly the validity of these preliminary field results.

Chapter 9

General discussion, conclusions and future research

9.1 Egg-laying of *Acraea acerata* on sweet potato plants in laboratory conditions

Plants are vital resources for phytophagous insects. However insects do not indiscriminately utilise all plants. Lepidoptera, especially butterflies are known to have a remarkable ability to use plant stimuli to distinguish their host plants (Calvet and Hanson, 1983, Chew and Robbins, 1984). The number of plant stimuli involved increases after landing but even before landing butterflies which have true colour vision (Kinoshita *et al.*, 1999) take advantage of not only plant visual cues but also plant olfactory stimuli (Feeny *et al.*, 1989, Bernays and Chapman, 1994, Hern *et al.*, 1996).

In wind tunnel bioassays, female SPBs were observed flying, landing and laying eggs on nothing else but sweet potato plants situated at about 1.50 m upwind (Chapter 3). Previous studies reported that female SPBs oviposited on host plants offered in rearing cages but egg-laying in a wind tunnel had never been demonstrated before (Lefèvre, 1948, Hill, 1983 and Subukino, 1987, Lugojja, 1996, Azerefegne, 1999). This was the first attempt to study the process of host plant finding by the SPB and egg-laying on sweet potato plants was the most reliable indication that the wind tunnel was an appropriate setting. In all 80 mated female SPBs observed none oviposited on pots with peat (control treatment), walls, floor or roof of the wind tunnel. This confirmed the outstanding ability of butterflies' sensory system to use host plant stimuli to distinguish host plants from non-hosts (Calvet and Hanson, 1983). However, in more confined and maybe crowded rearing spaces, female SPBs have been reported to lay eggs on the walls of rearing cages (Smit *et al.*, 1997, *personal observation*). Such 'mistakes' in oviposition site discrimination could be fatal for the first instar larvae which are not yet ready to walk searching for food. Such 'mistakes' are less likely to happen in real conditions. Maybe the unrealistic laboratory rearing conditions (e.g.: few oviposition site options) played an important role in 'pushing' the butterflies to accept oviposition sites which are not normally acceptable. The study of the pre-oviposition behaviour of *Chlosyne lacinia*, a

butterfly of the same family as the SPB showed that only the ablation of the foretarsi abolished the butterfly's discrimination of oviposition sites leading to 50% egg laying on non-hosts in a controlled environment chamber (Calvet and Hanson, 1983). Might it be that those female SPBs which laid eggs on the walls of cages had accidentally lost their fortarsi? Only pre-oviposition behavioural studies of the SPB would reveal more about such a lack of oviposition site discrimination as studies carried out on host plant finding by ovipositing Lepidopteran species agreed that ovipositional mistakes on non host plants are to be very much avoided as they would most probably be fatal for newly hatched larvae (Calvet and Hanson, 1983, Feeny, Rosenberry and Carter, 1983, Renwick and Chew, 1994). Furthermore, like other phytophagous insects, butterflies have, over time, acquired different cost-effective (like sequestration of host plant poisonous chemicals for their own defence), less risky (in terms of predation) strategies to interact with their host plants to maximise their fitness (Rothschild, 1973, Bell, 1990). They all rely on different senses which feed information gathered from plant stimuli into the insect's central nervous system to be processed and weighed against internal as well as external factors to produce behavioural responses leading (or not) to searching, selecting, accepting (or rejecting) their host plants (or non host plants) (Kennedy, 1977, Miller and Strickler, 1984, Prokopy, 1986 and Renwick and Chew, 1994).

The wind tunnel bioassay was designed to put sufficient distance between sweet potato plants and female butterflies to investigate how they would respond to host plant olfactory and visual stimuli. 10% of observed butterflies flew upwind (because sweet potato plants were located upwind), and landed on sweet potato plants on which they laid eggs. Upwind flight towards sweet potato plants, landing and egg laying on sweet potato plants all suggested that female SPBs were using both host plant olfactory and visual stimuli to locate their host plants. As the aim of this study was to gain understanding into the process of host plant finding by the SPB to suggest strategies to improve its management, it was therefore crucial to study how both host plant olfactory and visual stimuli were involved in attracting the SPB.

9.2 Role of host plant volatiles in attracting female *Acraea acerata*

Plant volatiles are carried by the wind far away from their sources where distant phytophagous insects use them to locate their host plants (Murlis, Elkinton and Cardé, 1992). They constitute the foremost distant cues for phytophagous insects. Outwith plant habitat, plant volatiles, especially the abundant common green leaf volatiles, are thought to attract insects to possible host plant habitats. Within a host plant habitat, specific host plant volatiles would direct insects to patches of host plants and within a patch, mostly visual cues would direct them to land on an individual resource item (Prokopy, 1986, Ramaswamy, 1988, Bell, 1990). The conditions for such a catenary process of host plant finding were not met in wind tunnel bioassays but rather an environment where female SPBs were exposed separately to host plant olfactory stimuli (volatiles), visual stimuli and their combination was created. SPBs moved up closer to the source of sweet potato plant volatiles and landed more often on the muslin-screen in the presence of host plant volatiles (muslin-screened sweet potato plants) compared to visual stimuli alone (glass-screened sweet potato plants) or even the combination of both olfactory and visual stimuli (non-screened sweet potato plants). These results which revealed an important role of host plant volatiles in attracting the ovipositing female SPB to its host plant seemed to be at odds with the general understanding of the process of host plant finding which upholds the integration of insect sensory modalities as advantageous to the host-seeking insect (Dethier, 1982, Miller and Strickler, 1984, Nottingham, 1988). *R. pomonella*, the apple maggot fly, was found to alight more on a host-tree mimicking visual stimuli than it did on natural host-fruit volatiles alone. However, the combination of both olfactory and visual stimuli elicited the most alightments (Prokopy, 1986). Other insects including Lepidopteran species like *P. demoleus*, *P. polyxenes* and *P. rapae* were reported to display a more positive response to the combination of olfactory and visual stimuli (Saxena and Goyal, 1978, Feeny *et al.*, 1989, Hern, 1997). Because of the hierarchical levels of resource distribution (habitat, patch, resource item), Prokopy (1986) suggested that it was important to recognise that the response of a resource-seeking insect to an olfactory or a visual stimulus may be predominant depending on the level of the resource. In

this regard, there was enough evidence to suggest that the response to visual stimuli was predominant for the host-seeking *R. pomonella* (Prokopy, 1986, Aluja and Prokopy, 1993). In the same way, it has also been suggested that the response to olfactory stimuli predominate in host plant finding by moths whereas butterfly responses to host plant visual stimuli may be predominant at least at one level of the resource distribution hierarchy. Chew and Renwick (1995) suggested that vision appeared to be the predominant sensory modality in the orientation phase of host finding in *Pieris* butterflies. In wind tunnel bioassays, the response of the SPB to host plant volatiles seemed to predominate.

The apparent negative effect of host plant visual stimuli on the response of the SPB to host plant volatiles in the combination of olfactory and visual stimuli might have been due to the internal physiological state of butterflies. It had been observed that the female SPB landed or attempted to land on sweet potato plants, only when about to lay eggs (Chapter 3) and only 10% of butterflies laid eggs. These results were therefore consistent with the low response of female SPBs to host plant visual stimuli which seemed to be used only by butterflies which were ready to lay eggs. As to why female SPBs would respond to host plant volatiles by moving closer to odour sources (muslin-screened plants) but did not behave the same way with un-screened plants, it has been suggested that a phytophagous insect which stays longer in a host plant patch would increase its efficiency of search (Evans, 1991). Moreover, it may well be possible, as has been observed in a field experiment in Ethiopia, that SPBs would use host plants not only for ovipositing but also for mating (Azerefegne, 1999). It is likely that butterflies would move into a patch of host plants searching for mates and would not necessarily be interested in landing on host plants. In this study however, the odour-induced upwind movement of mated female SPBs was most probably bringing the butterflies closer to their oviposition site.

The role of host plant volatiles in attracting SPBs was confirmed by the response of the female SPB to headspace host plant collected volatiles. However, the response of the female SPB to host plant headspace volatiles was not as large as in the case of

host plant volatiles emanating directly from whole sweet potato plants especially for the distances moved towards the source of volatiles. It is known that profiles of chemical components of volatiles emitted by plants vary according to the time of sampling and for some compounds, important differences can emerge especially between day and night (Loughrin, Hamilton-Kemp, Andersen and Hildebrand, 1990). The collection of volatiles which lasted 24 hours for each sample (photoperiod of 16L:8D) meant that the volatiles collected contained a large proportion which are normally emitted by sweet potato plants during the night. Sweet potato plant volatiles emitted during the night could have changed the profile of the chemical components of the sweet potato volatiles emitted during the day. Being a diurnal insect, the SPB would have found sweet potato plant volatiles which included volatiles collected during the night less attractive than those it had been exposed to during the day in the wind tunnel bioassay with whole plants. This might explain why butterflies did not show a strong response to headspace volatiles as they did with volatiles emanating from whole plants. Headspace volatiles collected for a number of hours during day time might have provoked similar responses to the wind tunnel experiments.

The sweet potato plant volatiles were found to be made up of a large number of chemical compounds of which alcohols and esters were the main components (Chapter 6). Like aldehydes, these leaf fatty acid derivatives are part of the green leaf volatiles which have been found to play an important role in host plant finding by a large number of phytophagous insects (Metcalf, 1987, Visser, 1986). Although EAG responses do not necessarily mean behavioural responses, both male and female SPBs had a significant EAG response to cis-3-hexen-1-ol. Nottingham and co-workers (1989), using a 'purge-and -trap' leaf extract, had identified copaene, *trans*-caryophyllene, α-humulene, γ-cadinene and γ-elemene as the main sweet potato leaf volatiles. Sweet potato plant volatiles obtained from headspace entrainment also revealed the presence of copaene in a very low proportion and *trans*-caryophyllene and 3-carene in relatively high proportions. EAG response to 3-carene was not high and the response to *trans*-caryophyllene was very low prompting one to think that the SPB might not be relying on the specific odour characteristics of these terpenes alone

to find its host plants. Considering the long list of sweet potato plant volatiles and the electrophysiological responses of SPBs to some of the major chemical components of sweet potato plant volatiles, a mixture of the different components of the host plant volatiles at specific proportions would probably elicit an optimum response from the host-seeking SPB than a single or few chemical components. This view was supported by Metcalf (1987) who argued that evolutionary pressures would favour insect responses to combinations of chemical components of host plant odours.

9.3 Interfering with *Acraea acerata* host plant finding

Researchers have suggested that olfactory and visual stimuli from non-host plants can interfere with host plant stimuli with the resulting effects of disrupting the behavioural responses of phytophagous insects in their process of host plant finding (Perrin and Phillips, 1978, Visser, 1986, Prokopy, 1986, Nottingham, 1988, Andow, 1991, Altieri, 1994, Bernays and Chapman, 1994). The mechanisms which are believed to act in disrupting insect host plant finding by non-host resources were summarised as physical barrier, visual camouflage, masking of host plant odours, altering the profiles of host plant odours, repelling chemicals and appropriate/inappropriate landings (Thiery and Visser, 1986, Andow, 1991, Altieri, 1994, Finch, 1996). As the SPB host plant finding (up to before landing) was found to be dominated by its responses to host plant volatile stimuli (Chapter 5), it was decided to investigate ways of interfering with host plant volatiles to disrupt the responses of the SPB in a bid to suggest some pest management strategies. Intercropping, an old major agricultural practice in the tropics especially in Africa where the SPB is increasingly becoming a threat to the food security of subsistence farmers, became one of the best options to explore (Perrin and Phillips, 1978, Vandermeer, 1989, Tukahirwa and Coaker, 1982, Abate, van Huis and Ampofo, 2000). Intercropping with strong smelling plants such as onion, tomato, garlic, molass grass have been reported as potentially repellent to some phytophagous insects (Tahvanainen and Root, 1972, Buranday and Raros, 1975, Nottingham, 1988, Khan *et al.*, 1997a, Khan *et al.*,1997b). Screening of potentially repellent plants to the SPB in an olfactometer and a wind tunnel suggested the mixtures of volatiles

from sweet potato + onion plants and sweet potato + *Desmodium* plants as being able to modify the behavioural response of the SPB to sweet potato plant volatiles (its major host plant).

The mechanism by which onion plant volatiles interfered negatively with the response of the SPB to sweet potato plant volatiles is not fully understood. The mixture of volatiles from sweet potato + onion plants could have been repellent to the SPB in which case its olfactory receptor systems should have receptors tuned to the repellent volatile chemicals from onion plants or the mixture of sweet potato + onion plants or onion volatiles could have masked sweet potato plant volatiles in which case SPB olfactory receptors tuned to specific sweet potato plant volatiles would not have been able to detect them (Visser, 1986, Nottingham, 1988, Theunissen, 1997). Andow (1991) argued that the sensory basis for odour masking was obscure because 'for a non-host plant chemical to mask a host odour, the non-host odour must interfere with the neural output from the host-odour receptor or affect central-nervous-system processing of the host-odour stimulus'. However, the masking of host plant volatiles might be happening before the insect central nervous system and its olfactory receptors are involved. In this regard, as the wind disperses the host plant volatiles, it would mix them with non-host plant volatiles and the insect would respond differently to the 'mixed' host plant volatile stimuli. The mixing of volatiles from host and non-host plants to produce a different volatile stimulus was confirmed by the mixtures of volatiles from sweet potato plants + *Desmodium* plants (a non-host plant) to which female SPBs responded more positively than sweet potato plant volatiles alone. *D. uncinatum* (Leguminosae) is neither taxonomically related to the sweet potato plant nor to any other known SPB host plants all of which belong to the Convolvulaceae family (Smit *et al.*, 1997, Azerefegne, 1999). It is however known that plant species from both Convolvulaceae and Leguminosae families contain alkaloids and cyanogenic compounds which are sequestered by many butterfly species of the Nymphalidae family (genera of Danainae, Ithomiini, Heliconiinae and Acraeinae) and used as defence chemicals (Bowers, 1988, Steward and Keeler, 1988, Raubenheimer, 1989). In particular, indole alkaloids have been found in plant species

of both *Ipomoea* and *Desmodium* (Ghosal, Srivastava, Banerjee and Dutta, 1971, Ghosal, Mazumder, Mehta, 1972a, Ghosal, Srivastava, 1973a, Marsh *et al.*, 1977, Smolenski and Kinghorn, 1981, Steward and Keeler, 1988). Indole was one of the chemical compounds identified by GC-MS in the sweet potato plant volatiles (Chapter 5). Although, previous studies had shown or suggested that the unpalatability of some of *Acraea* species originated from sequestration of cyanogenic compounds (Owen, 1971, Ackery and Vane-Weight, 1984, Bowers, 1988, Raubenheimer, 1989, Azerefegne, 1999) there is no reason to exclude the possibility of the SPB sequestering both cyanogenic and alkaloid compounds especially as *I. batatas*, its major host plant, contains both compounds. The utilisation of both pyrrolidizine alkaloids and cardiac glycosides is seen as a selective advantage for danaine butterflies which have to find these chemicals on host plant species from different families (Ackery and Vane-Weight, 1984). Pyrrolidizine alkaloids are used by the danaines and ithomiines (Nymphalidae) not only for defence purposes but also for the production of male pheromones such as danaidone (Ackery and Vane-Weight, 1984, Trigo and Motta, 1990). It is therefore hypothesised that the SPB, an aposematic, unpalatable butterfly, sequesters both alkaloids and cyanogenic compounds and its attraction to *Desmodium* plant species is linked to their presence in these plants. It might be even possible, as in the case of some species of danaine and ithomiine butterflies, that the SPB would also use the sequestered alkaloids as pheromone precursors.

From an evolutionary view point, the adoption of *Desmodium* plants as host plants by the SPB, even if it is not taxonomically related to its major host plant, would have increased its fitness by expanding and diversifying its sources of chemical defence compounds. This might have been the case for other butterflies, for instance, *Aeria olena* (Ithomiini) which was reported to visit pyrrolizidine alkaloid containing plants from the families of Asteraceae, Apocynaceae and Boraginaceae and acquire them by phamacophagy or checkerspot butterflies of the genus *Euphydryas* which feed on plant species of Scrophulariaceae, Plantaginaceae, Caprifoliaceae and Bignoniaceae to sequester iridoid glycosides (Bowers, 1988, Trigo and Motta, 1990, Trigo *et al.*,

1996). However, *D. uncinatum* unlike *I. batatas*, has hooked trichomes and a woody stem, anti-herbivore defences which would be expected to deter the SPB (Skerman, Cameron, Riveros, 1988, Steward and Keeler, 1988). *D. uncinatum* which originates from Brazil, might have developed these anti-herbivore characters before it came into contact with the SPB (an African butterfly). Maybe with time, the SPB might interact with *Desmodium* plants and develop mechanisms to overcome *Desmodium*'s defence and adopt it as another host plant.

9.4 Towards elaborating a management strategy for *Acraea acerata*

The behaviour of insects in confined laboratory settings can be often misleading especially with regard to what happens in fields (Wyatt, 1997). Field realities are very complex, very variable with time and the interactions of many biotic as well as abiotic factors impact on the behaviour of phytophagous insects. Nevertheless, the results of a preliminary field experiment in Uganda supported the laboratory findings discussed earlier. The trend of the effects of sweet potato + onion intercrop was to lower numbers of SPB larvae and to reduce the damage of sweet potato plants whereas the trend of the effects of sweet potato + *Desmodium* intercrop appeared to increase the numbers of SPB larvae and their damage to sweet potato plants. The overall damage score was very low and could arguably be partly attributed to the intercropping system of the field experiment as severe attacks were reported in the vicinity of the field experiments. However, many other factors were not monitored, in particular the effect of the intercropping on the parasitism of the SPB. Intercropping sweet potato with onions might have favoured SPB parasitoids and predators resulting in high death rate of SPBs as postulated in the 'enemy hypothesis' (Root, 1973). This could have led to the low incidence of the SPB on sweet potato plants intercropped with onions. Conversely, the intercrop sweet potato + *Desmodium* plants might not have favoured predators and parasitoids which might have resulted in relatively higher incidence of the SPB. Suggestions like these might well explain the trends of the results obtained in the field experiment but such effects of predators and parasitoids seemed unlikely for two reasons: i) all the studies carried out on the effects of predators and parasitoids of the SPB reported a low rate of

parasitoid attacks (Lefèvre, 1948, Lugojja, 1996, Azerefegne, 1999), ii) the SPB is an aposematic butterfly which is thought to sequester cyanogenic compounds and indole alkaloids (as hypothesised) which have one of the highest toxicity of all alkaloids (Steward and Keeler, 1988, Larsen, 1991, Azerefegne, 1999). Avian predation on adult SPBs and/or SPB larvae would therefore have little or no effect on the population of the butterfly. A significant effect of predators and parasitoids seemed therefore unlikely to have occurred.

A physical barrier is another possible cause for reduction of pests in intercropping systems (Perrin and Phillips, 1978, Karel, 1993, Finch, 1996). High plants can disturb the movement of adult butterflies as well as larvae. Both onion and *Desmodium* plants do not grow tall enough to have such an effect on the SPB. *D. uncinatum* is a rambling plant. Thus, the disruption of host plant finding by onion plant volatiles (non host plant volatiles), as suggested by laboratory results, seemed to be a plausible alternative explanation to the low incidence of the SPB in the case of the intercrop sweet potato + onions. In the same way, the mixture of volatiles from sweet potato + *Desmodium* plants which was found more attractive than sweet potato plant volatiles alone would explain the relatively high incidence of the SPB in the intercrop sweet potato + *Desmodium* plants.

The SPB, unlike the sweet potato weevils, a world-wide pest of sweet potatoes, is a tropical butterfly which has been reported only in Africa. East African countries which suffer most from SPB attacks are among the world's poorest countries and depend almost entirely on subsistence farming. A pest management strategy for the SPB suitable for resource-poor subsistence farmers of that region would be a step foward in the war against hunger.

The mixtures of sweet potato + onion plants and sweet potato + *Desmodium* plants as discussed above could be very interesting in elaborating management strategies for the SPB if they were validated by comprehensive field experiment results. They could offer at least four strategic options to control the SPB:

i) trap cropping: the mixture of sweet potato + *Desmodium* plants could be arranged to attract the ovipositing female SPBs away from the main sweet potato crop. Butterflies could find the mixture of sweet potato + *Desmodium* plants more attractive than the monocrop of sweet potato. As reviewed by Hokkanen (1991), trap cropping as a control strategy for herbivorous insects has known some success: for instance, the system cotton (main crop) and alfalfa (trap crop) was efficient in controlling *Lygus hesperus* and *Lygus elisus*; the system soybeans (main crop)/snap beans (trap crop) in controlling the Mexican bean beetle (*Epilachna varivestris*) and the system potato (main crop)/potato (trap crop) in controlling the Colorado beetle, *Leptinotarsa decemlineata*. There are also many proposed trap crop systems which include Lepidoptera species such as tomato (main crop)/corn (trap crop) to control *Heliothis zea*, sorghum (main crop)/corn (trap crop) to control the fall armyworm, *Spodoptera frugiperda* and cowpea (main crop)/*Crotalaria* spp.(trap crop) to control the legume pod borer, *Maruca testulalis*.

The system sweet potato (main crop)/mixture sweet potato + *Desmodium* plants (trap crops) would be more interesting. *Desmodium* plants could not be used as discard plants for the SPB because they are not host plants for SPBs, nevertheless, their dense hooked trichomes which earned some species names such as tick clover, tick trefoil and beggarweed (Allen and Allen, 1981) were reported to trap large insects like butterflies or moths (Sutherst and Wilson, 1986). Moreover, the trichomes of *Desmodium* plants with their 'Velcro® effect' were observed trapping aphids (personal observation) and would probably trap SPB larvae. There would be therefore no need to use pesticides to kill the trapped insects as in some other trap cropping systems (Hokkanen, 1991).

ii) Interfering with the attractiveness of sweet potato plant volatiles: the mixture of sweet potato + onion plants seemed to be repelling or to be avoided by the ovipositing female SPB. Practical field examples of the use of repellent plants against phytophagous insects are very scarce though many reports have suggested non host plant repellency as one of the possible explanations for pest reduction in

mixed cropping especially with strong smelling plants (Perrin and Phillips, 1978, Andow, 1991, Potts and Gunadi, 1991, Finch, 1996, Theunissen, 1997). The recent successful case of molass grass (*M. minutiflora*) used to repel maize and sorghum stem borers (*B. fusca* and *C. patellus*) in field experiments in Kenya (Khan *et al.*,1997b) has supported these reports. It is therefore more likely that the mixture of volatiles from sweet potato + onion plants would be repelling or disorienting for the host-seeking female SPB conferring in this way some level of protection to sweet potato plants.

iii) Combination of i) and ii): this strategy has been termed 'push-pull' or stimulo-deterrent diversionary strategy' (Foster and Harris, 1997, Khan *et al.*, 1997a, Khan *et al.*, 1997b, Pickett, Wadhams and Woodcook, 1997) in which the ovipositing host-seeking female SPB would be 'pushed' away from sweet potato plants by the mixture of volatiles from sweet potato + onion plants and 'pulled' (attracted) by the mixture of volatiles from sweet potato + *Desmodium* plants where they would be trapped by the trichomes of *Desmodium* plants. The 'push-pull' strategy has been successfully used in Kenya to protect maize against lepidopteran maize borers *C. partellus* and *B. fusca*. Molass grass was used to repel ('push') them away from maize crop while they were 'pulled' by Sudan grass (*Sorghum sudanensis*) and Napier grass (*Pennisetum purpureum*), wild graminaceous strong attractant plants for the borers and used as discard plants (Khan *et al.*, 1997a, Khan *et al.*,1997b).

iv) Identification of the chemical compounds responsible for repelling the SPB from the mixture of sweet potato + onion plants and the attracting chemical compounds from the mixture sweet potato + *Desmodium* plants. Insect repellents/or attractants can be used in pest management. Although insect repellents have been mostly limited to protect people against insect bites, some applications of repellents in pest management like verbenone (used against bark beetles), pine oil (used against bark beetles) and (*E*)-(β)-farnesene (used against *Myzus persicae*) have proven the effectiveness of using repellents to control insect pests (Schreck, 1977, Foster and Harris, 1997). On the other hand, insect attractants known as pheromones are used in

mating disruption strategies (Foster and Harris, 1997, McLaughlin, Mitchell and Kirsh, 1994). However, normal plant volatile attractants have been shown to be useful in pest management. For instance, the combination of a bait of methyl eugenol, an insect attractant, with an insecticide resulted in the eradication of the oriental fruit fly, *Dacus dorsalis* from Rota Island (Steiner, 1952, Steiner, Mitchell, Harris, Kozuma, Fujimoto, 1965). A water formulation of almond oil, an attractant of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae) sprayed on almond trees provided some level of protection to unharvested nuts (Van Steenwyk and Barnett, 1987). Concluding his review on plant volatiles as insect attractants, Metcalf (1987) wrote that 'the technology of use of kairomones (kairomone is defined by Whitman (1988) as a compound released by an organism which evokes a response beneficial to a member of another species but not the emitter) in integrated pest management of insect pests appears to have virtually limitless possibilities and can provide the impetus for development of new and novel methods of insect pest suppression', however, the potential uses of repellents/attractants to control the SPB which attacks a subsistence crop would be mostly limited by financial factors.

9.5 Conclusions and future research

The results of this study have revealed that prior to landing host plant olfactory stimuli play a predominant role in host plant finding by the SPB. Interfering with the process of host plant finding by intercropping sweet potato plants with onions or *Desmodium* plants appeared to offer promising environmentally friendly, pest management strategies for resource-poor farmers. Future research work should validate the findings of this study by carrying out comprehensive field experiments of the effects of intercropping sweet potato with onions and/or *Desmodium* plants on the SPB. In particular, the best ratio, planting time and spatial arrangement of the intercrops for optimum effect would have to be determined. Moreover the identification of repellents/attractants for the SPB in sweet potato, onion and *Desmodium* plants or the combination of each with sweet potato plants could open up more pest management opportunities. The suggested chemical similarities between sweet potato and *Desmodium* plants and their implications in evolutionary ecology of

the SPB require further investigations. Lastly, a study of the pre-oviposition behaviour of the SPB and the reaction of sweet potato plant to feeding by SPB larvae would give a more complete picture of the interactions between the SPB and its host plants.

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Appendices

Appendix 3.1 Analysis of variance of the number of butterflies resting, walking, flying and the average distances moved by butterflies in a wind tunnel

Genstat 5 Release 3.2 (PC/Windows/Win32s)

Genstat 5 Second Edition (for Windows)

Genstat 5 Procedure Library Release 3[3] (PL9)

Identifier	Values	Missing	Levels
Treatmen	96	0	2
Identifier	Values	Missing	Levels
Day	96	0	4
Identifier	Values	Missing	Levels
Time	96	0	12

Identifier	Minimum	Mean	Maximum	Values	Missing	
Resting	1.000	7.896	10.000	96	0	Skew
Identifier	Minimum	Mean	Maximum	Values	Missing	
Walking	0.000	1.385	7.000	96	0	Skew
Identifier	Minimum	Mean	Maximum	Values	Missing	
Flying	0.0000	0.5313	3.0000	96	0	Skew
Identifier	Minimum	Mean	Maximum	Values	Missing	
Landing	0.0000	0.5104	30.0000	96	0	Skew
Identifier	Minimum	Mean	Maximum	Values	Missing	
Egg_layi	0.0000	0.1771	1.0000	96	0	Skew
Identifier	Minimum	Mean	Maximum	Values	Missing	
Average_	1.000	2.829	4.400	96	0	
Identifier	Minimum	Mean	Maximum	Values	Missing	
Moving	0.000	1.917	9.000	96	0	Skew

***** Analysis of variance *****

Variate: Average_

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day stratum	3	8.8142	2.9381	4.22	
Day.Treatmen stratum					
Treatmen	1	10.9350	10.9350	15.69	0.029
Residual	3	2.0908	0.6969	3.40	
Day.Treatmen.Time stratum					
Time	11	7.7908	0.7083	3.45	<.001
Lin	1	6.1678	6.1678	30.05	<.001
Quad	1	1.0656	1.0656	5.19	0.026
Deviations	9	0.5574	0.0619	0.30	0.972
Treatmen.Time	11	1.8025	0.1639	0.80	0.641
Treatmen.Lin	1	0.8619	0.8619	4.20	0.044
Treatmen.Quad	1	0.1982	0.1982	0.97	0.329
Deviations	9	0.7425	0.0825	0.40	0.930
Residual	66	13.5450	0.2052		
Total	95	44.9783			

* MESSAGE: the following units have large residuals.

Day 3.00 Treatmen Clean air Time 11.00 1.092 s.e. 0.376

***** Tables of contrasts *****

Variate: Average_

***** Day.Treatmen.Time stratum *****

*** Time contrasts ***

Lin 0.073 s.e. 0.0134 ss.div. 1144.

Quad -0.0100 s.e. 0.00438 ss.div. 10677.

Deviations e.s.e. 0.160 ss.div. 8.00

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	-0.08	-0.04	0.11	0.10	0.02	0.00	-0.09
Time	8.00	9.00	10.00	11.00	12.00		
	0.05	-0.03	-0.13	0.00	0.10		

*** Treatmen.Time contrasts ***

Treatmen.Lin e.s.e. 0.0189 ss.div. 572.

Treatmen	Clean air	SP plants
	0.027	-0.027

Treatmen.Quad e.s.e. 0.00620 ss.div. 5339.

Treatmen	Clean air	SP plants
	0.0043	-0.0043

Deviations e.s.e. 0.227 ss.div. 4.00

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		0.15	-0.10	-0.12	0.06	-0.02	-0.14
SP plants		-0.15	0.10	0.12	-0.06	0.02	0.14

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		0.11	0.03	0.06	0.06	-0.02	-0.07
SP plants		-0.11	-0.03	-0.06	-0.06	0.02	0.07

***** Tables of means *****

Variate: Average_

Grand mean 2.829

Treatmen	Clean air	SP plants
	2.492	3.167

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	2.162	2.375	2.675	2.800	2.837	2.912	2.887

Time	8.00	9.00	10.00	11.00	12.00
	3.087	3.037	2.950	3.075	3.150

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		1.900	1.850	2.125	2.425	2.400	2.375
SP plants		2.425	2.900	3.225	3.175	3.275	3.450

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		2.625	2.775	2.800	2.775	2.875	2.975
SP plants		3.150	3.400	3.275	3.125	3.275	3.325

*** Standard errors of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
e.s.e.	0.1205	0.1602	0.2481
d.f.	3	66	36.50

Except when comparing means with the same level(s) of

Treatmen	0.2265
d.f.	66

*** Standard errors of differences of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
s.e.d.	0.1704	0.2265	0.3509
d.f.	3	66	36.50

Except when comparing means with the same level(s) of

Treatmen	0.3203
d.f.	66

***** Analysis of variance *****

Variate: Resting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day stratum	3	18.125	6.042	1.10	
Day.Treatmen stratum					
Treatmen	1	5.042	5.042	0.92	0.408
Residual	3	16.458	5.486	1.40	
Day.Treatmen.Time stratum					
Time	11	42.958	3.905	0.99	0.462
Lin	1	31.225	31.225	7.94	0.006
Quad	1	0.067	0.067	0.02	0.897
Deviations	9	11.667	1.296	0.33	0.962
Treatmen.Time	11	18.958	1.723	0.44	0.933
Treatmen.Lin	1	0.637	0.637	0.16	0.689
Treatmen.Quad	1	0.522	0.522	0.13	0.717
Deviations	9	17.799	1.978	0.50	0.867
Residual	66	259.417	3.931		
Total	95	360.958			

* MESSAGE: the following units have large residuals.

Day 3.00 Treatmen Clean air Time 12.00 -4.87 s.e. 1.64

***** Tables of contrasts *****

Variate: Resting

***** Day.Treatmen.Time stratum *****

*** Time contrasts ***

Lin -0.165 s.e. 0.0586 ss.div. 1144.

Quad 0.002 s.e. 0.0192 ss.div. 10677.

Deviations e.s.e. 0.701 ss.div. 8.00

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	0.02	-0.16	0.28	-0.04	0.13	-0.32	-0.03
Time	8.00	9.00	10.00	11.00	12.00		
	-0.37	0.78	-0.44	0.45	-0.28		

*** Treatmen.Time contrasts ***

Treatmen.Lin e.s.e. 0.0829 ss.div. 572.

Treatmen	Clean air	SP plants
	-0.024	0.024

Treatmen.Quad e.s.e. 0.0271 ss.div. 5339.

Treatmen	Clean air	SP plants
	-0.007	0.007

Deviations e.s.e. 0.991 ss.div. 4.00

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		0.14	0.47	-0.06	-0.58	-0.58	-0.45
SP plants		-0.14	-0.47	0.06	0.58	0.58	0.45

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		0.20	0.74	0.54	-0.02	0.06	-0.47
SP plants		-0.20	-0.74	-0.54	0.02	-0.06	0.47

***** Tables of means *****

Variate: Resting

Grand mean 7.90

Treatmen	Clean air	SP plants
	8.13	7.67

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	8.88	8.50	8.75	8.25	8.25	7.62	7.75
Time	8.00	9.00	10.00	11.00	12.00		
	7.25	8.25	6.87	7.62	6.75		

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		9.25	9.25	9.00	8.00	8.00	7.50
SP plants		8.50	7.75	8.50	8.50	8.50	7.75

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		8.25	8.25	9.00	7.00	7.75	6.25
SP plants		7.25	6.25	7.50	6.75	7.50	7.25

*** Standard errors of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
e.s.e.	0.338	0.701	1.007
d.f.	3	66	61.89

Except when comparing means with the same level(s) of

Treatmen	0.991
d.f.	66

*** Standard errors of differences of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
s.e.d.	0.478	0.991	1.425
d.f.	3	66	61.89

Except when comparing means with the same level(s) of

Treatmen	1.402
d.f.	66

***** Analysis of variance *****

Variate: Walking

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day stratum	3	24.365	8.122	1.31	
Day.Treatmen stratum					
Treatmen	1	0.010	0.010	0.00	0.970
Residual	3	18.615	6.205	2.75	
Day.Treatmen.Time stratum					
Time	11	24.115	2.192	0.97	0.480
Lin	1	12.693	12.693	5.63	0.021
Quad	1	1.046	1.046	0.46	0.498
Deviations	9	10.376	1.153	0.51	0.861
Treatmen.Time	11	16.865	1.533	0.68	0.752
Treatmen.Lin	1	0.923	0.923	0.41	0.524
Treatmen.Quad	1	0.364	0.364	0.16	0.689
Deviations	9	15.577	1.731	0.77	0.646
Residual	66	148.771	2.254		
Total	95	232.740			

* MESSAGE: the following units have large residuals.

Day 3.00	Treatmen Clean air	Time 11.00	3.15 s.e. 1.24
Day 3.00	Treatmen Clean air	Time 12.00	3.90 s.e. 1.24
Day 4.00	Treatmen Clean air	Time 5.00	3.65 s.e. 1.24
Day 4.00	Treatmen Clean air	Time 6.00	3.65 s.e. 1.24

***** Tables of contrasts *****

Variate: Walking

***** Day.Treatmen.Time stratum *****

*** Time contrasts ***

Lin 0.105 s.e. 0.0444 ss.div. 1144.

Quad -0.010 s.e. 0.0145 ss.div. 10677.

Deviations e.s.e. 0.531 ss.div. 8.00

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	-0.12	0.30	-0.51	0.07	0.18	0.55	-0.05
Time	8.00	9.00	10.00	11.00	12.00		
	-0.01	-0.45	0.00	-0.40	0.47		

*** Treatmen.Time contrasts ***

Treatmen.Lin e.s.e. 0.0628 ss.div. 572.

Treatmen	Clean air	SP plants
	0.028	-0.028

Treatmen.Quad e.s.e. 0.0205 ss.div. 5339.

Treatmen	Clean air	SP plants
	0.006	-0.006

Deviations e.s.e. 0.751 ss.div. 4.00

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		-0.21	-0.31	0.09	0.59	0.59	0.07
SP plants		0.21	0.31	-0.09	-0.59	-0.59	-0.07

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		-0.21	-0.87	-0.30	0.39	-0.06	0.23
SP plants		0.21	0.87	0.30	-0.39	0.06	-0.23

***** Tables of means *****

Variate: Walking

Grand mean 1.39

Treatmen	Clean air	SP plants
	1.40	1.37

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	0.50	1.12	0.50	1.25	1.50	2.00	1.50

Time	8.00	9.00	10.00	11.00	12.00
	1.62	1.25	1.75	1.37	2.25

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		0.25	0.75	0.50	1.75	2.00	2.00
SP plants		0.75	1.50	0.50	0.75	1.00	2.00

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		1.25	0.75	1.00	2.25	1.50	2.75
SP plants		1.75	2.50	1.50	1.25	1.25	1.75

*** Standard errors of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
e.s.e.	0.360	0.531	0.804
d.f.	3	66	43.39

Except when comparing means with the same level(s) of

Treatmen	0.751
d.f.	66

*** Standard errors of differences of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
s.e.d.	0.508	0.751	1.137
d.f.	3	66	43.39

Except when comparing means with the same level(s) of

Treatmen	1.062
d.f.	66

***** Analysis of variance *****

Variate: Flying

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day stratum	3	1.0313	0.3438	0.55	
Day.Treatmen stratum					
Treatmen	1	0.2604	0.2604	0.42	0.564
Residual	3	1.8646	0.6215	1.08	
Day.Treatmen.Time stratum					
Time	11	9.7812	0.8892	1.55	0.135
Lin	1	2.4093	2.4093	4.20	0.044
Quad	1	0.6145	0.6145	1.07	0.304
Deviations	9	6.7575	0.7508	1.31	0.249
Treatmen.Time	11	5.1146	0.4650	0.81	0.629
Treatmen.Lin	1	0.0964	0.0964	0.17	0.683
Treatmen.Quad	1	0.0100	0.0100	0.02	0.895
Deviations	9	5.0082	0.5565	0.97	0.473
Residual	66	37.8542	0.5735		
Total	95	55.9063			

* MESSAGE: the following units have large residuals.

Day 3.00 Treatmen SP plants Time 1.00 1.583 s.e. 0.628

Day 3.00 Treatmen SP plants Time 10.00 -1.667 s.e. 0.628

Day 4.00 Treatmen SP plants Time 11.00 1.833 s.e. 0.628

***** Tables of contrasts *****

Variate: Flying

***** Day.Treatmen.Time stratum *****

*** Time contrasts ***

Lin 0.046 s.e. 0.0224 ss.div. 1144.

Quad 0.0076 s.e. 0.00733 ss.div. 10677.

Deviations e.s.e. 0.268 ss.div. 8.00

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	0.08	-0.14	0.25	0.00	-0.26	-0.17	-0.09
Time	8.00	9.00	10.00	11.00	12.00		
	0.35	-0.35	0.56	0.07	-0.30		

*** Treatmen.Time contrasts ***

Treatmen.Lin e.s.e. 0.0317 ss.div. 572.

Treatmen	Clean air	SP plants
	0.009	-0.009

Treatmen.Quad e.s.e. 0.0104 ss.div. 5339.

Treatmen	Clean air	SP plants
	0.001	-0.001

Deviations e.s.e. 0.379 ss.div. 4.00

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		0.08	-0.16	-0.04	-0.04	-0.05	0.32
SP plants		-0.08	0.16	0.04	0.04	0.05	-0.32

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		0.18	0.17	-0.22	-0.48	-0.12	0.36
SP plants		-0.18	-0.17	0.22	0.48	0.12	-0.36

***** Tables of means *****

Variate: Flying

Grand mean 0.531

Treatmen	Clean air	SP plants
	0.479	0.583

Time	1.00	2.00	3.00	4.00	5.00	6.00	7.00
	0.500	0.250	0.625	0.375	0.125	0.250	0.375
Time	8.00	9.00	10.00	11.00	12.00		
	0.875	0.250	1.250	0.875	0.625		

Treatmen	Time	1.00	2.00	3.00	4.00	5.00	6.00
Clean air		0.500	0.000	0.500	0.250	0.000	0.500
SP plants		0.500	0.500	0.750	0.500	0.250	0.000

Treatmen	Time	7.00	8.00	9.00	10.00	11.00	12.00
Clean air		0.500	1.000	0.000	0.750	0.750	1.000
SP plants		0.250	0.750	0.500	1.750	1.000	0.250

*** Standard errors of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
e.s.e.	0.1138	0.2678	0.3800
d.f.	3	66	65.63

Except when comparing means with the same level(s) of

Treatmen	0.3787
d.f.	66

*** Standard errors of differences of means ***

Table	Treatmen	Time	Treatmen Time
rep.	48	8	4
s.e.d.	0.1609	0.3787	0.5374
d.f.	3	66	65.63

Except when comparing means with the same level(s) of

Treatmen	0.5355
d.f.	66

Appendix 4.1 Analysis of variance of the average distances moved and the time allocated to resting and moving (walking + flying or WFL) by butterflies in a wind tunnel.

Genstat 5 Release 3.2 (PC/Windows/Win32s)

Genstat 5 Second Edition (for Windows)

Genstat 5 Procedure Library Release 3[3] (PL9)

Identifier	Values	Missing	Levels
Olf_cues	80	0	2

Identifier	Values	Missing	Levels
Vis_cues	80	0	2

Identifier	Minimum	Mean	Maximum	Values	Missing
Resting	1.00	20.78	30.00	80	0

Identifier	Minimum	Mean	Maximum	Values	Missing
WFL	0.000	8.512	27.000	80	0

Identifier	Minimum	Mean	Maximum	Values	Missing	
d1	14.60	41.68	131.40	80	0	Skew

Identifier	Minimum	Mean	Maximum	Values	Missing
d2	-14.60	58.18	146.00	80	0

Identifier	Minimum	Mean	Maximum	Values	Missing
d3	-14.60	65.37	160.60	80	0

***** Analysis of variance *****

Variate: d1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Olf_cues	1	22946.2	22946.2	23.89	<.001
Vis_cues	1	7428.2	7428.2	7.73	0.007
Olf_cues.Vis_cues	1	7769.7	7769.7	8.09	0.006
Residual	76	72988.5	960.4		
Total	79	111132.7			

* MESSAGE: the following units have large residuals.

units 48 91.8 s.e. 30.2

units 74 77.7 s.e. 30.2

***** Tables of effects *****

Variate: d1

Olf_cues response 33.9 s.e. 6.93 rep. 40

Vis_cues response -19.3 s.e. 6.93 rep. 40

Olf_cues.Vis_cues response

-39.4 s.e. 13.86 rep. 20

***** Tables of means *****

Variate: d1

Grand mean 41.7

Olf_cues	no	yes
	24.7	58.6

Vis_cues	no	yes
	51.3	32.0

Olf_cues \ Vis_cues	no	yes
no	24.5	25.0
yes	78.1	39.1

*** Standard errors of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
e.s.e.	4.90	4.90	6.93

*** Standard errors of differences of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
s.e.d.	6.93	6.93	9.80

***** Analysis of variance *****

Variate: d2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Olf_cues	1	52523.	52523.	34.19	<.001
Vis_cues	1	3530.	3530.	2.30	0.134
Olf_cues.Vis_cues	1	7316.	7316.	4.76	0.032
Residual	76	116755.	1536.		
Total	79	180125.			

***** Tables of effects *****

Variate: d2

Olf_cues response 51.2 s.e. 8.76 rep. 40

Vis_cues response -13.3 s.e. 8.76 rep. 40

Olf_cues.Vis_cues response

-38.3 s.e. 17.53 rep. 20

***** Tables of means *****

Variate: d2

Grand mean 58.2

Olf_cues	no	yes
	32.6	83.8

Vis_cues	no	yes
	64.8	51.5

Olf_cues	Vis_cues	no	yes
no		29.6	35.5
yes		100.0	67.6

*** Standard errors of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
e.s.e.	6.20	6.20	8.76

*** Standard errors of differences of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
s.e.d.	8.76	8.76	12.39

***** Analysis of variance *****

Variate: d3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Olf_cues	1	52374.	52374.	31.24	<.001
Vis_cues	1	1131.	1131.	0.67	0.414
Olf_cues.Vis_cues	1	2000.	2000.	1.19	0.278
Residual	76	127395.	1676.		
Total	79	182900.			

* MESSAGE: the following units have large residuals.

units 26 -114.3 s.e. 39.9

units 64 122.1 s.e. 39.9

***** Tables of effects *****

Variate: d3

Olf_cues response 51.2 s.e. 9.15 rep. 40

Vis_cues response -7.5 s.e. 9.15 rep. 40

Olf_cues.Vis_cues response

-20.0 s.e. 18.31 rep. 20

***** Tables of means *****

Variate: d3

Grand mean 65.4

Olf_cues	no	yes
	39.8	91.0

Vis_cues	no	yes
	69.1	61.6

Olf_cues	Vis_cues	no	yes
no		38.5	41.0
yes		99.7	82.2

*** Standard errors of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
e.s.e.	6.47	6.47	9.15

*** Standard errors of differences of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
s.e.d.	9.15	9.15	12.95

***** Analysis of variance *****

Variate: Resting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Olf_cues	1	273.80	273.80	5.52	0.021
Vis_cues	1	2.45	2.45	0.05	0.825
Olf_cues.Vis_cues	1	20.00	20.00	0.40	0.527
Residual	76	3771.70	49.63		
Total	79	4067.95			

* MESSAGE: the following units have large residuals.

units 33	-18.60	s.e. 6.87
units 63	-19.30	s.e. 6.87

***** Tables of effects *****

Variate: Resting

Olf_cues response -3.70 s.e. 1.575 rep. 40

Vis_cues response -0.35 s.e. 1.575 rep. 40

Olf_cues.Vis_cues response

-2.00 s.e. 3.150 rep. 20

***** Tables of means *****

Variate: Resting

Grand mean 20.77

Olf_cues	no	yes
	22.62	18.92

Vis_cues	no	yes
	20.95	20.60

Olf_cues \ Vis_cues	no	yes
no	22.30	22.95
yes	19.60	18.25

*** Standard errors of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
e.s.e.	1.114	1.114	1.575

*** Standard errors of differences of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
s.e.d.	1.575	1.575	2.228

***** Analysis of variance *****

Variate: WFL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Olf_cues	1	221.11	221.11	5.62	0.020
Vis_cues	1	63.01	63.01	1.60	0.210
Olf_cues.Vis_cues	1	37.81	37.81	0.96	0.330
Residual	76	2992.05	39.37		
Total	79	3313.99			

* MESSAGE: the following units have large residuals.

units 15 15.25 s.e. 6.12

units 35 17.40 s.e. 6.12

***** Tables of effects *****

Variate: WFL

Olf_cues response 3.32 s.e. 1.403 rep. 40

Vis_cues response 1.78 s.e. 1.403 rep. 40

Olf_cues.Vis_cues response

2.75 s.e. 2.806 rep. 20

***** Tables of means *****

Variate: WFL

Grand mean 8.51

Olf_cues	no	yes
	6.85	10.17

Vis_cues	no	yes
	7.63	9.40

Olf_cues \ Vis_cues	no	yes
no	6.65	7.05
yes	8.60	11.75

*** Standard errors of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
e.s.e.	0.992	0.992	1.403

*** Standard errors of differences of means ***

Table	Olf_cues	Vis_cues	Olf_cues Vis_cues
rep.	40	40	20
d.f.	76	76	76
s.e.d.	1.403	1.403	1.984

Appendix 4.2 Genstat program for the randomisation test

```
variate [values=1...1000] n,vp, vs, vps
for vv=p1, p2, p3
calc rolf, rvisu=olef,visu
for i=0...1000
        calc p=sum(rolef*vv)
        &   s=sum(rvisu*vv)
        &   ps=sum(rolef*rvisu*vv)
        if i.eq.0
                calc xp, xs, xps=p, s, ps
                print xp, xs, xps
        else
                calc elem(vp, vs, vps; i) =p, s, ps
        endif
        randomize rolef, rvisu
endfor
sort vp
&   vs
&   vps
    hist vp
&   vs
&   vps
      print n, vp, vs,vps
calc ip, is, ips=0
for i=1...1000
         if xp.gt.elem(vp;i)
                calc ip=i
        endif
        if xs.gt.elem(vs;i)
                calc is=i
        endif
        if xps.gt.elem(vps;i)
                 calc ips=i
        endif
  endfor
print 'Point for olef', ip, Out of 1000'; dec=0
      &      'Point for visu', is, Out of 1000'; dec=0
&     'Point for olef,visu interaction', ips, 'Out of 1000'; dec=0
endfor
```

Appendix 8.1 Data recording sheet for the field experiment on the effects of the intercrops sweet potato/onions and sweet potato/*Desmodium* on *Acraea acerata*

STUDY NUMBER	NICK/PhD/96
STUDY TITLE	Effects of the intercrops sweet potato/onions and sweet potato/Desmodium on *Acraea acerata* (Lepidoptera: Nymphalidae)
SUPERVISOR	Dr Rod McKinlay
PhD STUDENT	Nicholas Hitimana

SAC CROPS DIVISION DATA SHEET	Page of
Study No:-	Date / /
Title:-	DD/MMM/YY
Assessor	Growth stage

Plot No	Plant No	Height	Total no. of Leaves	No. of damaged Leaves	No of Egg batches	Location: Upper (U) Middle (M) Lower (L)	No of eggs/ batch	No. of larvae	Damge score
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								
	9								
	10								

Damage score

Score 0 = No damage; all plants free from caterpillar feeding;
1 = Slight damage; few plants showing feeding symptoms;
2 = About 25 % of the plants showing pest feeding symptoms;
3 = About 50% of plants showing pest feeding symptoms;
4 = Damage serious with >50 of the plants damaged, but plants not yet dead;
5 =Plants completely defoliated bare to the vine; plants dead;

Comments:-

Signature:-

Published papers

Paper 1. *Proceedings of the 1998 Brighton Conference on Pests & Diseases* Vol.**1**: 309-310.

A study of olfactory and visual cues attracting the sweet potato butterfly, *Acraea acerata*, to its host plant

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ABSTRACT

The results of a bioassay in a wind tunnel using glass-screened, muslin-screened and non-screened sweet potato plants suggest that sweet potato volatiles play an important role in attracting *Acraea acerata* to its host plant. Both the distance moved by mated female butterflies towards muslin-screened plants (olfactory cues) and the percentage of butterflies which landed on the screen support this conclusion. Visual cues seemed to have a negative effect.

INTRODUCTION

The sweet potato butterfly (*Acraea acerata*) is a serious pest of sweet potato in East Africa. Its caterpillars defoliate sweet potato leaves reducing tuber yield by up to 70% (Tardif-Douglin & Rwalinda, 1993). Small subsistence farmers who rely on sweet potato as a staple crop cannot easily afford to buy pesticides with which to control the butterfly. Such farmers could potentially use intercropping, the most common cultural practice of the tropics (Altieri, 1994), to reduce damage from the butterfly. Intercropping tends to disrupt and/or mask plant olfactory and/or visual stimuli which attract the host plant seeking insect pests (Altieri, 1994).

With the aim of developing a pest management strategy for sweet potato butterfly using appropriate intercropped plants, we have initially examined the role of plant visual structures and olfactory volatiles in attracting *A. acerata* to its host plant.

MATERIALS AND METHODS

Sweet potato plants were grown in the glasshouse using vine cuttings planted in pots. All the plants used for the bioassay were from the same clone. Butterflies were kept between 23-27 °C with a photoperiod of L12:D12 and 70% relative humidity. Eggs

of *A. acerata* obtained from Uganda had been collected from a sweet potato field. Caterpillars were fed on sweet potato plants and butterflies on a 10% sugar solution.

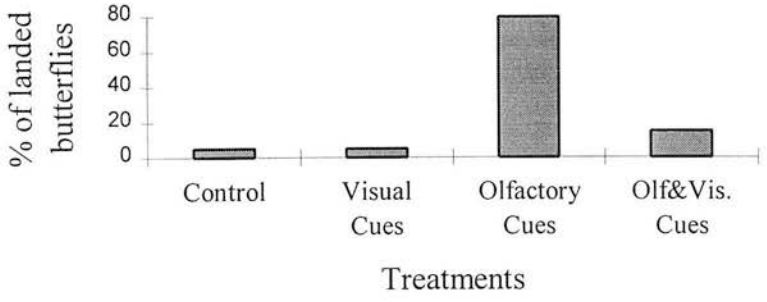
The experiment was carried out in the wind tunnel described by Hern (1997). Its experimental area measured 2.0 m wide, 1.75 m long and 1.0 m high. The lighting provided full spectrum light close to daylight. The average wind speed was 29 cm/s and the temperature interval was 26-30 °C. Treatments were introduced to the wind tunnel to allow the wind speed and the temperature to stabilise before releasing butterflies. There were 20 butterflies per treatment.

There were four treatments: pots with peat only (control), glass-screened (visual cues), muslin-screened (olfactory cues) and non-screened sweet potato plants (visual & olfactory cues). Six week old plants were used. For each treatment, three pots were placed at about 25 cm from the upwind wall of the tunnel. Individual two day old naive and mated female butterflies were released onto a thread through a small window at the height of 35 cm in the downwind wall of the tunnel. The main behavioural events recorded were resting, walking, flying and landing on the treatment for a total observation time of 30 minutes per individual butterfly. The positions of butterflies in wind tunnel were also recorded.

RESULTS AND DISCUSSION

The analysis of variance of the average distances (cm) moved by sweet potato butterflies towards the treatments suggests a very strong effect of sweet potato volatiles (33.87 ± 6.92, $P < 0.001$; 50.52 ± 8.93, $P < 0.001$; 51.10 ± 9.17, $P < 0.001$ respectively for the periods 1-10, 11-20 and 21-30 minutes) in attracting the butterflies towards their host plant. The effect of visual cues (-19.27 ± 6.92, $P = 0.007$) and subsequently the effect of the interaction of both visual and olfactory cues (-39.42 ± 13.87, $P = 0.006$) on movement towards the host plant seemed to be negative for the first 10 minutes of the observation period. These results, supported by the very high percentage of butterflies which landed on the muslin screen (Figure 1), suggest that female sweet potato butterflies are stimulated by host plant volatiles to orient and move upwind towards their host plants.

Figure 1. Landing of butterflies in wind tunnel



ACKNOWLEDGEMENTS

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Paper 2. *Proceeding of the International Symposium on lepidopteran pests in vegetable crops* (Skierniewice, Poland, 30/11-4/12/1988). In Press

Attractiveness of host plant volatiles to sweet potato butterfly, *Acraea acerata* (Lepidoptera: Nymphalidae)

N Hitimana[1], R G McKinlay[2] & E A Hunter[3]

ABSTRACT

Sweet potato volatiles obtained by the headspace entrainment method were used in a wind tunnel bioassay to test for their attractiveness to *Acraea acerata* the sweet potato butterfly. The butterflies reacted to the presence of sweet potato volatiles by increasing the time they spent walking and/or flying, and by increasing the percentage of landings and the number of landings per butterfly. The results confirmed the conclusion of a previous bioassay which used screened plants that sweet potato volatiles play an important role in attracting *Acraea acerata* to its host-plant.

INTRODUCTION

Sweet potato is mostly known for its tuber production. However, leaves and tips of sweet potato provide a very healthy vegetable consumed in some countries like China, India, Brazil, Tanzania and Liberia (Woolfe, 1992; Kapanga, Ewell, Jeremiah & Kileo, 1995; Hitimana, 1996). Apart from spinach, sweet potato leaves have much more protein content than the commonly consumed Western vegetables (Woolfe, 1992).

The sweet potato butterfly *Acraea acerata*, an African butterfly the larvae of which feed on sweet potato leaves, reduces both tuber and vegetable production of sweet potato. It is reported to be a serious pest of sweet potato in East Africa (Lefèvre, 1948; Hill, 1983; Tardif-Douglin & Rwalinda, 1993). Farmers of that region generally grow sweet potato as a subsistence crop. The methods of control of *A. acerata* will therefore be mostly limited to those which do not require some monetary investments such as hand-picking webs of young caterpillars and destroying them; and early planting and harvesting to escape heavy outbreaks (Skoglund & Smit, 1994). It is with a view to increasing the range of farmer's options for controlling *A. acerata* that a study of how the butterfly finds its host-plants was undertaken. A previous bio-assay using screened sweet potato plants had suggested that *A. acerata* is mostly attracted to its host-plant by volatiles (Hitimana, McKinlay & Hunter, 1998). This paper will present the results of a bio-assay in a wind tunnel which recorded the activities of sweet potato butterflies in the presence of host-plant volatiles.

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MATERIALS AND METHODS

Sweet potato plants: sweet potato plants were grown in the glasshouse using vine cuttings planted in pots. All the plants used for the bioassay were from the same clone. The clone was obtained by growing one sweet potato tuber bought from a local supermarket. Sweet potato tubers were first germinated by half submerging them in water and keeping them at 20 °C in an incubator. Once germinated , they were placed in pots containing peat in a glasshouse.

Butterflies: eggs of *A. acerata* were obtained from Uganda (Scottish Office Agriculture Environment and Fisheries Department Import licence no. PH/10/1998). The culture of butterflies was kept in the insectary at the Scottish Agricultural College in Edinburgh between 23-27 °C with a photoperiod of L12:D12 and 70% relative humidity. Caterpillars were fed on whole sweet potato plants and/or cuttings. Butterflies were fed on 10% sugar solution.

Sweet potato used for volatile collection: sweet potato used for volatile collection was grown without agro-chemicals. The pots of sweet potato were put on a tray made of a water-proof black plastic sheet and covered by a kind of cage. The tray was constantly covered by water which not only watered the plants but also stopped insects especially aphids walking onto the plants. The cage served as a physical barrier to flying insects.

Sweet potato volatile collection: headspace entrainment method

The entrainment system used was similar to that described by Robertson, Griffiths, Smith & Butcher (1993). Appendix 1 pictures a simplied diagrammatic representation of the entrainment system used. Ambient air was drawn by a vacuum pump through stainless steel puritubes filled with activated charcoal (air filter) (Cat. No. 900058, Phase Separations Ltd, Deeside Industrial Estate, Clwyd CH5 2NU, UK) and molecular sieve (to regulate the humidity of the air) (Cat. No.900046, Phase Separations Ltd, Deeside Industrial Estate, Clwyd CH5 2NU, UK) into a 2 litre volume airtight flask. The flask contained vine cuttings of sweet potato plants placed in water in a small glass container. Six to eight week old plants were used. A glass column filled with 0.3 g of Tenax Ta ([C6H4 0]X: poly (2,6-diphenyl-p-phenylene oxide)), a porous polymer, as a volatile trap was placed at the exit port of the flask to collect volatiles given off by the sweet potato plants. The Tenax Ta was conditioned by passing 3.5 ml (2 volumes of the column) of diethyl ether. The columns were then dried by passing through dry air for 30 minutes. The columns were heated in a gas chromatograph at 180 °C (heating rate: 8 °C/minute) for 3 hours with a stream of helium (BOC grade A) passing through at a rate of 20 ml/minute. To eliminate odour contamination, Teflon (PTFE) tubing was used and the sealing was done by a Teflon tape (PTFE tread seal tape, BS 7786: 1995 Grade L). The collection was done for 24 hours with a photoperiod of L16:D8 under sodium light (conditions of the glasshouse where the plants were grown). After 24 hours, the columns were removed and the trapped volatiles were eluted with 3.5 ml of diethyl ether in a glass sample tube in a

bath of ice/methanol. The volatiles were diluted to 1 gle (gram leaf equivalent) before using them in bio-assays.

The wind tunnel bioassay: the wind tunnel was described by Hern (1997). Appendix 2 shows a diagrammatic representation of the wind tunnel (the same as Figure 3.1). The assay was carried out in the same way as described by Hitimana *et* al.(1998). The average wind speed was 27cm/s and the temperature interval was 26-30 °C. Treatments were first introduced into the experimental area of the wind tunnel before releasing butterflies. There were 20 butterflies per treatment. There were three treatments: Ambient air(T0), solvent alone (diethyl ether)(T1) and solvent + sweet potato volatiles(T2). A 50 ml vial with a wick was used for T1 and T2. Individual two day old naive and mated female butterflies were released onto a thread through a small window at a height of 35 cm in the downwind wall of the tunnel and observed for a 30 minute period. The main behavioural events recorded were resting, walking, flying and landing on the treatment. The positions of butterflies along the length of wind tunnel were also recorded.

RESULTS AND DISCUSSION

Four parameters had been considered: distances moved by butterflies towards the treatments, activities of butterflies (resting vs. walking & flying), number of butterflies landed on treatments and the average landings per butterfly landed. The analysis of variance of the average distances moved by butterflies did not reveal any statistically significant difference ($P > 0.05$) between treatments. This might be explained by the relatively high activity (walking & flying) of butterflies in the presence of sweet potato volatiles. Figure 1 shows that butterflies spent more than 50% of the observation time (min) walking + flying (5 ± 2, 7 ± 2, and 16 ± 2 in the presence of ambient air, the solvent and sweet potato volatiles respectively; $P < 0.001$) in the presence of sweet potato volatiles. Conversely, in the presence of ambient air or solvent alone they spent about 80% of their time resting.

Figure 1: Activities of butterflies in wind tunnel

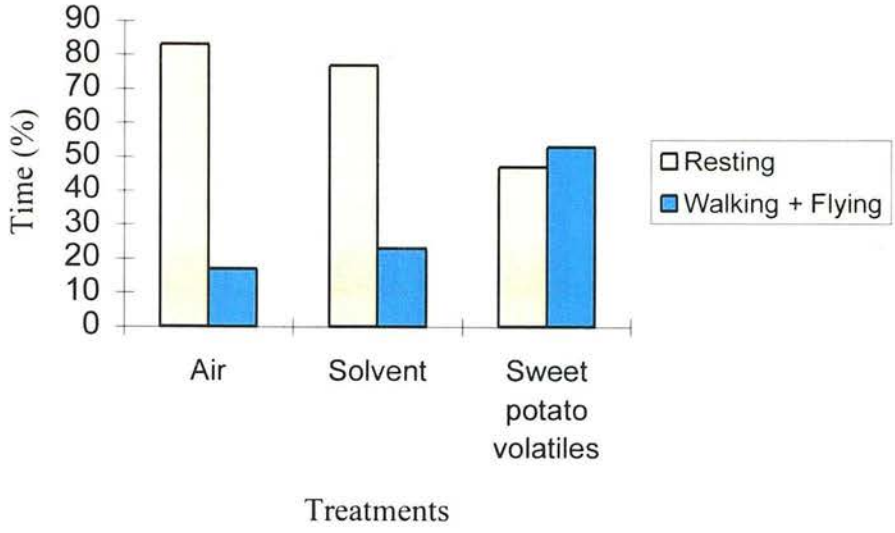
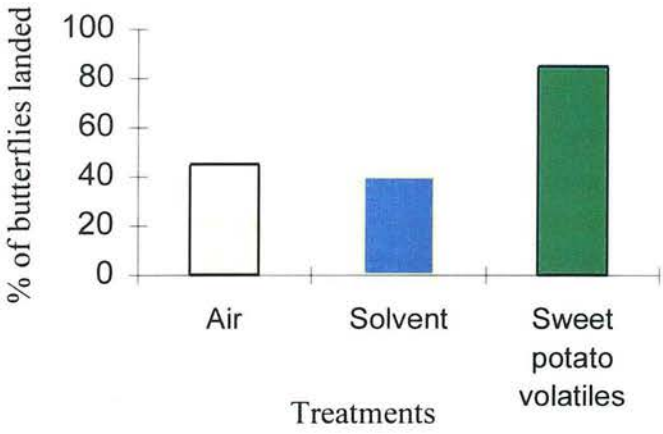
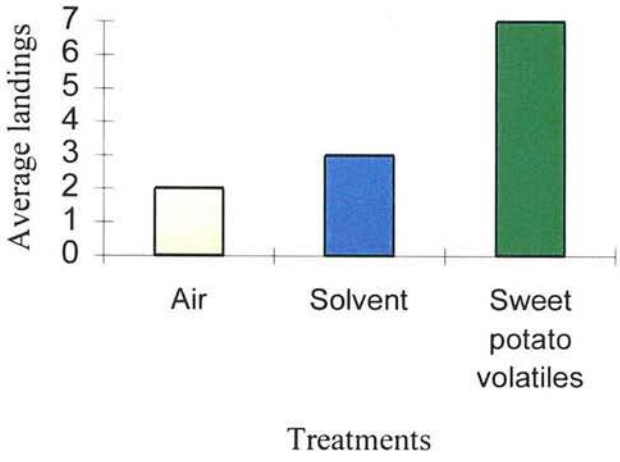


Figure 2: Landing of butterflies on different treatments



The attraction of *A. acerata* to sweet potato volatiles was also supported by the effect the volatiles had on the percentage of landings of butterflies (P = 0.006) and the average number of landings per butterfly landed (P = 0.001). In the presence of sweet potato volatiles, there were more than 80% of landings against less than 50% for the ambient air and solvent alone as shown in Figure 2. Moreover, in the presence of sweet potato volatiles, there were 2 to 3 times more landings/butterfly than in the other treatments as shown in Figure 3. There was one landing in about every 4 minutes in the case of sweet potato volatiles whereas for the control, there was one landing in every 10-15 minutes.

Figure 3: Average landings per butterfly



Conclusion

The results confirm the conclusion of a previous bio-assay which used screened sweet potato plants that sweet potato volatiles play a very important role in attracting *A. acerata* to the sweet potato plant (Hitimana *et* al.,1998). This opens up the possibility of investigating the potential for intercropping sweet potato with other plants which might mask the volatiles emitted by the host plant and so reduce the incidence of the sweet potato butterfly.

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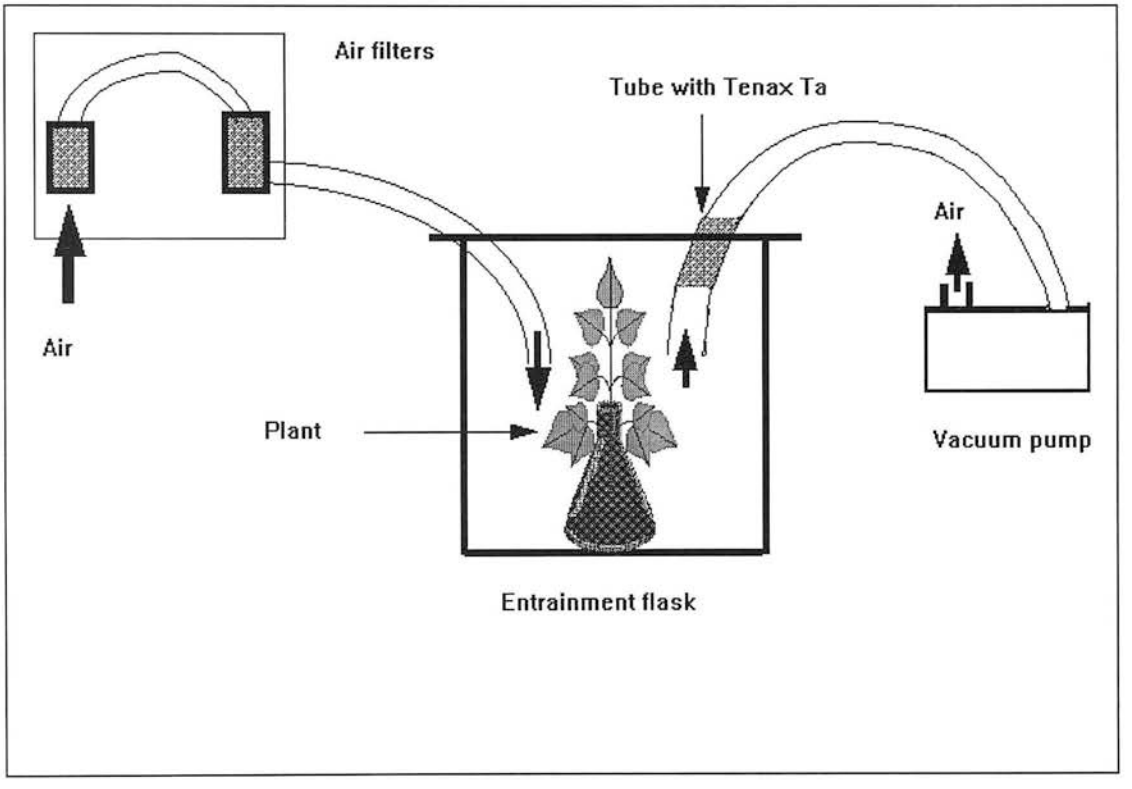
Appendix 1.Simplified diagrammatic representation of the headspace entrainment system



Paper 3. *Agroforestry Forum* **9**(2): 9-11, June 1988

The Effects of Intercropping on Phytophagous Pests: A review.

Intercropping, a very old agricultural practice, could also be a very useful, environmentally friendly pest management option of the future. The reduction of phytophagous pests by intercropping is, however, complex. Researchers should seek first to understand specific insect-host plant interactions in order to alter them using appropriate intercrops.

Introduction

Intercropping, still widely practised by small farmers in the tropics, is one of the oldest agricultural practices (Papendick, Sanchez and Triplett,1976; Francis,1986; Vandermeer, 1989; Innis, 1997). Intercropping is here referred to as growing two or more crops simultaneously in the same field during part or all of the life cycle of each crop (Francis,1986; Vandermeer, 1989). The spatial arrangement of the crops is a variable which contributes to the creation of a less/more 'competitive' or a less/more 'facilitative' environment to the intercrops (Vandermeer, 1989). If the balance of advantage resulting from the new environment created by a particular intercropping pattern is perceived positively by farmers, it will be adopted. One of the advantages of intercropping is a reduction in herbivore attack. There is now a growing interest in this advantage of intercropping as the problem of pollution of the environment by agricultural chemicals is becoming more and more a general public concern especially in industrialised countries. This paper presents an overview of the reduction of pest incidence by intercropping.

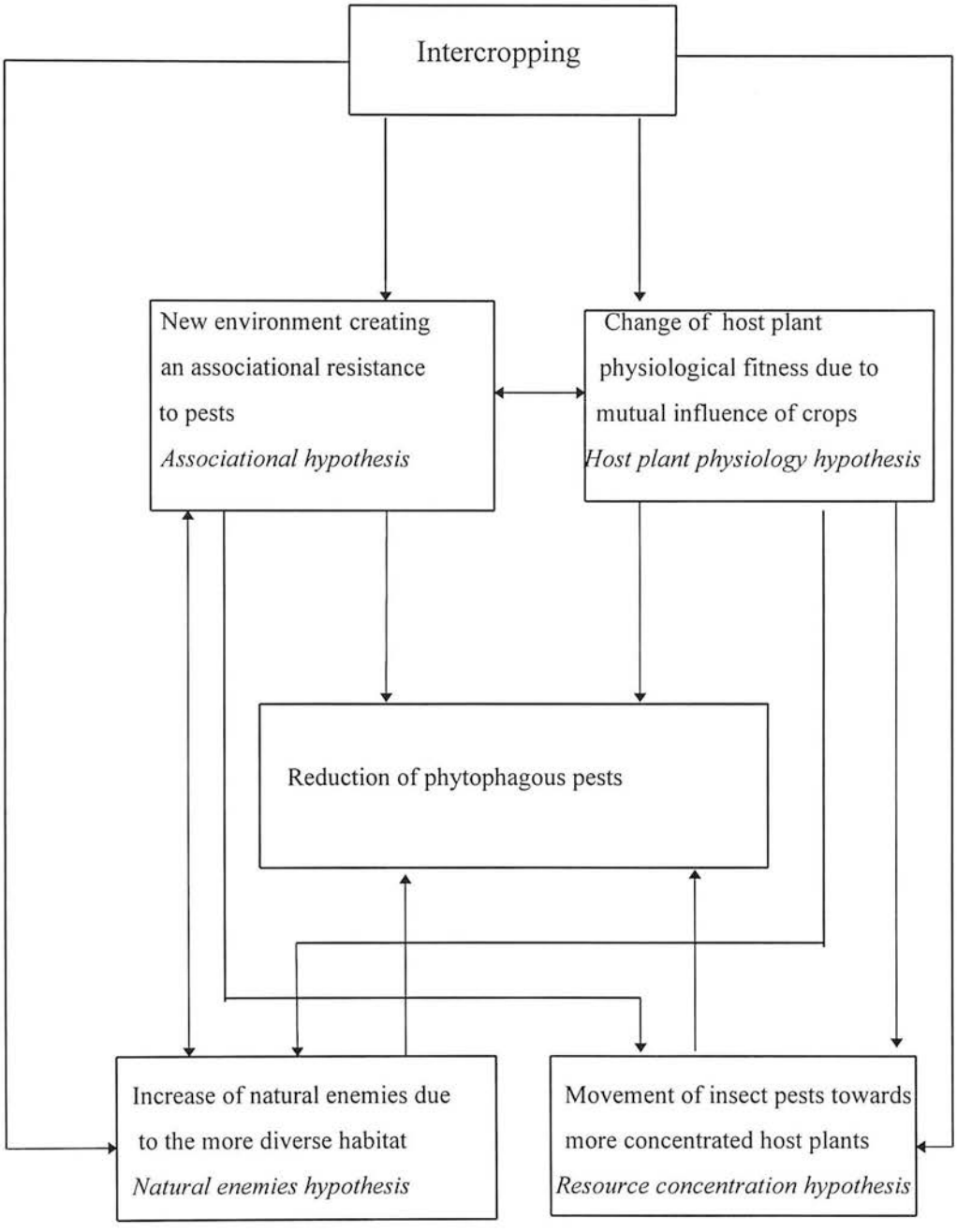
Reduction of pests by intercropping

Many researchers have been trying to understand the mechanisms explaining the reduction of pest damage in diverse habitats. Diagram 1 gives a condensed picture of existing hypotheses and the relationships between them which would support the reduction of insect pests by intercropping. Reviewing various explanatory hypotheses, Vandermeer (1989) summarized them as: the disruptive crop, the trap crop and the enemies hypotheses. Andow (1991) reviewed the responses of arthropod herbivores to mixtures of different plant species (polycultures). With 163 references, he discussed proposed hypotheses explaining the responses of arthropods to polycultures in order to elaborate a common theory which would account for all the different arthropod responses to polycultures. He found that many cases of herbivore responses to plant mixtures were mostly explained by the resource concentration hypothesis (Diagram 1). He underlined, however, that the response of polyphagous insects and the effects of natural enemies are still unpredictable. He suggested that there might be more than one ecological explanatory theory.

Altieri (1994) listed the factors involved in the reduction of insect pest incidence in mixed crops: increase of parasitoid and predator populations, higher availability of alternate food for natural enemies, decrease in pest colonisation and reproduction, chemical repellency, masking and/or feeding inhibition due to nonhost plants,

physical barriers to pest movement and/or emigration, '*and optimum synchrony between pests and natural enemies*'.

Diagram 1: The effects of intercropping on phytophagous pests



Discussing the mechanisms described by Altieri (1994), Finch (1996) agreed with the mechanism of physical interference but underlined the incompleteness of available experimental data. He pointed out the need for scientific experimental proofs for the other mechanisms apart from the alteration of the hostplant odour profiles which is

currently being studied. In opposition to the previous mechanisms, he presented another mechanism termed 'appropriate/inappropriate landings' to describe why host plants grown in bare soil become populated with more insect pests than when host plants are undersown with another plant. All landings on host plants growing on bare soil will be 'appropriate' as insects were said to land solely on host plants, being the only green objects available. Conversely, 'inappropriate' landings occurred when host plants were undersown because 'pest species do not discriminate between host and non-host plants when both are green'(Finch, 1996). It was suggested that this mechanism might apply to phytophagous insects on the whole. However, landing by a phytophagous insect on a host plant is only one event in an involved behavioural repertoire associated with host plant location and not all pest species do not discriminate between host and non-host plants when both are green. Lepidoptera, for instance, are able to discriminate between green host and non-host plants (Bernays and Chapman, 1994). Discovering a unique explanatory mechanism that accounts for all different reactions of all insect pests to intercropping seems unlikely at the moment and, as consequence, consideration should be given to uncovering mechanisms with more specific types of insects.

Conclusion and research fields

Hill (1983) has described agricultural insect pests of the tropics and their control. For instance, details on host plants, damage, pest status, life history, distribution and control of 90 species of tropical lepidopterous pests are given and about 68 were presented as major pests. However, intercropping is not listed among control methods used by farmers in the tropics. Fortunately, there is a growing research interest into the effects of intercropping on insect pests but there is a lack of a holistic research approach into this complex field. This leads to many trials and errors in studying the effects of intercropping on insect pests. It is however worth noticing that a number of researchers have now embarked on a more consistent research approach to intercropping studies which considers and tries to understand the phytophagous insect population dynamics in order to interfere purposefully with its host plant location behaviour and subsequent population development and survival (Perrin and Philips, 1978; Theunissen, 1997). Therefore, any serious study of intercropping as a pest management strategy should first find out how the insect locates its hosts and then study its population development and survival. These studies should open up a set of possible actions using intercropping to alter the behaviour of phytophagous insects and/or to attract insect natural enemies to protect the crop(s).

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