The pre-oviposition behaviour of *Pieris rapae* **L.**

by

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DECLARATION

I hereby declare that the contents of this thesis are, except where otherwise stated, my own original work.

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ABSTRACT

This thesis reports the results of investigations on aspects of the pre-oviposition behaviour of *Pieris rapae* **L.** (Lepidoptera: Pieridae) in relation to host plant finding and acceptance. Investigations were carried out into various aspects of the influence of sensory cues on host plant finding including the effect of colour and host plant extracts. The results showed that the number of landings was strongly influenced by colour ($P<0.001$) and that extracts containing contact stimulants also increased the number of landings ($P<0.05$).

Volatile extracts were also tested in a wind tunnel and the results showed that the extracts increased the number and duration of flights as compared to the solvent controls ($P<0.05$). However, there was no evidence that the volatile extracts tested acted as attractants. Volatile extracts applied to non-host plants increased the proportion of landings as compared to the controls ($P<0.05$).

The role of different sensory cues (volatile extracts, colour contact stimulants), from three host plants with differing acceptabilities were investigated in order to assess the relative importance of each cue. The results showed that the variety tested had a significant influence on the number of landings and eggs laid. Volatile extracts from the three varieties were prepared and their effect on landing in a choice and no-choice test with model plants was carried out. The volatile extracts significantly increased ($P<0.05$) the number of landings on the treated plants as compared to the controls.

The effect of prior experience was also investigated using three host plants with differing acceptabilities to *P. rapae*. The results showed that the effect of prior experience ($P>0.05$) itself was not a significant explanatory variable for the number of eggs laid, however, the host plant variety used was a significant explanatory variable ($P<0.001$). Additionally, there was a significant interaction between these two factors ($P<0.05$). In a separate experiment, more detailed behavioural observations of the females were made of ovipositing females. The results once again showed that the prior experience treatment was not a significant explanatory variable ($P>0.05$) as was the host plant varieties used ($P>0.05$). The interaction between these two factors was statistically significant ($P<0.001$) for all of the behaviours considered. However, when the analysis of the data was repeated with the number of landings used as a covariate in the analysis, the interaction between prior experience and host plant variety was found to be non-significant (P>0.05) for all post-alighting behaviours. Therefore, it would appear that the main effect of prior experience on *P. rapae* is on the choice of landing site and other ovipositional behaviours are not affected.

Finally, an artificial life model of the pre-oviposition behaviour of *P. rapae* is proposed.

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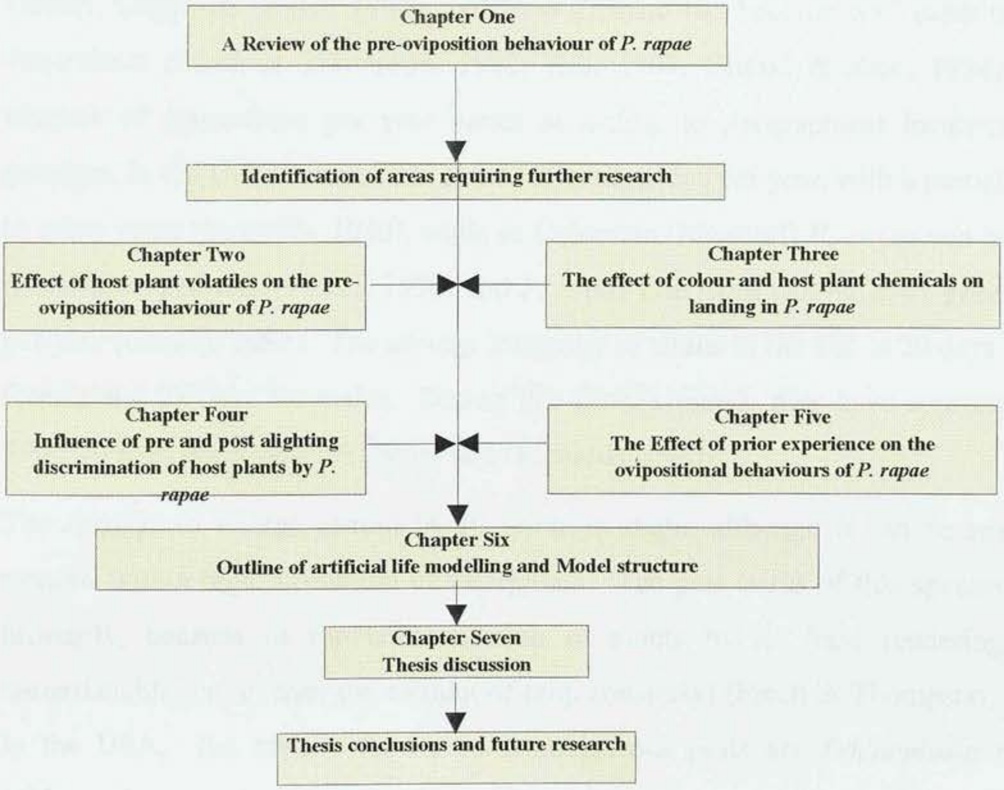
Chapter One
A review of the pre-oviposition behaviour of *P. rapae* (Lepidoptera: Pieridae)[1]

INTRODUCTION TO THESIS

The aims of the research presented in this thesis were twofold. The main aim of this research was to develop an artificial life model of the pre-oviposition behaviour of the Small Cabbage White Butterfly (*Pieris rapae* **L.** Lepidoptera: Pieridae). In order to create such a model accurately, experimental work was carried out on aspects of the behaviour of this butterfly. This introduction lays out the structure that will be followed in this thesis and is shown diagramatically in Figure 1.1.

Figure 1.1 Organisation of the thesis



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[1] This chapter is based largely on a paper by Hern, Edwards-Jones & McKinlay, 1996a

This chapter reviews the current literature regarding the pre-ovipositional behaviour of *P. rapae* to identify areas which require further study in order to fully develop the model. Chapters Two to Five present the results of these further experiments. Chapter Six presents an overview of the application of Alife modelling techniques and the type of data needed to implement such a model (Section A). The structure of the model is presented in sufficient detail to allow implementation at a later date (Section B). Chapter Six also presents the rationale for developing an artificial life (Alife) model of the pre-oviposition behaviour of *P. rapae*. The final chapter (Chapter Seven) discusses the findings of the current research and attempts to place them into a wider context.

INTRODUCTION

P. rapae is a cosmopolitan species which is distributed throughout the Northern hemisphere, between the Arctic Circle and the Tropic of Cancer. It is widespread throughout Europe, Asia and North Africa. It has been introduced into Canada, USA, Hawaii, Japan, Mexico and since its introduction into Australia, in 1939 (Jones, Gilbert, Guppy & Nealis, 1980), and New Zealand has become well established in Australasia (Finch & Thompson, 1992; Hill, 1987; Ohsaki & Sato, 1994). The number of generations per year varies according to geographical location. For example, in the UK., *P. rapae* has three full generations per year, with a partial fourth in some years (Richards, 1940), while in Columbia (Missouri) *P. rapae* can have six generations per year (Parker, 1970) and *P. rapae crucivora* can have six generations per year (Ohsaki, 1982). The average longevity of adults in the UK is 20 days for the female and 23 days for males. During this time, a female may have approximately 450-500 eggs ripen with 350 being laid (Richards, 1940).

The damage to foliage caused by *P. rapae* is slight, although it can be severe in seasons with a high infestation of caterpillars. The pest status of this species arises primarily, because of the contamination of plants by its frass rendering them unmarketable, rather than the amount of crop consumed (Finch & Thompson, 1992). In the USA, the main collard crop lepidopterous pests are *Trichoplusia ni* (the cabbage Looper; Lepidoptera: Noctuidae); *P. rapae* and *Plutella xylostella* (the diamond back moth; Lepidoptera: Plutellidae), with their percentage occurrences being 71 per cent *P. rapae*; 28 per cent *P. xylostella* and *T. ni* 1 per cent in 1985 and in 1986 91 per cent *P. rapae* and 9 per cent *P. xylostella* (Stewart and Sears, 1988).

This review considers the searching behaviour of *P. rapae* and the sensory cues utilised in the decision-making process of a female when searching for ovipositional sites. Studies utilising other butterflies are used for comparison or to suggest possible proxies suitable for use in a model for *P. rapae* in the absence of data for this species. This chapter is structured according to the sequence of events characterising the host finding and egg laying behaviours of *Pieris* spp., namely generalised searching behaviour, specific searching behaviour and the role of visual and olfactory cues, post-alighting discrimination and contact chemoreception. The review considers prior learning and experience and finishes by identifying the areas subjected to experimental investigation.

The sensory cues that elicit or inhibit oviposition play an important part in the survival of most phytophagous insects (Renwick & Chew, 1994). This is particularly true for the Lepidoptera, as the larvae of most species grow and survive on a limited number of host plant species belonging to a single family. The newly hatched larvae of most species are incapable of locating a new host and are dependent on the host plant location 'skills' of their mothers (Feeny, Rosenberry & Carter, 1983). Singer (1986) points out that observed larval distributions are a result of various effects including initial adult distribution and density; oviposition preferences and plant acceptability; egg survival patterns; larval movements, preference, performance and host plant suitability, including differential predation between host plants. The term preference as defined by Painter (1951) "is used to denote the group of plant characters and insect responses that lead to or from the use of a particular plant or variety, for oviposition, for food or shelter, or for a combination of the three. Anthropomorphic connotations should not be read into this terminology" (Painter, 1941; 1951). Preference can be used to refer to the behaviour of an insect towards parts of an individual plant, particular sets of plants of the same species, or towards a specified set of plant species" (Singer, 1986).

PRE-ALIGHTING BEHAVIOUR

Searching behaviour

Searching behaviour has been defined by Bell (1990) as the active movements by which an insect seeks a resource. As the resources utilised are usually essential to the survival and fecundity of the individual, an efficient searching mechanism and accurate assessments of located resources are deemed to be crucial to an organism's individual fitness. In addition, searching for a resource incurs costs over and above the energetic cost of locomotion, such as increased risk of predation and the time lost to other activities (Bell, 1990). A female's searching behaviour determines the subset of all possible patches which are encountered, which in combination with her response to these patches determines the observed egg distributions (Ives, 1978). It may be expected, therefore, that host plant selection is a complex process subject to considerable selection pressures.

A substantial quantity of work has been undertaken on the searching behaviour of insects, for example, Courtney (1983) and Cain (1985) have developed general models of herbivorous insect search mechanisms; Jones (1977); Jones *et al.* (1980); Root & Kareiva (1984); and Fahrig & Paloheimo (1987; 1988) have worked specifically on *P. rapae*. Studies of the search behaviour of other insects include MacKay's (1985) work on *Euphydryas editha* (the checkerspot butterfly; Lepidoptera: Nymphalidae); and Kareiva (1982) worked on *Phyllotreta cruciferae* and *P. striolata* (flea beetles; Coleoptera: Chrysomelidae).

Host plant density and flight behaviour

Pre-alighting search consists of the behaviours the female exhibits up to the moment of alighting on a plant, and is dominated by the requirement to locate a suitable habitat and then to find patches of vegetation that contain potential host plants. Pre-alighting discrimination, defined by Mackay (1985) as "the responses of flying insects to habitat variation resulting in the alighting of the insect on various types of ground cover, with frequencies different from their abundance in the habitat, occurs as a result of the insect's pre-alighting search strategy". At this gross level, the behaviour of the insect may be affected by the spatial arrangement and density of plant patches, and in this situation, Cain's (1985) model for a generalised herbivore suggests that searching success decreases as plant density decreases, and increases as the size of patches increase. At high plant densities, insects locate host plants more easily when the plants are uniformly dispersed than when they are clumped. Increases in movement directionality do not alter an insect's ability to locate a host plant when plants are hard to find (i.e. when the radius of detection[2] is low, with short-move lengths), but when plants are easy to find (i.e. high radius of detection and long-move lengths) increased movement directionality decreases insect searching success (Cain, 1985).

The predictions of the model have not been specifically tested for *P. rapae,* but extensive field work has revealed some interesting patterns in the searching behaviour of this species. Root & Kareiva (1984) report that unlike many insects, *P. rapae* does not alter its turning behaviour upon encountering dense concentrations of host plants; and in all the situations they observed, ovipositing females tended to move along linear flight paths. Further, the time taken by a female to approach and locate a suitable host plant was not influenced by collard density. However, move length was affected by density, and average move length decreased as collard density increased, but this decrease was not sufficient to concentrate eggs on collards in dense plantings. For example, when collard density was increased by 400 per cent, the butterfly's move length decreased by 20 per cent. Consequently, increases in collard density resulted in a net decrease in the number of eggs each plant received (Root & Kareiva, 1984).

These results support the general conclusion of Jones (1977) who reported a significant negative correlation between local collard density and move length. Jones (1977) also reported that the direction of flight, flight length, frequency of visits to a plant, returns to the same plant and the number of eggs per visit varied with the spatial distribution, species and age of the host plants. However, the frequency distribution of turning angles was similar between different gardens, and a predominance of turning angles greater than 45° was observed. This result agrees with the robust tendency towards linear flight reported by Root & Kareiva (1984). Jones (1977) reached these general conclusions despite observing that populations of

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[2] The radius of detection refers to the distance from which an insect can detect and be influenced by a plant. As such it measures the ability of an insect to perceive plant characteristics and then to act upon that information.

Canadian and Australian *P. rapae* showed different movement patterns. Australian *P. rapae* turned less and had a lower frequency of returns to the same host plant than Canadian butterflies.

The model of *P. rapae* movement patterns and egg distributions developed by Jones (1977) was later extrapolated to predict movement over several days (Jones *et al.,* 1980). The directionality of *P. rapae* movement noted by Jones (1977) was found to continue, and although individuals demonstrated different directions, each individual flew with sufficient directionality to keep within an angle of 45 ° over one kilometre. Each day a new direction was adopted by an individual and the overall pattern of egg distribution was to spread in all directions. However, occasionally there was a northward bias in the egg distribution. The authors postulated that the direction adopted by the individuals was not itself ecologically significant as such but was simply a mechanism for covering more ground which may be ecologically significant. Day-to-day movement was a random walk with a step length of 450 metres (Jones *et al.*, 1980). Fahrig & Paloheimo (1987) found that the residence time of a butterfly in a patch was 1.3 days and out of 102 butterflies marked in a patch over 17 days, the percentage of butterflies not observed in that patch on days following marking was 74.

Flight behaviour and weather

The current and immediately previous weather appears to have a major impact on the ovipositional behaviour of *P. rapae.* For example, Root & Kareiva (1984) report that, generally, *P. rapae* flies only in moderate weather conditions, restricting nectar feeding and oviposition to sunny, warm (18-24 °C) periods and concentrating most egg laying in the late morning or early afternoon. Individual variations on this pattern were observed, with some individuals nectar feeding for uninterrupted bouts and ovipositing for extended periods, while other flight sequences involved frequent alternations between limited oviposition bouts and nectar feeding. However, the weather had a major impact on this pattern, and intensive oviposition bouts were most common following prolonged periods of cloudy or rainy weather.

In addition to influencing the apportionment of time between oviposition and feeding, Root & Kareiva (1984) also observed that the weather influenced the acceptance rate of host plants. Normally, during pre-alighting flights *P. rapae* females flew over apparently suitable host plants, often passing over more than forty collards, while ovipositing fewer than 10 eggs. However, following two consecutive days of rain the ratio of plants passed over to eggs laid was reduced, and individuals passed over comparatively few plants per laid egg. A similar effect was reported by Gossard & Jones (1977), who observed that overcast days inhibited oviposition by not allowing flight, but if the next day was sunny then the number of eggs laid increased by 78 per cent. When *P. rapae* was subjected to several successive cloudy days, then egg laying was increased further on the next sunny day.

Egg laying, patches and background vegetation

Root (1973) formulated the resource concentration hypothesis following a study of the insect fauna of collards in simple and complex habitats. This hypothesis suggests that many herbivores, especially those with a narrow host range, are more likely to find host plants that are concentrated in dense or 'pure' stands. Those species whose requirements are met in these stands will remain there the longest and reproduce. Conversely, those species which require a resource not found within the stand will migrate. *P. rapae* is an exception to this hypothesis because on 88 per cent of sampling occasions abundance was higher in perimeter rows than in pure stands (Root, 1973). Only during population peaks was abundance higher in the pure stands as compared to the perimeter rows.

Subsequent studies have confirmed this effect. For example, Maguire (1983) demonstrated that *P. rapae* lays its eggs in patches of small numbers of host plants rather than in a single large patch. Similarly, in a comparative study, Cromartie (1975) found a significant negative relationship between plot size and the number of *P. rapae* eggs per plant; regardless of plant size, time of year or background. The plot background exerted a significant effect on colonisation by other major collard herbivores, such as cabbage aphids and flea beetles. Only *P. rapae* invaded uncultivated plots to any extent; aphids and flea beetles were virtually absent. To exclude the possibility that this result arose from a lack of potential colonists of the other species, the experiment was repeated on a farm with a direct comparison between cultivated and uncultivated plots. Three of the four herbivores colonised the cultivated plots, with *P. rapae* again colonising uncultivated plots confirming the earlier result (Cromartie, 1975).

Mangel (1987) postulated that clutch sizes vary with host distribution. When suitable hosts were likely to be encountered clutch sizes fell compared to when the probability of encountering suitable hosts was lower. Host deprivation led to larger clutch sizes. Similarly, Courtney *et al.* (1989) suggest that host plants are arranged in a hierarchical fashion with the acceptability of a host plant depending upon its ranking and the insect's physiological state i.e. an individual which will accept a low ranking host will accept all hosts with a higher ranking and a female with a high egg-load (or other reason for a low threshold of acceptance) should accept the first host plant she encounters which has an acceptability above her current behavioural (motivational) threshold.

These points are suggestive that clutch size may vary with host plant availability. When host plant deprivation occurs, the insect's motivation to oviposit rises leading to females becoming less discriminating in their choice of oviposition sites. Experimental work with *P. rapae* (Jones, 1977) has confirmed that a female with a high egg-load would lay most of her daily egg complement on the first host plant or group of hosts found. However, as egg-load decreases, an individual leaves hosts more readily as do individuals with few eggs initially.

The response of *P. rapae* to host plants and its general search strategy are different from other butterflies so far studied. Particular differences in *P. rapae's* searching behaviour include its tendency to exhibit directional flight paths as noted by Jones (1977), which are responsible for the widespread distribution of eggs; the apparent by-passing of host plants and linked to this the finding of Fahrig and Paloheimo (1987) that the presence of host plants does not affect flight orientation and also the lack of counter-turning in a host plant patch are significant departures from the behaviour of other butterflies.

These differences between *P. rapae's* search behaviour and that of other butterflies result in a greater investment in ovipositional flights than if the female laid her eggs singly and exploited clusters of host plants. This point is important as the two major factors limiting butterfly populations are predation and the failure of the female to lay her full complement of eggs (Dempster, 1983). A central axiom of evolutionary and behavioural ecology is that animals optimise behavioural decisions to maximise their fitness. The costs and benefits of an animal's actions are ultimately measured in terms of Darwinian fitness (survival and reproduction). The rationale behind this approach is a Darwinian one, since selection is an iterative and competitive process which tends to produce phenotypes which represent the best available balance of costs and benefits over evolutionary time scales (Krebs & Kacelnik, 1991).

The costs of *P. rapae's* searching behaviour are the increased metabolic costs from the increase in flight, increased time in oviposition bouts with a commensurate loss of time to spend on alternative activities and an increased risk of predation. The main hypothesis put forward to explain the differences in the pre-alighting behaviour of *P. rapae* as compared to other butterflies studied is Root & Kareiva's (1984) "spreading of risk syndrome". The advantage to *P. rapae* of spreading eggs is that the unpredictable nature of the environment is evened out by depositing eggs widely.

Courtney (1986b) has suggested that "risk spreading, although, popular amongst insect ecologists, is unlikely to operate in insect populations". He proposes that density-dependent processes offer a better explanation of dispersal behaviours. However, in a reply to Courtney's article, Root & Kareiva (1986) argue that "although, Courtney (1986b) raises interesting points, we believe that he misinterprets our original paper (Root & Kareiva, 1984)", and they go on to defend their hypothesis of risk spreading as the determinant of dispersal behaviour in *P. rapae*. Specifically, Root & Kareiva (1986) argue that there is no evidence of density dependent mortality in *P. rapae* populations and that the fact that when ovipositing *P. rapae* does not avoid hosts with eggs or larvae already, and that this alone would suggest that the spreading of eggs does not occur as a mechanism to avoid density dependent mortality (Root & Kareiva, 1986).

Ohsaki and Sato (1994) have argued that *P. rapae* uses hosts in ephemeral habitats and disperses from habitats in order to avoid parasitoids, an argument not incompatible with Root and Kareiva (1984). Additionally, Jones (1981) reports that the community of parasitoids shows large variations even in a small geographic area. For example, Ohsaki and Sato (1990; 1994) showed that in a newly established population of *P. rapae* rates of parasitism by *Apanteles glomeratus* are generally low since *A. glomeratus* can usually only colonise a habitat after *P. rapae*, which allows the larvae to "escape" as the parasitoid prefers to attack the first two larval instars (Ohsaki & Sato, 1990). In contrast, in a permanent population of *P. rapae* the rate of attack by *A. glomeratus* is much higher (van Driesche, 1988; Ohsaki & Sato, 1990). These results would appear to suggest the dispersal of females may be an attempt to secure "enemy free space" which is considered by some authors to be very important in determining observed patterns of host use (Bernays & Graham, 1988 and references therein).

For example, although cabbage failed to show wound induced herbivore defence (Coleman, Barker & Fennman, 1996). They found weak evidence that a lower leaf area was consumed by *P. brassicae* caterpillars in herbivore damaged plants, and concluded "that there are few wound induced effects of herbivory in cabbage". However, they went on to argue that the lack of effects in their experiments did not necessarily imply the absence of such effects which may simply be more subtle and only become apparent in conjunction with other features of the ecology of the plant-herbivore system. An example of such an effect has been demonstrated (Mattiaci, Dicke & Posthumus, 1994). It was reported that damaged brussels sprout plants are more attractive to *Cotesia glomerata* than undamaged plants. Mattiaci *et al.* (1994) showed that mechanically damaged leaves were less attractive to the parasitoid than those leaves damaged by herbivores or mechanically damaged leaves treated with larval regurgitant. Analysis of the volatiles produced by the leaves showed that if plants were infested with caterpillars or if caterpillar regurgitant was applied to damaged leaves, the emission of green leaf volatiles was enhanced (Mattiaci *et al.*, 1994). Therefore, we may assume that when brassica plants are attacked by herbivores the plants respond by emitting volatile cues which are highly attractive to parasitoids. This link with a higher trophic level may be of immense importance in the ecology of the plant-herbivore interactions.

Courtney (1986b) raises the question of whether once a female has exhausted her compliment of mature eggs, time spent dispersing between patches may not readily be counted as lost search time since no reduction in offspring number occurs (Courtney (1986b). Root & Kareiva (1986) also suggest that this argument does not explain the why *P. rapae* engages in straight line flights between host plant patches since it is more energetically efficient to stay in a host plant patch and wait for the development of more eggs than to engage in a prolonged flight between patches (Root & Kareiva, 1986). This argument is further developed below.

Theoretical models of host plant utilisation suggest that the observed pattern of host use demonstrated by a phytophagous insect is inherently 'plastic', for example Courtney *et al.*, 1989; Jaenike, 1978. The observed pattern of host use can be thought of as been a trade-off between host plant findability and host plant quality. Most studies of *P. rapae* have concerned themselves with how a female responds to a plentiful host supply and from the theoretical models developed and optimal foraging theory the answer is to use only the best hosts and then move on. On the other hand, when host plants are not in a plentiful supply or egg-load is high it may be advantageous to lay many eggs quickly. This is what is clearly observed in the field. Therefore, the extra costs associated with the longer flights must be weighed against the increased fitness of the progeny from either better host plants or from a lower rate of attack from parasitoids.

This point can be illustrated with a thought experiment. If a clump of hosts plants with equal acceptabilities are imagined, the optimal strategy for encountering all of these plants is area concentrated search (counter-turning). However, as the insect oviposits on the host plants her motivation to oviposit falls and then a straight line flight would 'spread the risk' while the insect's motivation increases. An analogy can be drawn with *P. rapae's* behaviour when nectar-feeding which is really a diversion from reproduction in the sense that it is necessary only to provide energy to search for hosts. During nectar-feeding, females alter their behaviour when they enter a flower patch, abandoning their linear flight path and exhibiting tight turning behaviour (Root & Kareiva, 1984). Thus when entering a small patch, they often visit every inflorescence in the patch, sometimes stopping at each inflorescence several times as they patrol through the area. In addition, nectar-feeding females visit on average 72 per cent of the flowering stems per patch while ovipositing females by contrast land on only 33 per cent of the collards per patch. This type of behaviour is the most efficient way to maximise encounter and thereby nectar uptake. Therefore, the contrast between the flight patterns of ovipositing individuals of *P. rapae* and females engaged in nectar-feeding is not surprising.

As *P. rapae* has only a limited number of eggs to lay on any given day one explanation for individuals by-passing hosts is as follows. Given that a field of cabbages contains more suitable host plants than a female could utilise in a day, the most efficient way to use this resource is to lay an egg. Once laid, the motivational state of the female falls i.e. it becomes more discriminating. Now if the motivational threshold is still relatively high, then the female may oviposit again on another nearby host. Once the motivational threshold drops below the current acceptabilities of the available hosts, then *P. rapae* should leave this host plant patch. Once the motivational threshold for accepting a host plant falls the female should seek a shot and utilise it, and so forth. This straightforward "rule of thumb" can explain the apparently maladaptive behaviour of *P. rapae*.

Clearly, the exact reasons behind *P.* rapae's dispersal behaviours are complex. Incomplete data and understanding of the processes of both adult and juvenile survival along with the possibility of a parent-offspring conflict in ovipositional behaviours muddy the costs and benefits of risk spreading and dispersal in *P. rapae* and prevent the identification of the true causal factors responsible. My personal view is that a number of factors will operate, some of which favour dispersal and some of which select against the dispersal of females with the observed dispersal behaviours occurring as a result of these complex interactions which are currently poorly understood.

SENSORY MODALITIES USED IN SEARCHING BEHAVIOUR

Visual cues

Vision is undoubtedly important in host plant location by phytophagous insects (Miller & Strickler, 1984; Prokopy & Owens, 1983) and it is unlikely that *P. rapae* is an exception to this rule. Although leaf size and shape play no role in the discrimination of hosts, the colour of the plant has been shown to be important in host plant location (Renwick & Radke, 1988). For almost all phytophagous insects, the preferred colour is green, or shades thereof (Moericke, 1969).

As *P. rapae* performed a rapid and direct flight to green objects in experiments, and since there is no experimental evidence of responses to host plant odours, Traynier (1979) concluded that vision alone was sufficient for host plant location. In experiments investigating the effect of plant fertilisation on the acceptability of cabbage plants for oviposition by *P. rapae*, it was found that the butterflies initially approached both fertilised and un-fertilised plants with the same frequency, but laid more eggs on fertilised plants. Three to four days later when the fertilised plants became greener females were more likely to approach them from a distance (Myers, 1985).

Similarly, in other experiments it was found that *P. rapae* preferred to lay its eggs on yellow-green substrates. The effect of blue and green in differing ratios was also investigated and higher numbers of eggs were found with mixtures of blue and green than either colour alone (Hovanitz & Chang, 1964). Unpublished results, referred to in Renwick & Radke (1988), indicate that *P. rapae* showed a preference for artificial leaves with a maximal reflectance at 550 nm.

The studies detailed above clearly show that *P. rapae* responds to visual cues and that these stimuli are central to host plant location. However, because of the great inter- and intra-specific variation between plants, colour and shape are unlikely to be able to account entirely for the discrimination of host plants. Bernays & Chapman (1994) suggest that olfactory cues and visual cues operate in unison to guide an insect to a suitable host.

Volatile odour cues

The volatile compounds emanating from plants are thought to play a major role in the orientation of insects to their host plants and in the avoidance of unsuitable plants. However, very few reports of the specific chemicals responsible for the attraction of gravid female insects exist. Those that do include long range orientation of *Delia antiqua* (the onion fly) to n-propyl mercapton, the attraction of *D. radicum* (the cabbage root fly) and *Phyllotreta* spp. by mustard oils and allyl isothiocyanate respectively (Renwick, 1989; Visser, 1986).

In the Lepidoptera, host specific volatiles have been shown to cause gravid female insects to approach odour sources. For example, the attraction of *Acrolepiopsis assectella* (leek moth) to propylthiosulphinate and *Ctenucha virginica* (meadow caterpillars (Arctiidae)) to dihydroxydanaidal. The olfactory attraction by host plant extracts and host plants has been shown in: *Agraulis vanillae*, (gulf fritillary); *Helicoverpa subflexa* (Noctuidae); *H. virescens* (tobacco budworm; Noctuidae); *Manduca sexta* (tobacco hornworm); *T. ni* and *P. xylostella* (Renwick, 1989; Bernays & Chapman, 1994).

The role of volatile chemical cues in host plant location by butterflies is best documented in the Papilionidae. Two species have been studied viz. the Citrus butterfly (*Papilio demoleus*) by Saxena & Goyal (1978) and the black swallowtail (*P. polyxenes*) by Feeny, Stadler, Ahman & Carter (1989). Female *P. demoleus* are attracted by both visual and olfactory cues. When leaves of their host plant were offered in a choice experiment with one set of leaves screened by glass (visual stimuli only) and the other unscreened (visual and olfactory cues), the unscreened leaves elicited a higher level of response than the glass screened leaves at the opposite end of the chamber. It would appear, therefore, that both olfactory and visual cues are important in host plant location and selection in *P. demoleus* (Saxena & Goyal, 1978).

Similarly, treating experimental leaves with carrot volatiles alone did not increase the number of eggs laid by *P. polyxenes* on these leaves. However, the addition of both volatiles and contact stimulants resulted in significantly more eggs being laid on the treated leaves. In the presence of model plants, the number of flutter bouts, landing rates and eggs laid were higher in the presence of volatiles (Feeny *et al.*, 1989).

Although there is no evidence to suggest that volatile chemicals play any role in the orientation of *P. rapae* to its host plant (Renwick & Radke, 1983), there is circumstantial evidence for a role of volatiles influencing host plant location in Pierids. *Mathania leucothea* (Lepidoptera: Pieridae) appears to examine rosaceous shrubs for its host plants, parasitic *Loranthacea* spp. (mistletoes), which grow deep within the shrubs foliage and are, therefore, not readily detected using visual stimuli. Females spend longer searching shrubs with mistletoes than those without even though it may be 90 seconds or more before they contact the host. Similar observations of *P. napi* (the Green Veined White) in England revealed that females were able to detect a host plant growing in an isolated position completely overtopped by dense bracken in heath land, an unusual habitat for this butterfly. The female's behaviour was observed as they crossed the heath with rapid flight, arrested in the vicinity of the patch and began searching with the slow flight, and high wing beat amplitude, typical of ovipositing females (Courtney, 1986a). Similarly, Mitchell (1977) reports results which may be interpreted as circumstantial evidence for the role of volatile compounds, particularly allyl nitrile, a sinigrin derivative, emanating from cabbage attracting *P. brassicae*.

Volatile chemicals from macerated cabbage have been shown to deter oviposition by *P. rapae* (Renwick & Radke, 1983). Non-host volatiles can act as oviposition deterrents when applied to cabbage plants by decreasing the frequency of alightings (Tabashnik, 1987). Therefore, it may be concluded from studies investigating behaviour and egg distributions that the role of olfaction is limited to the avoidance of non-host plants (Renwick & Radke, 1988).

POST-ALIGHTING DISCRIMINATION

Once a female has alighted on a plant, the chemistry of that plant is probably central to acceptance and oviposition (Jones, 1991; Schultz, 1988), although other factors such as predators and parasitoids may well affect a plant's overall acceptability (Bernays & Graham, 1988; Ohsaki & Sato, 1994).

Contact chemoreception

Studies on the effect of host plant chemistry and host plant acceptability for oviposition in *P. rapae* have been of two kinds: either investigations of the effect of host plant chemistry in stimulating oviposition (for example, Traynier & Truscott, 1991; Renwick Radke, Sachdev-Gupta & Stadler, 1992) or of chemicals which act as deterrents to oviposition (for example, Tabashnik, 1987; Sachdev-Gupta, Renwick & Radke, 1990; Dimock & Renwick, 1991; Renwick & Radke, 1985). The effect of oviposition stimulants and deterrents in mediating host plant selection have also been studied by Huang & Renwick (1993).

Ovipositional stimulants

A common experimental design for investigating the influence of host plant chemicals on oviposition by *P. rapae* utilises a green card bioassay. Here the different chemical(s) or concentrations are offered to the butterflies singly or in tandem, and the number of eggs laid is assumed to reflect the acceptability of the chemical. One study which utilised this approach found that, when offered a choice between ether and water extracts of cabbage at two concentrations of 1 g/ml and 5 g/ml, the water extracts were preferred (Renwick & Radke, 1983).

The effect of the concentration of the water extract was investigated over the range 0.01-10 g/ml. *P. rapae* could not distinguish between cards treated with the 0.01 or 0.1 g/ml with the same number of eggs being laid at each concentration. However, when 0.1 g/ml and 1.0 g/ml were offered simultaneously, there was an order of magnitude difference between the number of eggs laid, which were 30 and 311 respectively. Similar results were obtained when 0.1 g/ml was compared with 10 g/ml with 45 and 412 eggs respectively. When 1.0 and 10 g/ml were compared, the ability of the butterflies to distinguish between these two high concentrations appeared to be lowered with the number of eggs laid being 104 and 210 respectively (Renwick & Radke, 1983). Following these experiments, Renwick & Radke (1983) concluded that the ovipositional stimulants are polar and the effect is concentration dependent.

The same experiment also tested the role of sinigrin as an oviposition stimulant; and when compared with 5 g/ml extract of cabbage, sinigrin at 1 mg/ml was less active. When two concentrations of sinigrin were compared simultaneously, the higher concentration was always preferred (Renwick & Radke, 1983).

The leaf surface oviposition stimulants to *P. rapae* in cabbage plants have been investigated. Chromatography showed that the oviposition stimulants were found in a limited number of fractions in which the major glucosinolates were glucobrassicin, sinigrin and glucoiberin. The results of comparative bioassays of these glucosinolates with a crude extract of cabbage showed that at concentrations physiologically relevant, i.e. similar to the concentration found in plants of 0.2 mg/plant glucobrassicin, oviposition was higher than an equivalent amount of cabbage extract with the number of eggs laid being 1576 and 493 respectively. The activity of sinigrin was lower than glucobrassicin, even at concentrations higher than those found in plants. Glucoiberin was inactive as a stimulant (Renwick *et al.*, 1992), therefore, they concluded that glucobrassicin alone could account for the stimulation of *P. rapae* to oviposit on cabbage.

Further research into the activities of different chemical classes of glucosinolates has shown that, in general, aromatic glucosinolates are more stimulatory than a post-butanol cabbage extract[3] (Huang & Renwick, 1994). However, the post-butanol cabbage extract was more stimulatory than all of the aliphatic glucosinolates tested (sinigrin, glucocapparin, glucoerucin and glucoiberin). Of these four, the least stimulatory were glucoerucin and glucoiberin which have a sulphur atom in the side chain.

Therefore, all glucosinolates are not equally effective in stimulating oviposition by *P. rapae* and the identification of glucobrassicin as the most active stimulatory compound in cabbage indicated that this indole glucosinolate could account for the recognition/acceptance of cabbage by *P. rapae* (Renwick *et al.*, 1992). Later studies have, however, shown other aromatic glucosinolates to be as stimulatory as glucobrassicin (Huang & Renwick, 1994).

These results contrast with the findings of Feeny *et al.* (1983) who found that for swallow tail butterflies, mixtures of plant constituents are necessary to stimulate oviposition and single compounds or fractions of a plant extract are less stimulatory than the whole or a blend of plant extracts. Furthermore, the concentration of the glucosinolate also affects its activity as an ovipositional stimulant. Each glucosinolate has an optimal concentration at which the stimulation of oviposition is greatest, above this concentration there is either no further increase or a decline in oviposition (Huang & Renwick, 1994,).

Oviposition deterrents

Tabashnik (1987) studied the effect of the application of non-host phyto-chemicals, coumarin and rutin, to cabbage plants on *P. rapae* oviposition . Coumarin is present in several plant families (including, the Compositae, Lauraceae, Leguminosae and Umbelliferae) as the major aromatic constituent, but does not occur in crucifers at substantial concentrations. Rutin is a non-volatile flavonoid pigment found in many plant families. In choice tests with coumarin at concentrations of 0.1 M and 0.01 M, both concentrations acted as oviposition deterrents. Although rutin deterred oviposition at a concentration of 0.1 M, at a concentration of 0.01 M there was no significant effect.

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[3] The post-butanol extract is prepared by boiling leaves in ethanol and homogenising the leaves. The homogenate is filtered and the filtrate is evaporated to dryness. The residue is dissolved in water and extracted in butanol. The post-butanol fraction is the remainder of the extract after partitioning in butanol.

Both compounds acted as ovipositional deterrents by affecting the pre-alighting rather than the post-alighting behaviour of *P. rapae* through a decrease in the number of contacts with plants (Tabashnik, 1987). Although once alighted, females were less likely to oviposit on treated as compared to untreated plants, this effect was small. The mechanism by which these two chemicals decrease the number of alightings on plants appears to differ and while the action of coumarin is thought to be primarily olfactory, rutin, a non-volatile pigment, was postulated to deter alighting by altering the visual stimuli emanating from the plant, presumably by the absorption of light by the pigment, thereby altering the reflectance of the substrate.

The effect of 0.1 M rutin in a no choice test depended upon the length of the test. The deterrent effect was strongest in a five minute test and intermediate in 15 and 60 minute tests and in a 24 hour test the deterrence was weakest. Furthermore, in this situation there was no significant difference in the rate of oviposition between an untreated plant with no eggs and an untreated plant with eggs (Tabashnik, 1987).

Therefore, the increased acceptability of rutin-treated plants with time may have occurred because the females were deprived of the opportunity to oviposit on untreated plants. The time available for oviposition may be a limiting factor for butterflies which lay their eggs singly. As the time between ovipositions increases, they may then accept non-host plants, or less acceptable host plants, more readily than butterflies that lay their eggs in clusters (Tabashnik, 1983). Thus it would appear from these results that the discrimination phase of *P. rapae* to the plants utilised and treated in this experiment is between 5 and 15 minutes (Tabashnik, 1987). The results described above show that non-host plant chemicals deter oviposition by *P. rapae*. However, the deterrence is not absolute and the application of the negative stimuli does not completely mask the positive stimuli of the treated cabbage but merely lowers its acceptability.

A different approach to investigating the role of oviposition deterrents was taken by Renwick & Radke (1985) who found that the application of macerated cabbage, an ether extract of cabbage leaves and non-polar extracts (hexane) of acceptable cruciferous host plants deterred oviposition by *P. rapae*. Similarly, non-polar extracts of the non-acceptable crucifers *Capsella bursa-pastoris* (shepherd's purse) and *Erysimum cheiranthoides* (treacle mustard) and the non-acceptable non-crucifers

Lycopersicon esculentum (tomato); *Solanum tuberosum* (potato) and *Glycine max* (Soya bean) deterred oviposition. The water extracts of non-acceptable crucifers and non-crucifers were all highly deterrent, whilst water extracts of acceptable crucifers had very little impact on oviposition, exhibiting slight deterrence. However, *Brassica juncea* (mustard) had a small stimulatory effect (Renwick & Radke, 1985) and this was also noted in a series of experiments in which *P. rapae* was offered a choice between cabbage and plants belonging to the families Cruciferae, Capparidaceae and Tropaeolaceae. *P. rapae* laid significantly more eggs on cabbage than *Alyssum saxatile*; yellow alison, Cruciferae, *E. cheiranthoides*; Cruciferae, *Iberis amara*; wild candy tuft, Cruciferae, and *Lunaria annua*; honesty, Cruciferae. Similar numbers of eggs were laid on cabbage as compared to *Isatis tinctoria*; woad, Cruciferae, *Tropaeolum majus*; nasturtium, Tropaeolaceae, *Conringia orientalis*; hares ear cabbage, Cruciferae, and *Cleome spinosa*; spider flower, Capparidaceae. The only stimulatory plant as compared to cabbage was *B. juncea* (Huang & Renwick, 1993).

Butanol extracts of these plants were prepared and their effect on oviposition by *P. rapae* tested. The butanol extracts from *A. saxatile*, *B. oleracea*, *C. spinosa* and *C. orientalis* had no significant effect on oviposition. The other butanol extracts all had a significant deterrent effect on oviposition as compared to the methanol control, except *B. juncea* which stimulated oviposition by *P. rapae*, resulting in 223 eggs being laid on the treated plant and 116 on the control plant (Huang & Renwick, 1993).

In a comparison of the post-butanol extracts of these plants and a cabbage post-butanol extract, the effects varied. For example, *I. tinctoria*, *B. juncea*, *I. amara* and *C. orientalis* all stimulated oviposition to a greater extent than the cabbage extract whilst *L. annua* was slightly less stimulating and *C. bursa-pastoris* and *E. cheiranthoides* exhibited low stimulatory activity receiving 4 and 12 per cent of the number of eggs laid as compared to the cabbage extracts. *T. majus*, *C. spinosa* and *A. saxatile* showed no significant difference from the cabbage extract (Huang & Renwick, 1993).

In a study of the influence of oviposition stimulants and deterrents from the non-acceptable host plants, *C. bursa-pastoris* and *E. cheiranthoides*, Renwick & Radke (1987) found that *E. cheiranthoides*, as well as containing oviposition deterrents, also contained oviposition stimulants. However, these fractions were less stimulating than the corresponding extracts of cabbage, whilst those of *C. bursa-pastoris* showed no significant stimulatory activity (Renwick & Radke, 1987).

These results suggest that the chemical basis for the avoidance of these two Crucifers is different. As *C. bursa-pastoris* extracts are non-stimulatory to *P. rapae*, this evidence suffices as an explanation for the non-acceptability of this plant. However, as *E. cheiranthoides* contains compounds which stimulate oviposition, this plant would be acceptable for oviposition if it were not for the negative stimuli from the oviposition deterrents which effectively mask the positive stimuli of the ovipositional stimulants (Renwick & Radke, 1987).

The ovipositional deterrents of *E. cheiranthoides* to *P. rapae* have been further studied by Renwick, Radke & Sachdev-Gupta (1989); Sachdev-Gupta *et al.* (1990) and in field studies by Dimock & Renwick (1991). Extracts of the leaves of *E. cheiranthoides* were taken using n-butanol. The oviposition deterrent index[4] (ODI) of 3 gram leaf equivalent per plant was found to be 84.7. The extract was then fractionated and the fractions with the highest ODI's were studied further. The u.v. spectra of the prominent peaks in these fractions were almost identical with an absorption maximum at 216-219 nm typifying a butenolide ring. Further identification suggested that the specific compounds were cardenolides (Renwick *et al.*, 1989).

The cardenolides reported as being the major ovipositional deterrents by Renwick *et al.* (1989) have been isolated, identified and bioassayed. The three compounds are erysimoside, erychroside and erycordin. These three compounds were then bioassayed for their effect on oviposition, along with the hydrolysis products of erysimoside and erychroside, helveticoside and stophanthidin respectively. The results of the assays indicated that the diglycosides of stophanthidin containing a 2,6,-dideoxy sugar are deterrents; i.e. erysimoside (ODI 53.9) and erychroside (ODI 55). However erycordin, which is a diglycoside of cannogenol and lacks a 2,6,-dideoxy

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[4] The oviposition deterrent index (ODI) is a useful and robust measure of the effect of different treatments on the acceptability of an ovipositional substrate and is calculated by (C-T/C+T) x 100, where C and T are the number of eggs laid on control and treated plants respectively. An ODI rating of 100 indicates that the treatment acts as a complete deterrent to oviposition, i.e. all of the eggs were laid on the control treatment; an ODI of 0 indicates that there is no difference between the treatment and the control whilst a negative ODI indicates that the treatment is an ovipositional stimulant as compared to the control.

sugar, is relatively inactive (ODI 19). The hydrolysis products helveticoside, and stophanthidin, are inactive as oviposition deterrents (Sachdev-Gupta *et al.*, 1990).

The effect of applying butanol extracts of *E. cheiranthoides* to cabbage plants on oviposition by *P. rapae* in a field experiment showed that the extracts had a significant effect on oviposition. However, the effect was not uniform for all spray periods. Fewer eggs were found on plants in treated plots than in control plots 24 hours after spray application. Similarly, in field cage experiments, 85 per cent or more of the total number of eggs laid were laid on untreated plants as compared to treated plants. However, in the field experiments, on subsequent sampling occasions, both the total number of eggs and the difference in the egg counts between treated and untreated plots decreased (Dimock & Renwick, 1991).

Thus, it would appear that ultimately host plant acceptability is mediated by a balance of sensory inputs from both negative and positive stimuli in the plant as outlined by Dethier (1982) and further expanded into the "rolling fulcrum" model of host plant selection suggested by Miller & Strickler (1984).

In this model, as in Dethier's, there is a balance between the external excitatory and inhibitory inputs from the peripheral receptors of an insect, including visual, olfactory and mechanical receptors (Miller & Strickler, 1984). For example, the relative concentrations and activities of oviposition stimulants and deterrents in a potential host plant interact to produce the plant's overall chemical acceptability to the insect. However, the relative balance between these opposing cues is weighted by the internal state of the insect. This internal state weighting is derived from the balance between internal excitatory and inhibitory inputs. This balance reflects an insect's 'motivational threshold' and thereby, takes into account the changes in host acceptability depending upon the individual's physiological state (Miller & Strickler, 1984), such as egg-load (Minkenberg *et al.*, 1992; Jones, 1977).

Oviposition Deterring Pheromones

Rothschild & Schoonhoven (1977) reported that the eggs of *P. brassicae* lowered the acceptability of host plants for oviposition by *P. rapae* and that, in choice experiments, *P. rapae* discriminated against cabbage leaves which had had *P. rapae* eggs removed from them as compared to control leaves which had not had *P. rapae* eggs. Following these experiments, Rothschild & Schoonhoven (1977) concluded that a mechanism exists in this species to prevent the overloading of host plants and that the mechanism involves detection of an oviposition deterring pheromone which is detected by the ovipositor of *P. rapae*.

However, in field experiments which utilised butterflies from both Australia and Canada, neither type of butterfly discriminated between leaves with and without eggs. In fact, leaves with eggs already present were more likely to receive more eggs than leaves without eggs. This result was considered to be a response to particular leaves rather than to the eggs *per se* (Ives, 1978). Similarly, in laboratory studies of the response of both naive and experienced *P. rapae* females to the presence of eggs on test discs, no difference was found between the numbers of eggs laid on discs with eggs and those without eggs for both naive and experienced females (Traynier, 1979).

In common with Ives (1978), Klijnstra (1985) showed that the presence of *P. rapae* eggs on cabbage leaves (250 egg equivalents in 1 ml methanol to each side of the leaf) was not a deterrent to ovipositing females. Females laid 2289 eggs on control leaves and 1702 eggs on treated leaves resulting in a per cent deterrence (calculated by the same formulae as for the oviposition deterrent index, page 26) by a putative oviposition deterring pheromone of 14.7 per cent. However, the response of *P. rapae* to the oviposition deterring pheromone of *P. brassicae* is a decrease in the number of eggs laid on treated as compared to control leaves, the mean number of eggs being 54 on the treated leaves (250 egg equivalents in 1 ml methanol per side of leaf) and 616 on control leaves (Klijnstra, 1985). Therefore, it would appear that *P. rapae* oviposition is not seriously affected by its own oviposition deterring pheromone whilst that of *P. brassicae* is highly deterrent to *P. rapae*.

Klijnstra (1985) suggests that the oviposition deterring pheromone of *P. brassicae* reduces the level of both intra and inter-specific competition, as females of *P. rapae*, detecting the infochemical, become informed of the degree of occupation of a potential host plant by the eggs of *P. brassicae*. Therefore, the ensuing reduction of inter specific competition between the larvae of *P. brassicae* and *P. rapae* may favour both species, however, the major advantage probably accrues to *P. rapae* (Klijnstra, 1985).

Thus it appears that although *P. rapae* can certainly perceive its own oviposition deterring pheromone, with tarsal and antennal sensilla (Schoonhoven, Beerling, Braaksma & van Vught, 1990), and thereby the degree of occupation of a host plant by its own eggs, the role of its own oviposition deterring pheromone is of minor importance in the overall decision to accept or reject a host plant.

Plant Nutrient Status

The importance of nitrogen in insect plant interactions and host plant suitability is well known. A survey of the literature of the last fifty years, carried out by Scriber (1984) showed that over 70 per cent of studies indicated that insect damage, growth, fecundity or abundance increases with increasing plant nitrogen.

Jansson, Leibee, Sanchez & Lecrone (1991) reported that the abundance of lepidopterous pests on crucifers was significantly and positively correlated with the rate of nitrogen applied to the plant as fertiliser. In similar experiments investigating the effect of fertiliser on the oviposition of *P. rapae*, Wolfson (1980) reported a clear preference for heavily fertilised plants over those fertilised with a lower nitrogen concentration and a lower overall dose of fertiliser. However, no difference was found between plants receiving varying amounts of sulphur as fertiliser. In addition to changing the relative ovipositional preference between plants, added fertiliser also altered the distribution of eggs within a plant. On those plants receiving a low fertiliser dose, the egg distribution shifted from a pattern where densities were highest on fully expanded middle aged leaves, and lowest on very young and old leaves, to one where the very young leaves received over 25 per cent of the total number of eggs.

In a study of the effect of nitrogen fertilisation on oviposition by *P. rapae* using potted kale, the mean number of eggs laid was higher on fertilised plants by two orders of magnitude reflecting differences in fertiliser treatments; leaf nitrogen concentration explained over 90 per cent of the variation in oviposition rate (Letourneau & Fox, 1989).

Myers (1985) found that, not only do fertilised cabbage plants receive significantly more eggs than unfertilised plants, but the application of fertiliser affects several physiological parameters of the plant. A significant correlation between per cent nitrogen of the foliage and the colour of the plant was found; plants receiving more fertiliser were greener (as measured by the ratio of red to infra red reflectance). The greenness of the plants was also significantly correlated with the number of eggs a plant received. There was a consistent trend for fertilised plants to have higher transpiration rates. Higher transpiration rates also tended to increase the number of eggs a plant received. Fertilisation increased the concentration of nitrogen and phosphorus in the cabbage plants after the first day.

Effects of prior experience and learning

Learning has been shown to occur in *P. rapae* (Traynier 1984; 1986; and Lewis, 1986). Traynier (1984; 1986) and Traynier & Truscott (1991) have shown that *P. rapae* can associate the colour of a disc with the presence of host phytochemicals (sinigrin or glucobrassicin). In addition, a preference was observed in subsequent tests for the visual stimuli (colour) learnt by association. However, Traynier (1987) reported that from preliminary experiments of 24 hours duration, *P. rapae* did not associate leaf shape characteristics with the presence of sinigrin.

Furthermore, *P. rapae* is unable to learn to associate negative stimuli with colour. Traynier (1987) conducted experiments in which *P. rapae* was offered a choice between six white discs for oviposition, on three different occasions. In the first and third tests, two discs were treated with sinigrin solution and the remainder with water, while in the second test two discs were treated with water, two with sinigrin solution and two with chlorogenic acid (a polyphenol found in many plant families). The egg distributions from the first and third trials were similar with most eggs laid on sinigrin-treated discs and the remainder evenly distributed on water discs. In the second test, the chlorogenic acid discs failed to elicit oviposition, receiving very few eggs, whereas, the water and sinigrin discs both received eggs. Therefore, Traynier (1987) concluded that chlorogenic acid influenced behaviour only instantaneously as a deterrent and failed to influence learning.

Population differences

In comparative studies of the pre-oviposition behaviour of *P. rapae* from different countries, the populations of *P. rapae* in Canada and Australia share preferences for age, size and species of host plant (Jones & Ives, 1979). For example, when offered cultivars of *B. oleracea*, radish and species of wild mustard, they preferred cabbage to other varieties for both landing and oviposition. They were more likely to alight on a large host plant than a small one, but once alighted they were more likely to oviposit on a young plant, resulting in well grown middle aged plants receiving most eggs. The Australian butterflies spread their eggs over a larger area and produce a less aggregated egg distribution, both plant to plant and patch to patch than Canadian butterflies.

In a similar comparison of the oviposition behaviour of *P. rapae* from the UK and Australia, it was found that UK females were more likely to resettle on the same host plant, rather than a new one; they made more alightings per minute; during an alighting, a UK female was more likely to oviposit; a UK female was less likely to alight on a non-host plant and was less likely to visit a small host plant (Jones, 1987). The result of this behavioural pattern is that UK females laid their eggs much more rapidly but utilised a smaller subset of available host plants. In these respects, they are similar to Canadian *P. rapae* females previously studied by Jones (1977).

These differences in the behaviour of *P. rapae* populations from different geographical localities indicate that there is a high degree of inherent plasticity in the insect's host plant finding behaviour on which selection may act to optimise the overall strategy employed in different biogeographic regions.

CONCLUSIONS AND AREAS FOR RESEARCH IN THIS THESIS

This review has demonstrated that host plant chemistry is considered to be central to the ovipositional decisions made by female *P. rapae* in rejecting or accepting host plants. However, the importance of other factors in mediating the decision-making process cannot be ignored, neither can the role of the insect's internal physiological state. The behaviour of an insect with respect to a host plant is obviously more complex than a simple chemically-mediated interaction. The evolutionary process shaping this interaction reflects a balance between host and insect herbivore; natural enemies may also influence this interaction.

The major knowledge gaps which have been identified in this review and which therefore, have been chosen as areas for experimental investigation are shown below:

Role of volatile odour cues in oviposition behaviour.

Role of colour in landing behaviour.

Integration of sensory modalities in the decision making process.

Influence of pre-and post alighting discrimination in host plant acceptance.

The role of prior experience in influencing host plant choice and pre-ovipositional behaviours.

The role of olfaction in host plant location in *P. rapae* has not been well investigated; no published systematic studies of the role of olfaction in host plant location in any Pierid exist. The circumstantial evidence cited and the role of volatiles in other species indicate that it is likely that *P. rapae* makes use of volatile cues emanating from host plants to aid their location. This is probably the major knowledge gap in the ovipositional behaviour of *P. rapae*.

The effect of colour has been investigated and statistical relationships between wavelengths reflected and oviposition have been recorded (Myers, 1985). The aim of this research is to further elucidate how spectral reflectance affects landing and which areas of the spectrum are most important.

At present most research on the cues utilised by females in selecting plants for oviposition have been studied independently. This type of research reveals very little about the relative importance of each sensory modality or any interactions between them. The models of host plant selection developed suggest that the balance of sensory inputs is critical in decision-making. However, there is little evidence to suggest the relative importance of the different sensory modalities.

An equally important and challenging area is to investigate the role of prior experience on the ovipositional choices and preferences of females. Learning has been shown to increase the searching efficiency of phytophagous insects. This may be a major factor in influencing a female's alighting preferences during an ovipositional bout.

Overall, the factors affecting oviposition for *P. rapae* have been investigated in some detail. However, areas have been neglected resulting in a bias of the understanding of the full oviposition behaviour of *P. rapae*, e.g. the role of volatiles, host plant physiology and insect learning are under-represented in the literature. By concentrating this research on the factors which affect pre-alighting discrimination such as the role of plant volatiles in host plant selection, the pre-dominance of research in post-alighting discrimination may be redressed.

Chapter Two
Effect of host plant volatiles on the pre-oviposition behaviour of *P. rapae*.

INTRODUCTION

Volatile compounds leaving plant surfaces spread through the air by diffusion. The olfactory receptor systems of phytophagous insects are able to perceive some of these compounds enabling them to act as chemical messengers (Visser, 1988). Host plant volatiles have been shown to be important olfactory cues affecting the orientation of many phytophagous insects (Renwick, 1989; Visser, 1986).

For *P. rapae*, electroantennography (EAG) indicates that the perception of volatile compounds, including general plant odours and isothiocyanates by *P. rapae* occurs (van Loon, Frentz & van Eeuwijk, 1992; Topazzini, Mazza, & Pelosi, 1990). The results of these studies indicate that the compounds producing the greatest effect were fatty acid derivatives, linalool, terpinol, 1-octen-3-ol, 1-phenylethanol and r-cresol, benzaldehyde and eugenol. Of the crucifer-specific compounds tested, phenylacetonitrile elicited a higher response than allyl isothiocyanate, benzyl isothiocyanate and 2-phenylethyl isothiocyanate (van Loon *et al.*, 1992; Topazzini *et al.*, 1990). Allyl isothiocyanate showed a difference in the strength of the EAG response between male and female preparations, with the relative response being 2.5 % and 15 % for males and females respectively (van Loon *et al.*, 1992). EAG is considered a useful technique in the examination of volatile stimuli for biological activity. The technique, which has been widely used assumes that volatile stimuli which elicit a strong electrophysiological response are more likely to be active elicitors of behavioural responses by the insect than compounds which produce a lower response (Marion-Poll & Thiery, 1996). However, it should be noted that since the EAG measures the response of an insect's olfactory sense cells located on the antennae to test odours, a response to an odour does not constitute evidence that the compound is behaviourally active, but merely demonstrates perception of that compound by the insect (Hern *et al.*, 1996a).

Evidence of behavioural effects on the Lepidoptera by volatile compounds is available (Ramasamy, 1988; Renwick, 1989; Bernays & Chapman, 1994; Saxena & Goyal, 1978; Feeny *et al.*, 1989). For *P. rapae*, it has been shown that volatile chemicals from macerated cabbage deterred oviposition by *P. rapae* (Renwick & Radke, 1983). In a review of the sensory inputs involved in oviposition behaviour Renwick & Radke (1988) concluded that the role of olfaction is limited to the avoidance of non-host plants. Similarly, Fahrig and Paloheimo (1987) concluded that as *P. rapae's* flight orientation was not affected by the presence of cabbage it is "unlikely that females can use olfactory detection to find host plants". However, Traynier (1979) was more circumspect in his assertion that vision alone could account for host plant location and warned against concluding that olfaction plays no part in host location by *P. rapae*.

The role of volatiles in host plant selection is unclear (Chew & Renwick, 1995) with some circumstantial evidence suggesting that such cues may be important to some species, for example, *Pieris napi* L. (Courtney, 1986a) and *Pieris brassicae* L. (Mitchell, 1977).

Given this incomplete understanding of the role of volatiles in the pre-oviposition behaviour of *P. rapae*, the overall aim of the work reported in this chapter was to experimentally investigate the role host plant volatiles play in *P.* rapae's host plant finding behaviour in order to test for any behavioural effect of volatiles in pre-ovipositional behaviours.

In order to assess whether or not volatiles affected the search for host plant patches, the effect of host plant volatiles on the number and duration of flight behaviours of *P. rapae* in a wind tunnel was investigated (Section A). In addition, the position of the butterflies in the wind tunnel relative to the odour sources was noted. To investigate whether or not volatiles may play a role in pre-alighting discrimination the effect of baiting non-host plant with host plant derived volatile cues on the decision to alight on such plants by *P. rapae* was carried out (Section B).

SECTION A

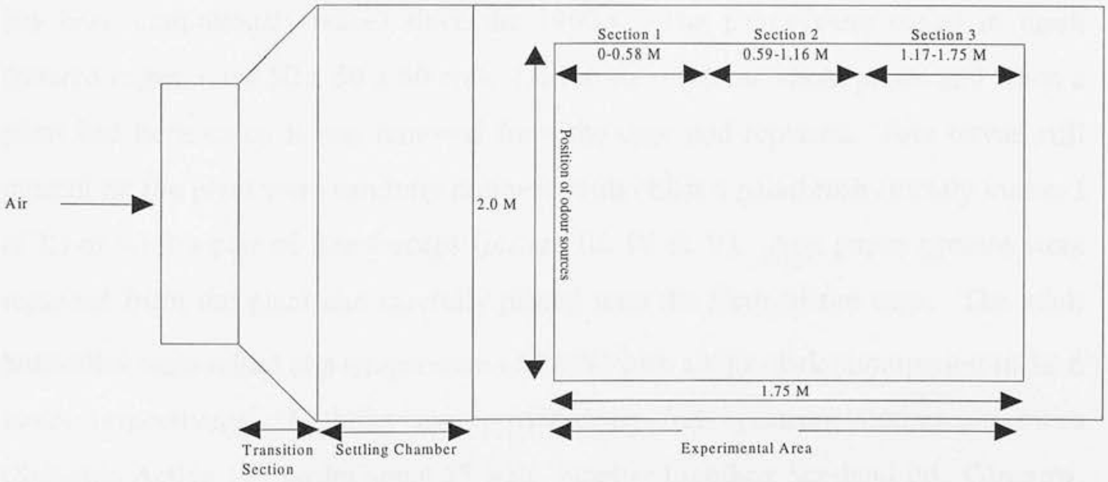
THE EFFECT OF VOLATILES ON THE FLIGHT BEHAVIOUR OF *P. RAPAE* FEMALES

METHODS

Wind tunnel description

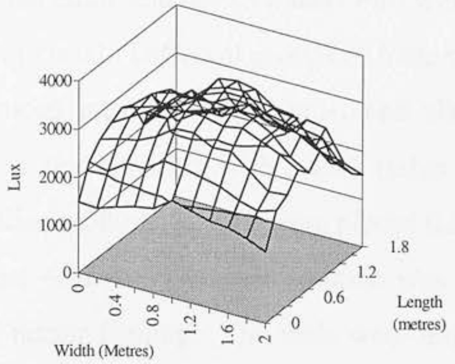
The wind tunnel was comprised of four main sections; the transition section, the settling chamber and the test section which also incorporated the experimental area (Figure 2.1). The transition section consisted of a wide angle diffuser fitted with a combination of adjustable air splitters and guide vanes to control the boundary layer. The settling chamber, was fed by a non-overloading centrifugal blower fan, with minimum disturbance to airflow. The settling chamber contained a combination of screens and honeycombs to provide a laminar air flow to the entrance of the test section (P. J. Bowden, unpublished).

Figure 2.1 Diagrammatic representation of the wind tunnel used in these experiments.



The wind tunnel experimental area was 2.0 metres wide, 1.75 metres in length and 1 metre high. The airflow within this section of the windtunnel has previously shown to be laminar (P.J. Bowden unpublished). Lighting was provided by eight evenly spaced fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland ltd., Glasgow, UK) providing full spectrum light with an emission spectrum close to that of daylight with additional reflectance in the ultra violet. The wind tunnel showed variation in the levels of light reaching the floor and this is illustrated in Figure 2.2. Ambient air is drawn from outside and electric heaters provide heating to 22 °C for experiments (P. J. Bowden unpublished).

Figure 2.2 Variation in light intensity within the wind tunnel experimental area.



Butterflies

All experiments utilised captive bred *P. rapae*. The culture used was started from eggs obtained from a culture maintained at HRI (Wellesbourne, UK). This culture has been continuously reared since the 1960's. The larvae were caged in mesh covered cages (size 50 x 50 x 50 cm). Larvae were fed on whole plants and when a plant had been eaten it was removed from the cage and replaced. Any larvae still present on the plant were carefully removed with either a paintbrush (mostly instars I & II) or with a pair of fine forceps (instars III, IV & V). Any pupae present were removed from the plant and carefully placed onto the floor of the cage. The adult butterflies were raised at a temperature of 22 °C with a light-dark photoperiod of 18:6 hours respectively. Lighting was provided by full spectrum fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland ltd., Glasgow, UK). The plants used for larval rearing and adult oviposition were glasshouse grown without the use of pesticides. Upon eclosion from the pupae, butterflies were transferred to another cage (usually 95 x 50 x 50 cm) with a host plant. An artificial nectar solution (15 per cent (w/v) sucrose with a dash of egg-yellow food colouring) was provided in a 'feeder'. The 'feeder' comprised a white perspex sheet (15 x 15 cm) with six vials attached by silicon sealer (non-toxic aquarium type). In between generations the cages were washed thoroughly with Decon solution (5 per cent) and wiped with a 70 per cent ethanol solution. This was done to minimise the risk of infection from microbial pathogens (particularly viral and fungal pathogens). The host plants used for rearing and oviposition was as follows Spring Cabbage var. Golden Acre Primo (II).

Group Assay

Groups consisting of ten adult females (5-6 days old) were treated as one trial. Three trials were carried out in total. Different groups of butterflies were used for each trial. Females were introduced into the wind tunnel and allowed to acclimatise for 30 minutes. Three vials of test chemical with a wick (ether or 1.0 gram leaf equivalents (gle[5]) host plant volatiles (volume 60 ml)) were placed 0.5 m apart across the width of the tunnel. A 20 % per cent (w/v) sucrose solution was positioned at the rear of the wind tunnel to allow nectar feeding. The vials were introduced 25 minutes into the acclimatisation period. After the full 30 minutes of the acclimatisation period was complete, behaviours were recorded at 10 min intervals for 90 min duration. The wind tunnel was split into three arbitrary sections lengthways (see Figure 2.3 for details of the layout of the windtunnel during these experiments) with the distance being the distance from the odour source, i.e. section 1 (0-0.58 m); section 2 (0.58-1.16 m) and section 3 (1.16-1.75 m). The activity and position of the butterfly was recorded as either flying, resting or feeding (nectar feeding on the sucrose solution) in each of the three sections.

Wind speed and temperature were recorded at each sampling interval using a low velocity flow analyser (DISA 54N50) with a combined wind speed and temperature probe (Dantec 9054R0102). The wind speed and temperature were recorded over a three minute period prior to each sampling time and expressed as the mean speed and temperature over the three minute period. The average and S.E. of the wind speed and temperature measurements for the treatments were calculated. For ether, the mean wind speed was 1.01 m/s (S.E. 0.0024) and the mean temperature was 21.9 ^{O}C

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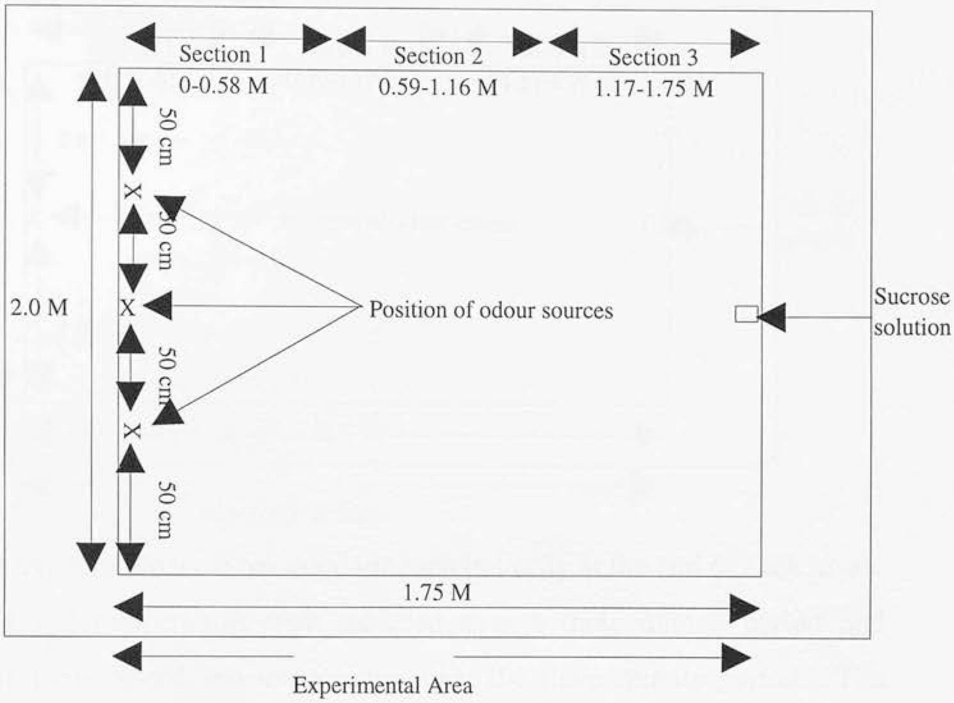
[5] Gram leaf equivalent is the amount of extract expressed in terms of the amount of leaf tissue extracted and the amount of solvent used. For example, 50 grams of leaf extracted in 50 ml of solvent would be expressed as 1 gle/ml or 50 grams of leaf in 25 ml of solvent would be 2 gle/ml. Therefore, to calculate the gle the amount of tissue extracted is divided by the amount of solvent in the final extract.

(S.E. 0.1). For volatiles the mean wind speed was 1.01 m/s (S.E. 0.004) and the mean temperature was 22.2 ^{O}C (S.E. 0.1).

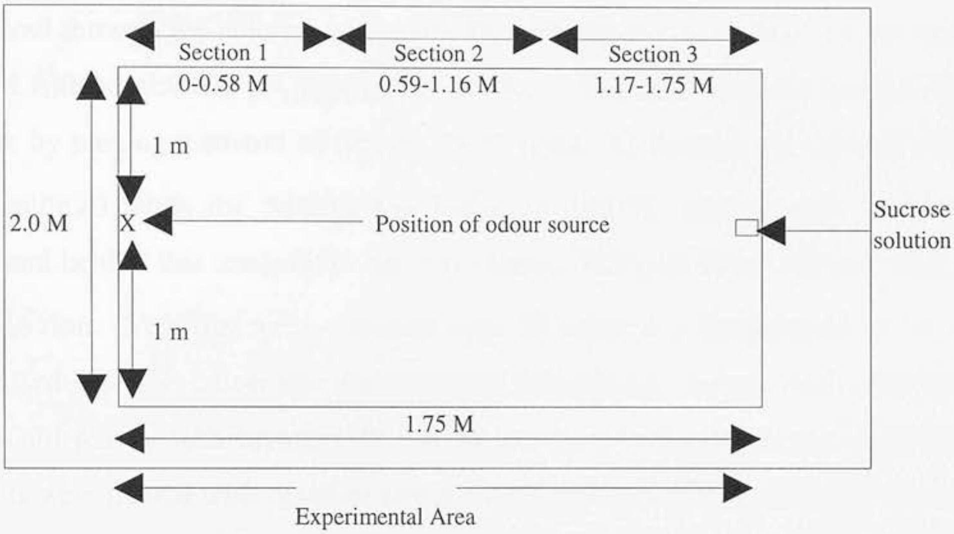
Figure 2.3 Layout of the windtunnel experimental area for the group assay experiments.



Individual Assay

Individual females (5-6 days old) were marked (with a two dot code with permanent markers) and exposed to either a single vial of 1.0 gle host plant volatiles or to ether with a wick (see Figure 2.4). The number, duration of flights and resting periods were recorded. In addition, the position of flight activities of individuals were recorded (as above) along with the positions and duration of resting. A 20 % per cent (w/v) sucrose solution was positioned at the rear of the wind tunnel to allow nectar feeding. The female was introduced into the wind tunnel and given a 15 minute acclimatisation period. Behaviour was recorded for 15 minutes. Five females were used in each trial and a total of three trials was carried out. Behaviours were recorded using the Observer software package, version 3.0 (Noldus Information Technology, Wageningen. The Netherlands). Upon completion of the assay butterflies were placed into polythene bags and placed in freezer (-20 ^{O} C for twenty minutes to kill the butterflies) after which the wing length of the female was measured as described by Jones, Hart & Bull (1982).

Figure 2.4 Layout of the wind tunnel experimental area for the individual assay.



Wind speed and temperature were recorded for each butterfly at the end of each assay. The wind speed and temperature were recorded over a three minute period and expressed as the mean speed and temperature over the three minute period. The average and S.E. of the wind speed and temperature measurements for the treatments were calculated. For ether, the mean wind speed was 1.06 m/s (S.E. 0.018) and the mean temperature was 22.1 ^{o}C (S.E. 0.19). For volatiles, the mean wind speed was 1.07 m/s (S.E. 0.01) and the mean temperature was 22.8 ^{o}C (S.E. 0.22).

Collection of volatiles

Cabbage volatiles (var. Golden Acre Primo (II)) were collected by dynamic headspace analysis using a laboratory entrainment system. The equipment used is similar to that described by Robertson *et al.* (1993). The major difference in the two systems is that in the present system air was pulled through the system rather than pushed as in the system of Robertson *et al.* (1993). The construction of the system was glass, stainless steel and PTFE tubing. All joints were wrapped in PTFE tape to provide an airtight seal. Excised leaves were placed in a 2 litre glass container. Air entering the system was filtered through activated charcoal and dried before entering the container with the excised leaves. Air was drawn over the leaves for 20 hours and the volatiles were trapped onto Tenax-Ta. The polymer was contained in a glass tube. All glassware was cleaned in distilled water and methanol and oven baked prior to use.

The Tenax-Ta ((mesh size 60-80) Alltech Associates, Lancashire, UK) was conditioned prior to the collection of volatiles. 3.5 ml of diethyl ether (HPLC grade) were passed through the column of Tenax. The column was then dried by passing a stream of filtered ambient air through the column. The final stage of conditioning was done by passing a stream of helium (BOC Grade A) through the column (flow rate 20 ml/min) while the column was heated to 180 ^{O}C (heating rate 8 ^{O}C per minute) and held at this temperature for three hours. 0.3 g of Tenax-Ta was used in each collection. Volatiles were collected over 20 hours at a temperature of 18 ^{O}C from excised leaves at a flow rate not exceeding 200 ml/min. Leaves used were from 12 week old plants, with between 30 and 50 grams of leaf material per collection. The plants were treated with systemic insecticide granules at sowing (Temik 1 granule per square inch of compost surface Rhone-Poulenc Agriculture, Ongar Essex UK) in an attempt to limit the damage caused by herbivorous insects and any subsequent effect this damage may have had on the volatiles released from the leaves. Elution of volatiles was achieved with 3.5 ml of diethyl ether into a glass sample tube in a bath of ice/methanol. Further dilution to 1.0 gle was undertaken prior to use with diethyl ether.

RESULTS

Group Assay

The data from this assay was analysed with a generalised model using the GLIM command in Minitab for Windows (Release 11.1). Prior to analysis, the data for each treatment (ether and volatiles) was combined and tested for normality using a Ryan-Joiner test (Shapiro-Wilk). If the data was not normally distributed ($P<0.05$), the data was transformed by $\text{Log}_{10}(\text{data}+1)$ (Table 2.1). This transformation was carried out to improve the additivity of the model and the normality of the residuals and thus more closely satisfy the assumptions of the analysis of variance with its significance tests. The results of this analysis are shown in Table 2.2. As can be seen sampling time was not a significant factor for any of the behaviours ($p>0.05$).

Table 2.1. The result of Ryan-Joiner tests for normality on the data for each behavioural class, data from volatile and ether treatments combined. ($P>0.05$ indicates that the data follow a normal distribution).

Behavioural class	Mean	Standard Deviation	N	W-test for normality		Data normally distributed	Data transformed for GLIM analysis
				R	P-value		
Flying section 1	0.9	0.97	60	0.996	0.1	Yes	No
Flying section 2	1.35	1.23	60	0.994	0.1	Yes	No
Flying section 3	0.63	0.78	60	0.998	0.1	Yes	No
Resting section 1	1.8	1.11	60	0.998	0.1	Yes	No
Resting section 2	3.53	2.02	60	0.979	0.044	No	Log$_{10}$(data+1)
Resting section 3	1.533	1.10	60	0.995	0.1	Yes	No
Feeding Behaviours	0.25	0.47	60	0.999	0.1	Yes	No

Figure 2.5. Mean number of *P. rapae* females in a group of ten exhibiting each type of behaviour when exposed to volatiles and ether over 90 minutes. Behaviours were recorded at 10 minute intervals (Mean and standard error of the mean shown are from three trials). Columns with different letters in the volatile and ether groups are significantly different from each other. n=30 (10 measurements per trial x 3 trials).

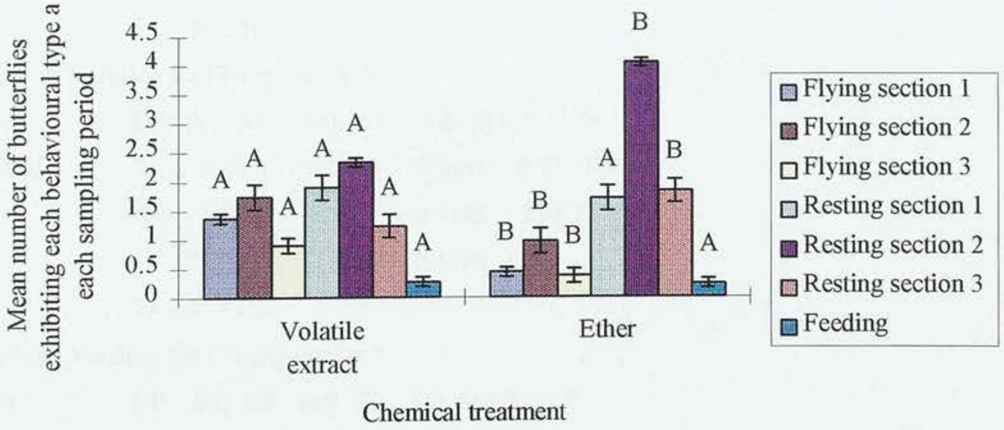


Table 2.2. Summary of Generalised linear model for the effect of volatiles on various behaviours with sampling time and chemical treatment as factors.

Factor	Levels	Values
Chemical	2	(volatile ether)
Time	10	(0 10 20 30 40 50 60 70 80 90)

Analysis of Variance for Feeding behaviours

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.0167	0.0167	0.0167	0.08	0.778
Time	9	3.0833	3.0833	0.3426	1.65	0.126
Error	49	10.1500	10.1500	0.2071		
Total	59	13.2500				

Analysis of Variance for Flying-section 1

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	13.0667	13.0667	13.0667	15.90	0.001
Time	9	2.0667	2.0667	0.2296	0.28	0.977
Error	49	40.2667	40.2667	0.8218		
Total	59	55.4000				

Analysis of Variance for Flying-section 2

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	8.817	8.817	8.817	6.20	0.016
Time	9	11.150	11.150	1.239	0.87	0.557
Error	49	69.683	69.683	1.422		
Total	59	89.650				

Analysis of Variance for Flying-section 3

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	4.2667	4.2667	4.2667	8.93	0.004
Time	9	8.2667	8.2667	0.9185	1.92	0.070
Error	49	23.4000	23.4000	0.4776		
Total	59	35.9333				

Analysis of Variance for Resting-section 1

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.600	0.600	0.600	0.43	0.516
Time	9	4.267	4.267	0.474	0.34	0.958
Error	49	68.733	68.733	1.403		
Total	59	73.600				

Analysis of Variance for Log_{10}(data+1) Resting-section 2

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.49302	0.49302	0.49302	17.97	0.001
Time	9	0.49405	0.49405	0.05489	2.00	0.059
Error	49	1.34450	1.34450	0.02744		
Total	59	2.33157				

Analysis of Variance for Resting-section 3

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	5.400	5.400	5.400	4.57	0.038
Time	9	7.600	7.600	0.844	0.71	0.693
Error	49	57.933	57.933	1.182		
Total	59	70.933				

Figure 2.5. shows the mean number of the behaviours observed, when exposed to the volatile extract, the number of females in flight in all three sections of the wind tunnel (section 1, section 2 and section 3) was significantly higher than the ether control ($p<0.001$; $p<0.01$; $p<0.01$; for section 1, section 2 and section 3 of the wind tunnel respectively). For resting behaviours, the number of females resting in section 2 and 3 of the wind tunnel was significantly lower when exposed to the volatile extract as compared to ether ($p<0.001$ and $p<0.05$ respectively). No other statistically significant differences between experimental and control groups were found (Table 2.2.).

Individual Assay

The data from this assay was analysed as for the group assay except that the factors used in the Glim analysis were chemical treatment (volatile or ether) and the individual butterflies wing length. The results of the normality tests are shown in Table 2.3.

Table 2.3. The result of Ryan-Joiner tests for normality on the data for each behavioural class for the individual assay, data from volatile and ether treatments combined. ($P>0.05$ indicates that the data follow a normal distribution).

		W-test for normality					
Behavioural class	Mean	Standard Deviation	N	R	P-value	Data normally distributed	Data transformed for GLIM analysis
Number of flights	2.83	3.19	30	0.815	0.01	No	$\text{Log}_{10}(\text{data}+1)$
Duration of flights	174	194.6	30	0.904	0.01	No	$\text{Log}_{10}(\text{data}+1)$
Number of resting periods	2.5	1.2	30	0.978	0.1	Yes	No
Duration of resting periods	719	196.9	30	0.904	0.01	No	$\text{Log}_{10}(\text{data}+1)$

Figure 2.6. The mean number of flights exhibited by female *Pieris* rapae on exposure to a volatile extract or ether. Error bars indicate the standard deviation. Columns with different letters are significantly different from each other. N=15

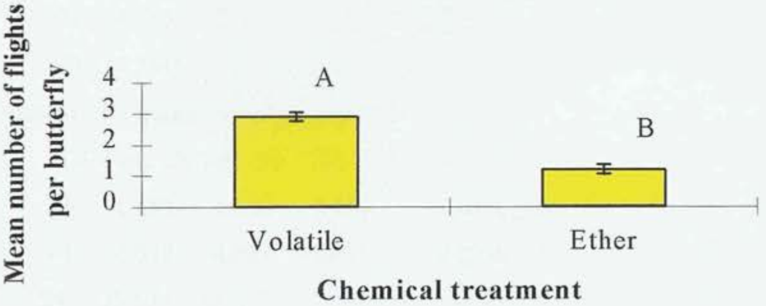


Figure 2.7. The mean duration (seconds) of flights exhibited by female *Pieris rapae* on exposure to a volatile extract or ether. Error bars indicate the standard deviation. Columns with different letters are significantly different from each other. N=15

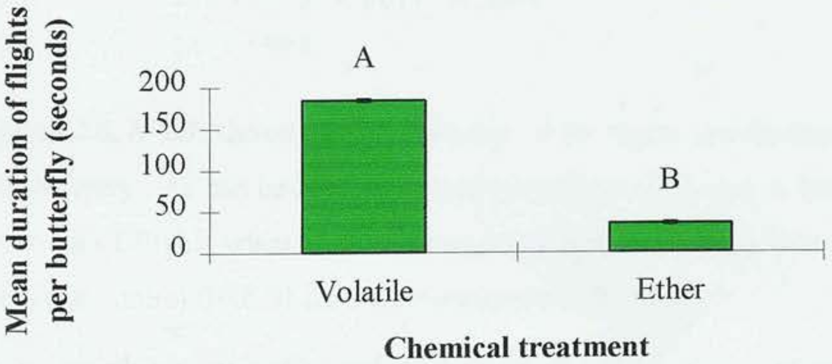


Table 2.4. Summary of Generalised linear model for the effect of volatiles on the various behaviours with female wing length and chemical treatment as factors.

General Linear Model

Factor	Levels	Values
Treatment	2	(Volatile Ether)
Female wing length	5	(18 20 21 22 23)

Analysis of Variance for Log_{10} (data+1) number of flights

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	0.44837	0.44837	0.44837	11.45	0.002
Wing length	4	0.46058	0.46058	0.11514	2.94	0.041
Error	24	0.93957	0.93957	0.03915		
Total	29	1.84851				

Analysis of Variance for Log_{10} (data+1) duration of flights

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	3.7531	3.7531	3.7531	10.16	0.004
Wing length	4	1.7460	1.7460	0.4365	1.18	0.344
Error	24	8.8657	8.8657	0.3694		
Total	29	14.3649				

Analysis of Variance for number of resting periods

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Wing length	4	1.833	1.833	0.458	0.31	0.869
Treatment	1	4.033	4.033	4.033	2.72	0.112
Error	24	35.633	35.633	1.485		
Total	29	41.500				

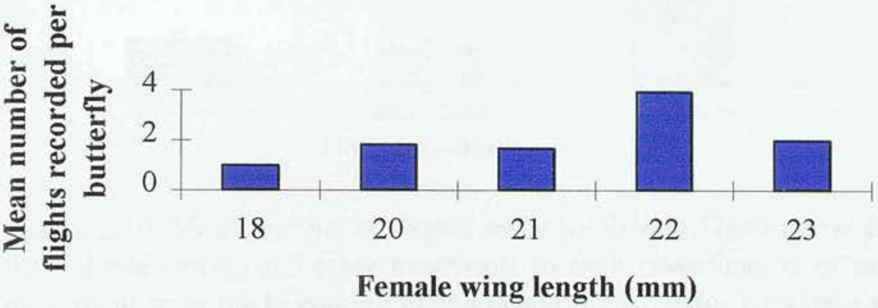
Analysis of Variance for Log_{10} (data+1) duration of resting periods

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Wing length	4	0.03381	0.03381	0.00845	0.40	0.804
Treatment	1	0.06159	0.06159	0.06159	2.94	0.099
Error	24	0.50313	0.50313	0.02096		
Total	29	0.59853				

Figure 2.6. & 2.7. shows the mean number of the flights and the duration of the flights respectively. As can be seen there was a significant increase in both the number and duration of flights when females were exposed to the volatile extract as compared to the ether control ($P<0.01$ for both behaviours Table 2.4.).

Wing length was measured and used as a factor in the statistical analysis of this data because Jones *et al.* (1982) have shown that the size of a female can be used as a proxy for its lifetime fecundity. This arises as a consequence of the fact that pupal weight is a strong determinant of female size and larger pupae also have a larger number of eggs. Female wing length exhibited a significant effect on the number of flights made by females ($p < 0.05$) (Table 2.4.), and the effect of female wing length on the number of flights is illustrated in Figure 2.8.

Figure 2.8. Effect of wing length on the number of flights made by *Pieris rapae* females (mean of volatile and ether treatments combined). N=30.



In order to investigate whether the volatile extract acted as an attractant to *P. rapae* females the flights recorded, were classified as resulting in a net movement upwind or downwind, or in no net change of position. The data from this classification was analysed using the Glim command in Minitab for Windows (release 11.1). Prior to analysis the data was tested for normality as before and was found to be not normally distributed W-test for normality (R 0.8057; $P<0.01$). To improve the normality of the data a Log_{10} (data+1) transformation was carried out which resulted in a normal distribution for the data (R 0.9896; $P>0.05$).

The results of this analysis indicate that chemical treatment, volatile extract or ether had a significant effect on the number of flights made ($P<0.01$). Similarly, the net-movement of the flights also showed significant differences ($P<0.01$)(Figure 2.9.). However, there was no evidence to suggest that in the presence of volatiles the number of upwind flights was significantly increased as compared to ether ($P>0.05$)(Figure 2.10.). Therefore, it would appear that volatile extracts do not act as attractants to *P. rapae* females, as defined by Dethier, Barton-Browne & Smith (1960) who defined attraction as "drawing from a distance". If the volatile extracts tested in these experiments were acting as attractants to the females it would be expected that there would be a significant increase in the number of flights which resulted in a net-movement upwind as when exposed to volatile extracts as compared to ether.

Figure 2.9. Mean number of flights made by female *Pieris rapae* in each classification of net-movement from the beginning to the end of the flight. Error bars indicate standard deviation and letters with different letters are significantly different from each other. Mean of all flights made by 15 individuals for both volatile extracts and ether. (N=77 flights made in total).

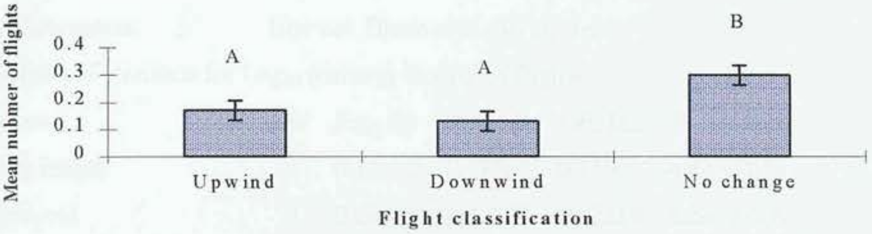


Figure 2.10. Mean number of flights made by female *Pieris rapae* for the volatile extract and ether treatments in each classification of net-movement from the beginning to end of the flight. Error bars indicate standard deviation and letters with different letters are significantly different from each other. Mean of all flights made by 15 individuals. (N=77 flights made in total).

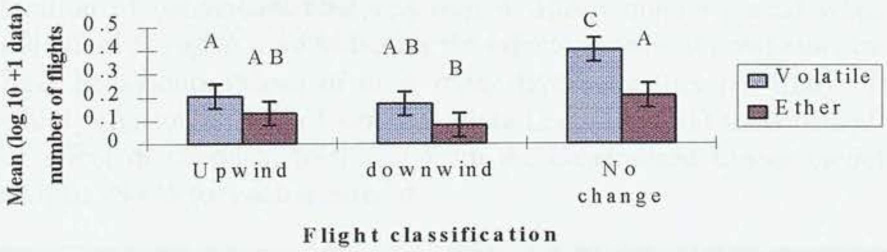


Table 2.5. The results of a Generalised linear model analysis on the number of flights made by *Pieris rapae* females resulting in an upwind, downwind or no change in the position of the female at the end of the flight. As female wing length was previously showed to be important in affecting the number of flights made by females this variable was included as a covariate in the Glim analysis.

General Linear Model

Factor	Levels	Values
Chemical	2	Volatile Ether
Net-movement	3	Upwind Downwind No change

Analysis of Variance for Log$_{10}$ (data+1) number of flights

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Wing length	1	0.17602	0.17602	0.17602	4.41	0.039
Chemical	1	0.32219	0.32219	0.32219	8.06	0.006
Net-movement	2	0.52215	0.52215	0.26107	6.53	0.002
Chemical * Net-movement	2	0.06542	0.06542	0.03271	0.82	0.445
Error	83	3.31629	3.31629	0.03996		
Total	89	4.40207				

Table 2.6. Mean and standard error of the mean for the number and duration of behaviours observed over a fifteen minute period when individual *P. rapae* were tested for the effects of volatiles and ether on flight behaviours (Mean of three trials; five butterflies per trial). P values refer to analysis of variance (data Log$_{10}$ (data+1) transformed) for effect of chemical treatment from the Generalised Linear model analysis. N=15 for each treatment.

	Frequency			Total duration (seconds)		
Behavioural class	Volatile	Ether	p	Volatile	Ether	p
Flight section 1	12.07	4.73	<0.01	36.60	19.13	<0.01
± SE	2.70	2.20		8.70	8.80	
Flight section 2	22.47	14.07	<0.01	168.10	97.30	<0.05
± SE	4.00	4.90		41.40	40.60	
Flight section 3	12.40	8.53	<0.05	41.30	28.30	<0.05
± SE	2.20	3.00		10.70	11.40	
Rest section 1	0.60	0.27	>0.05	114.50	73.30	>0.05
± SE	0.16	0.12		56.50	49.80	
Rest section 2	1.64	1.53	>0.05	320.30	417.50	>0.05
± SE	0.30	0.27		82.00	93.40	
Rest section 3	0.73	0.60	>0.05	221.40	260.90	>0.05
± SE	0.30	0.20		75.90	91.80	

In addition to the number and duration of flights being recorded the section of the wind tunnel in which each flight passed was recorded. The data is summarised in Table 2.6. Prior to analysis the data were tested for normality as before and the result of this analysis is summarised in Table 2.7. a & b.

Table 2.7a. The result of Ryan-Joiner tests for normality on the data for the frequency of behavioural events for each behavioural class, data from volatile and ether treatments combined. (P>0.05 indicates data is normally distributed.

			W-test for normality				
Behavioural class	Mean	Standard deviation	N	R	P-value	Data normally distributed	Transformation used
Flying section 1	8.4	9.95	30	0.91	0.01	No	Log_{10} (data +1)
Flying section 2	18.3	17.6	30	0.94	0.01	No	Log_{10} (data +1)
Flying section 3	10.5	10.2	30	0.93	0.01	No	Log_{10} (data +1)
Resting section 1	0.43	0.57	30	1	0.1	Yes	None
resting section 2	1.53	1.1	30	0.99	0.1	Yes	None
resting section 3	0.67	0.88	30	0.98	0.1	Yes	None

Table 2.7b. The result of Ryan-Joiner tests for normality on the data for the duration (seconds) of behavioural events for each behavioural class, data from volatile and ether treatments combined. (P>0.05 indicates data is normally distributed.

			W-test for normality				
Behavioural class	Mean	Standard deviation	N	R	P-value	Data normally distributed	Transformation used
Flying section 1	27.8	34.4	30	0.9	0.01	No	Log_{10} (data +1)
Flying section 2	132.7	160.2	30	0.9	0.01	No	Log_{10} (data +1)
Flying section 3	34.8	42.7	30	0.85	0.01	No	Log_{10} (data +1)
Resting section 1	93.9	203.6	30	0.84	0.01	No	Log_{10} (data +1)
Resting section 2	368.9	338.2	30	0.96	0.045	No	Log_{10} (data +1)
Resting section 3	241.1	321.2	30	0.97	0.1	Yes	None

Table 2.8. Summary of the generalised linear model analysis for the effect of chemical treatment and the females wing length on behaviours exhibited in each section of the wind tunnel.

General Linear Model

Factor	Levels	Values
Chemical	2	Volatile Ether
Wing length	5	18 20 21 22 23

Analysis of Variance for Log$_{10}$ (data+1) number of flights in section 1 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	2.0210	2.0210	2.0210	11.46	0.002
Wing length	4	0.6852	0.6852	0.1713	0.97	0.441
Error	24	4.2321	4.2321	0.1763		
Total	29	6.9383				

Analysis of Variance for Log$_{10}$ (data+1) number of flights in section 2 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	1.2454	1.2454	1.2454	5.80	0.024
Wing length	4	1.2944	1.2944	0.3236	1.51	0.232
Error	24	5.1536	5.1536	0.2147		
Total	29	7.6934				

Analysis of Variance for Log$_{10}$ (data+1) number of flights in section 3 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.7469	0.7469	0.7469	5.48	0.028
Wing length	4	1.0150	1.0150	0.2538	1.86	0.150
Error	24	3.2687	3.2687	0.1362		
Total	29	5.0306				

Analysis of Variance for the number of resting periods in section 1 of the wind tunnel.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.8333	0.8333	0.8333	2.76	0.110
Wing length	4	1.2833	1.2833	0.3208	1.06	0.397
Error	24	7.2500	7.2500	0.3021		
Total	29	9.3667				

Analysis of Variance for the number of resting periods in section 2 of the wind tunnel.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.000	0.000	0.000	0.00	1.000
Wing length	4	3.383	3.383	0.846	0.63	0.644
Error	24	32.083	32.083	1.337		
Total	29	35.467				

Analysis of Variance for the number of resting periods in section 3 of the wind tunnel.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.1333	0.1333	0.1333	0.15	0.699
Wing length	4	1.6667	1.6667	0.4167	0.48	0.751
Error	24	20.8667	20.8667	0.8694		
Total	29	22.6667				

Analysis of Variance for log 10 (data+1) duration of flights in section 1 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	2.9174	2.9174	2.9174	8.59	0.007
Wing length	4	2.3196	2.3196	0.5799	1.71	0.181
Error	24	8.1508	8.1508	0.3396		
Total	29	13.3877				

Analysis of Variance for log 10 (data+1) duration of flights in section 2 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	2.6485	2.6485	2.6485	4.88	0.037
Wing length	4	3.2209	3.2209	0.8052	1.48	0.238
Error	24	13.0267	13.0267	0.5428		
Total	29	18.8962				

Analysis of Variance for log 10 (data+1) duration of flights in section 3 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	1.0701	1.0701	1.0701	4.92	0.036
Wing length	4	1.6293	1.6293	0.4073	1.87	0.148
Error	24	5.2238	5.2238	0.2177		
Total	29	7.9232				

Analysis of Variance for log 10 (data+1) duration of resting periods in section 1 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	1.477	1.477	1.477	1.32	0.261
Wing length	4	3.337	3.337	0.834	0.75	0.569
Error	24	26.787	26.787	1.116		
Total	29	31.601				

Analysis of Variance for log 10 (data+1) duration of resting periods in section 2 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	0.855	0.855	0.855	0.52	0.479
Wing length	4	1.588	1.588	0.397	0.24	0.913
Error	24	39.631	39.631	1.651		
Total	29	42.075				

Analysis of Variance for duration of resting periods in section 3 of the wind tunnel

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Chemical	1	11682	11682	11682	0.10	0.751
Wing length	4	263819	263819	65955	0.58	0.678
Error	24	2716100	2716100	113171		
Total	29	2991601				

Table 2.6. shows the mean number of the behaviours observed and the standard error of the mean for the three trials. In the presence of volatiles females flew significantly more often in sections 1, 2 & 3 of the wind tunnel as compared to the ether control (section 1: $p<0.01$; section 2: $p<0.01$; section 3: $p<0.05$ respectively)(Table 2.8.). There were no significant differences in the number of resting behaviours between host plant volatile and control samples (Table 2.8.). Additionally, females spent significantly longer periods of time in flight in sections 1, 2 & 3 of the wind tunnel when exposed to host plant volatile extracts as compared to the ether control (section 1: $p<0.01$; section 2: $p<0.05$; section 3: $p<0.05$)(Table 2.8.).

SECTION B

METHODS

Windtunnel description

See Section A

Butterflies

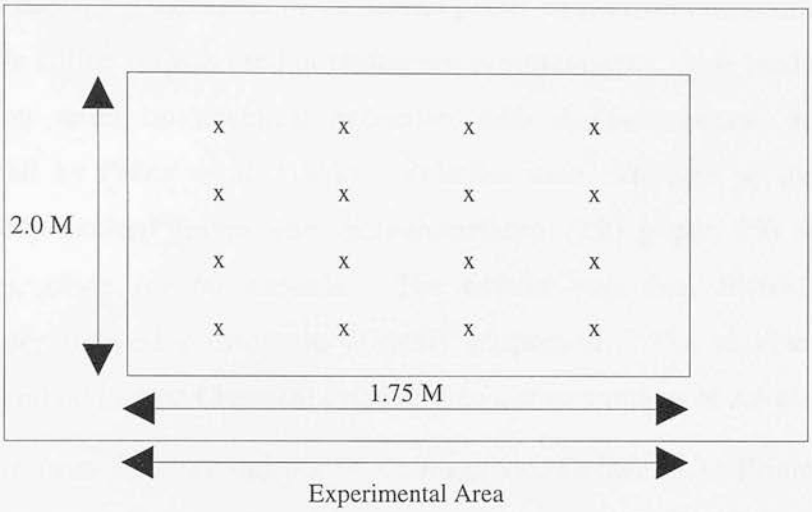
See Section A

No-choice assay

16, eight week old Iceberg lettuce plants were used in each test. The lettuce plants were grown without the use of pesticides in a greenhouse under natural lighting conditions. The plants were sprayed with either 2.5 gle volatile extract or mineral oil on both sides of all of the leaves using an air brush. The 16 plants were then arranged in a 4 x 4 grid in the wind tunnel with a 20 cm gap between plants, see Figure 2.11. 10 butterflies were introduced into the wind tunnel and left to acclimatise for 15 minutes and after this period the butterflies behaviours were recorded for three one hour periods, with a five minute interval between each sample.

Behaviours recorded were the frequencies of flights and alightings. Two trials of this experiment were carried out.

Figure 2.11. Diagrammatic representation of the wind tunnel used in these experiments. x = position of plants.



Wind speed and temperature were recorded at each sampling interval. The wind speed and temperature were recorded over a three minute period at the end of each sampling time and expressed as the mean speed and temperature over the three minute period. The average and S.E. of the wind speed and temperature measurements for the treatments were calculated. For oil, the mean wind speed was 1.04 m/s (S.E. 0.004) and the mean temperature was 21.8 ^{O}C (S.E. 0.37). For volatiles, the mean wind speed was 1.05 m/s (S.E. 0.007) and the mean temperature was 22.5 ^{O}C (S.E. 0.3).

Choice assay

The experimental procedure was as above except the 16 lettuce plants used in each test were randomly split into two groups of 8. One group of 8 was sprayed with mineral oil and the other group of 8 plants was sprayed with 2.5 gle volatile extract. The 16 plants were then arranged randomly in a 4 x 4 grid, see figure 2.11. 10 butterflies were introduced into the wind tunnel and left to acclimatise for 15 minutes and after this period the butterflies behaviours were recorded for three one hour periods, with a five minute interval between each sample. The frequencies of flights and alightings was recorded. Two trials of this experiment were carried out.

The mean wind speed was 1.07 m/s (S.E. 0.004) and the mean temperature was 22.8 °C (S.E. 0.34).

Collection of volatiles

In order to avoid damaging the leaves of the lettuce plants with solvent an alternative method of volatile collection was used in preference to entrainment. This method of volatile collection relies on chemical extraction with dichloromethane and is described in detail by Feeny *et al.* (1989). Volatiles were extracted by dipping bunches of freshly excised leaves into dichloromethane (250 g per 750 ml of dichloromethane), twice for 60 seconds. The extract was then filtered and concentrated under reduced pressure in a rotary evaporator. The residue was dissolved in mineral oil (Sigma Chemical Company) to a concentration of 2.5 gle.

Leaves used were from 12 week old plants, Cabbage var. Golden Acre Primo (II). The plants were treated with systemic insecticide granules at sowing (Temik 1 granule per square inch of compost surface Rhone-Poulenc Agriculture, Ongar Essex UK) in an attempt to limit the damage caused by herbivorous insects and any subsequent effect this damage may have had on the volatiles released from the leaves.

RESULTS

No-Choice Assay

The data from this assay was analysed using the GLIM command with chemical treatment as a factor. Prior to the analysis the data was transformed using an arcsine squareroot on the proportion of behaviours for volatile and oil. The results of this assay are shown in Table 2.9.

Table 2.9. Mean number of flights and alightings per trial made on non-host plants baited with volatiles or oil in a no-choice test in each sampling period by *P. rapae* (mean of 2 trials; 10 females per trial). N=2.

Time	Flights		Alightings	
(Min)	Volatile	Oil	Volatile	Oil
0	48 ± 14	33 ± 8	31.5 ± 7.5	15 ± 5
60	69.5 ± 11.5	62.5 ± 28.5	49.5 ± 7.5	30 ± 16
120	105 ± 42	88 ± 11	70 ± 23	49 ± 2

There was no significant increase in the number of flights in the presence of lettuce plants baited with volatiles as compared to the number of flights in the presence of plants baited with mineral oil ($p>0.05$; Table 2.10.). However, there was a significant increase in the number of alightings on lettuce plants baited with volatiles as compared to the oil controls ($p<0.05$; Table 2.10.).

Table 2.10. Summary of output from the GLIM analysis for the effect of volatiles on the number of flights and alightings made by *P. rapae* females in the presence of lettuce plants baited with either volatiles or oil.

Factor	Levels	Values
Treatment	2	Volatile Oil

Analysis of Variance for the number of Flights made in the presence of lettuce plants baited with volatile or oil.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	0.02175	0.02175	0.02175	1.67	0.226
Error	10	0.13054	0.13054	0.01305		
Total	11	0.15230				

Analysis of Variance for the number of alightings made in the presence of lettuce plants baited with volatile or oil.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	0.14052	0.14052	0.14052	9.09	0.013
Error	10	0.15464	0.15464	0.01546		
Total	11	0.29516				

Choice Assay

Table 2.11. Mean number of flights and alightings per trial made on non-host plants baited with volatiles or oil in a choice test in each sampling period by *P. rapae* (mean of 2 trials; 10 females per trial). N=2.

Time	Flight	Alightings	
(Min)		Volatile	Oil
0-60	23 ± 20	15 ± 14	5.5 ± 4.5
60-120	48 ± 36	24.5 ± 20.5	9.5 ± 8.5
120-180	68.5 ± 30.5	26.5 ± 4.5	20 ± 13

The results of the choice assay for lettuce plants baited with oil or volatiles are shown in Table 2.11. There was a significant increase in the number of alightings on plants baited with volatiles as compared to oil (p>0.01 Table 2.12.).

Table 2.12. Summary of output from the GLIM analysis for the effect of volatiles on the number of alightings made by *P. rapae* females in the presence of lettuce plants baited with volatiles or oil.

Factor	Levels	Values
Treatment	2	Volatile Oil

Analysis of Variance for Alighting

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	0.23520	0.23520	0.23520	17.06	0.002
Error	10	0.13790	0.13790	0.01379		
Total	11	0.37310				

The effect of sampling time on the behaviours recorded in the choice and no-choice tests.

The effect of sampling time (data shown in Tables 2.9., no-choice assay; and 2.11. choice assay) was analysed using linear regressions in Minitab for windows (Release 11.1). For all of the behaviours there was a significant linear regression between sampling time and the number of behaviours observed (ANOVA DF1,6, P>0.05; Table 2.13.). Line fit plots for each behaviour are shown in figures 2.12.-2.15.

Table 2.13. Summary of output from regression analysis of the effect of sampling time on the number of behaviours observed in the choice and no-choice assays.

Number of flights made in the choice test

Source	DF	SS	MS	F-Value	P
Regression	1	15043	15043	14.3	0.013
Error	5	5260	1052		
Total	6	20303			

Number of Volatile Alightings made in the choice test

Source	DF	SS	MS	F-Value	P
Regression	1	2941.8	2941.8	10.61	0.023
Error	5	1386.3	277.3		
Total	6	4328			

Number of Oil Alightings made in the choice test

Source	DF	SS	MS	F-Value	P
Regression	1	1020	1020	9.38	0.028
Error	5	544	108.8		
Total	6	1564			

Number of Volatile Flights made in the no-choice test

Source	DF	SS	MS	F-Value	P
Regression	1	36001	36001	39.98	0.001
Error	5	4502	900		
Total	6	40503			

Number of Oil Flights made in the no-choice test

Source	DF	SS	MS	F-Value	P
Regression	1	25441	25441	62.59	0.001
Error	5	2032	406		
Total	6	27473			

Number of Volatile Alightings made in the no-choice test

Source	DF	SS	MS	F-Value	P
Regression	1	16563	16563	58.94	0.001
Error	5	1405	281		
Total	6	17968			

Number of Oil Alightings made in the no-choice test

Source	DF	SS	MS	F-Value	P
Regression	1	7040.6	7040.6	60.55	0.001
Error	5	581.4	116.3		
Total	6	7622			

Figure 2.12. Line fit plot of the observed and predicted, from regression, number of flights made by ten female *P. rapae* in the presence of lettuce plants baited with a volatile extract or oil in a choice test, Original data in table 2.11.

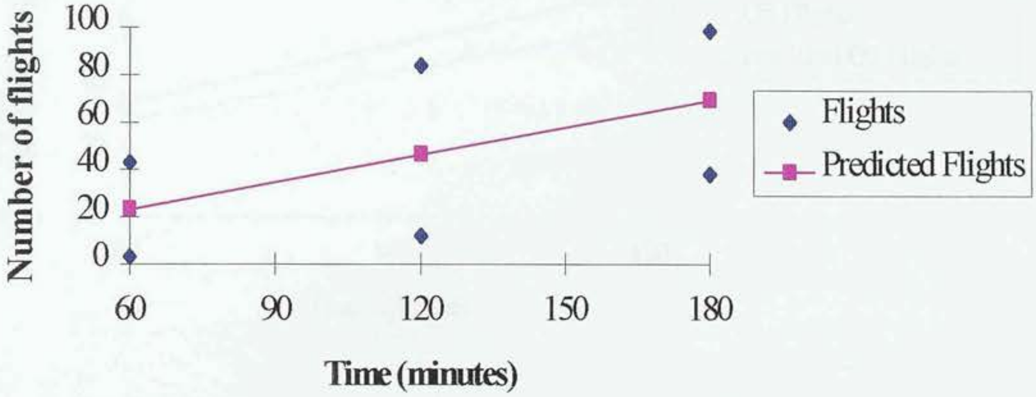


Figure 2.13. Line fit plot of the observed and predicted, from regression equation, number of alightings made by ten female *P. rapae* in the presence of lettuce plants baited with a volatile extract or oil in a choice test, original data in Table 2.11.

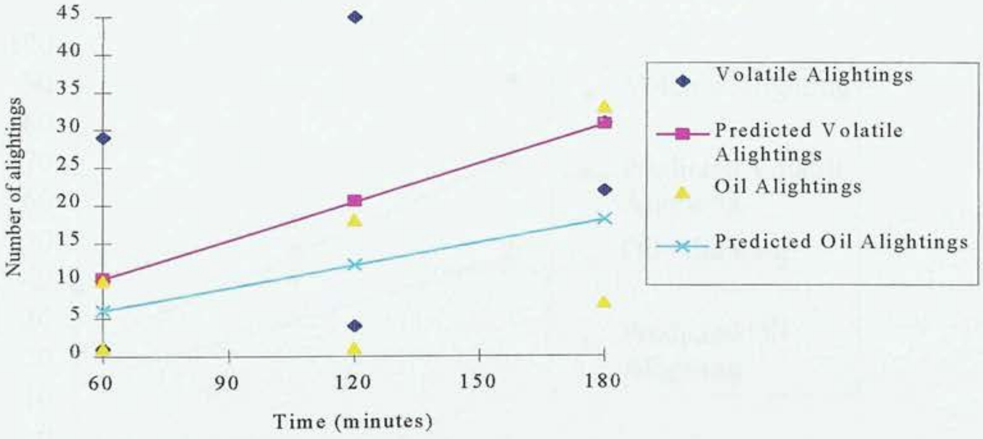


Figure 2.14. Line fit plot of the observed and predicted number, from regression equation, of flights made by ten female *P. rapae* in the presence of lettuce plants baited with a volatile extract or oil in a no-choice test, original data in Table 2.9.

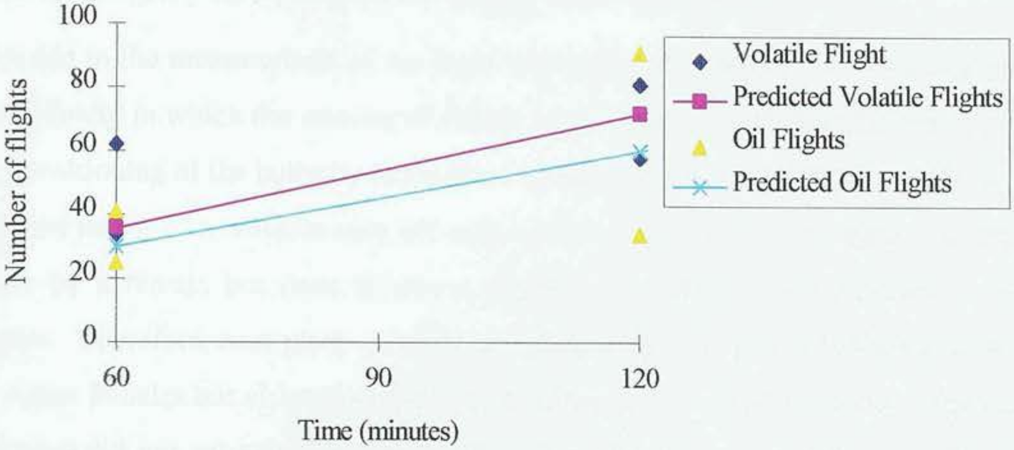
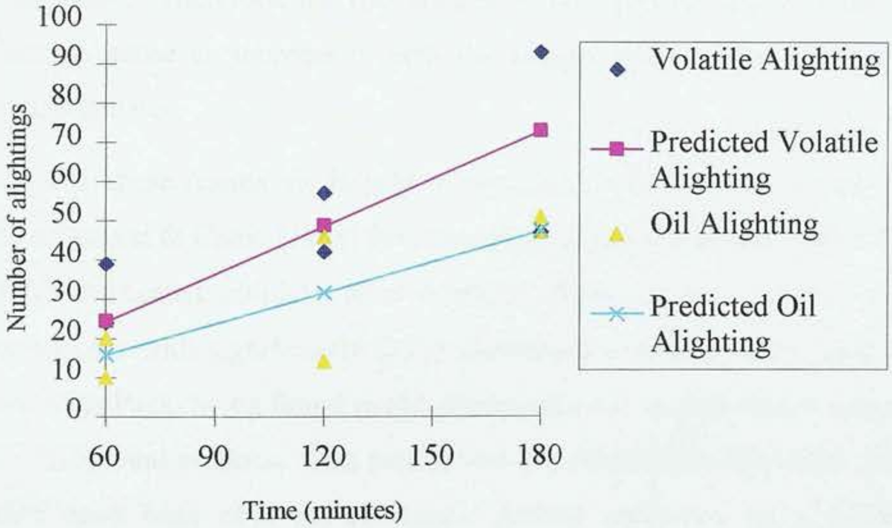


Figure 2.15. Line fit plot of the observed and predicted, from regression equation, number of alightings made by ten female *P. rapae* in the presence of lettuce plants baited with a volatile extract or oil in a no-choice test, original data in Table 2.9.



DISCUSSION

The results of the group assay show that for the duration of the experiments there was a greater number of *P. rapae* females engaging in flights in the presence of host plant volatile extracts as compared to the solvent controls. This effect may be due to either an increase in the number of flights made or the number of flights may be the same for volatiles and ether treatments but the duration of flights in volatiles was longer. As the behaviour of the butterflies was sampled at intervals it is impossible to say from these results which of these two factors cause the increases observed.

This led to the measurement of the flight behaviour of *P. rapae* females being made individually in which the number of flights was recorded. Additionally, the duration and positioning of the butterfly in the wind tunnel was recorded. These results clearly indicate that in fact, volatile cues not only lead to an increase in the number of flights made by a female but there is also a significant increase in the duration of such flights. Therefore, host plant volatiles are effective in not only stimulating flight by *P. rapae* females but also prolong flights as compared to solvent controls. However, volatiles did not act as attractants, as defined by Dethier *et al.* (1960), to female *P. rapae*.

It may be argued that the results from these experiments are due to the suppression of the butterflies behaviour by ether, however, this ignores the fact that the volatile extracts were extracted in ether. Therefore, even if the behaviours observed are suppressed by ether, then the volatile extracts present also must also be acting as stimulants. Therefore, the conclusions, in my opinion, remain valid, that is volatile extracts cause an increase in both the number and duration of flights made by *P. rapae* females.

Overall, these results are largely in agreement with the findings of Aluja, Prokopy, Bounaccorsi & Carde (1993) for *Rhagoletis pomenella* Walsh. which flew more often in air permeated with host plant volatiles. However, Evans (1991) noted an upwind movement with significantly fewer *Dasineura brassicae* Winn. and *Ceutorhynchus assimilis* Payk. being found in the downwind section of the wind tunnel as compared to the upwind sections. This pattern was not observed in this study. However, odour cues have been observed to induce random movement in a number of species including *Cydia pomonella* (Wearing, Connor & Ambler, 1973) and in *D. radicum* (Traynier, 1967). For *D. radicum* host plant odour increased the activity of the females but no directed responses to the odour were observed (Traynier, 1967) and Wearing *et al.* (1973) suggested that this type of increase in random flight (i.e. nondirected) may increase the chances of an insect contacting the source of the volatile odour.

The flight patterns observed in this study were random, that is, there was no evidence of either attraction or repulsion in the presence of volatile extracts. This result may be an accurate reflection of the behaviour of *P. rapae*. Alternatively, it may be due to either an inappropriate dosage of volatiles, either too high or too low, or may be a reflection of the constraints imposed on the flight behaviour of this insect in the confined area of the windtunnel. Clearly, it is not possible with the present data to attribute the causal factor underlying this result.

In the choice and no-choice assay, there was a significant increase in the number of alightings made on volatile treated plants as compared to the control plants. This suggests that volatile cues play a role, not only in the orientation phase of a female's host plant finding behaviour, but also in the acceptance phase of pre-alighting search behaviour.

An interpretation of the significant linear regressions between behaviour and time may be sought in the effect that the physiological state of the insect has on ovipositional behaviours. The physiological state of an insect is an important component in host acceptance behaviour (Miller & Strickler, 1984; Dethier, 1982). It is assumed that the insect's behavioural responses are triggered when the stimuli perceived from a potential host exceed some threshold. The threshold is variable and declines, for example as searching time for an oviposition site and current egg-load increase (Jaenike, 1990; Renwick & Chew, 1994). In conceptualised models, the decision to accept a plant is mediated by the balance of sensory inputs the insect receives from a potential host plant, some of which have a positive and others have negative impact on ovipositional behaviours (Dethier, 1982; Miller & Strickler, 1984). The conceptual models of Miller & Strickler (1984) and Dethier (1982) share the idea that the decision made by a female in response to sensory inputs is modified by its current motivational state. This internal state weighting is derived from the balance between internal excitatory and inhibitory inputs. This balance reflects an insect's 'motivational threshold' and takes into account the changes in host acceptability depending upon the individual's physiological state (Miller & Strickler, 1984). The current egg-load of a female is known to influence host acceptance thresholds and a high egg-load acts centrally to increase the tendency to accept any host (Courtney *et al.*, 1989). Therefore, when host deprivation occurs, the insect's motivational state rises leading to females becoming less discriminating. Experimental work with *P. rapae* (Jones, 1977; Gossard & Jones, 1977; Root & Kareiva, 1984) has confirmed that a female with a high egg-load would lay most of her daily egg complement on the first host plant or group of hosts found. As egg-load declines, an individual leaves hosts more readily, as do individuals with a lower initial egg-load.

We may assume that at the beginning of these experiments, each female's motivational state was low since it had access to host plants suitable for oviposition. As the experiments progressed, the motivational state may have risen as there was no oviposition on the baited plants. During the experiments, there was an increase in the number of behaviours with time i.e. females not only searched (as measured by the number of flights made) more intensively, they also alighted more often in the no-choice test. The influence the motivational state exerts on searching behaviours has not been investigated for *P. rapae*, but if the initial assumption that the motivational state increased over time were true, then there would appear to be a relationship between motivation and number of behaviours. From these results, it may be deduced that the main effect of increasing host plant deprivation on the responsiveness appears to be an increase in both the number of flights and the number of alightings. The linear relationship between the number of alightings made and the sampling period add credence to the proposition that as the motivational state of *P. rapae* increases there is an increase in the number of searching behaviours.

The use of the motivational state as discussed provides a convenient conceptual framework for integrating the effect of host plant deprivation described in these experiments into a coherent theoretical framework. However, the evidence presented here for such an effect is at best circumstantial and should be treated with caution.

Overall, these experiments have shown that volatile chemicals emanating from host plants influence the searching behaviour of female *P. rapae* and during the pre-alighting search for hosts it would appear that olfactory cues are utilised. However, the results from previous studies suggest that the prominent cue involved in searching behaviour is probably visual, with other studies clearly demonstrating that *P. rapae* responds to visual cues alone (Renwick & Radke 1988; Traynier, 1979). As plants show great inter-and intra-specific variation between plants, colour and shape are unlikely to be able to account entirely for the discrimination of host plants. Bernays & Chapman (1994) suggest that olfactory and visual cues operate in unison to guide an insect to a suitable host. Volatile cues may act at a greater distance and bring the female within visual range of the host plants. Therefore, it may be that volatiles act as a host recognition cue and that the discrimination of host plants is dominated by visual cues particularly colour.

CONCLUSIONS

This study has clearly shown that *P. rapae* responds to host plant derived volatile cues with an increase in the number of flight and a total duration of time spent in flight. When exposed to plants treated with volatiles there was an increase in the number of alightings on such plants as compared to controls.

Chapter Three
The effect of colour and host plant chemicals on landing in *P. rapae*

INTRODUCTION

Visual cues are important in host plant location by phytophagous insects (Miller & Strickler, 1984; Prokopy & Owens, 1983). However, the visual aspects of host plant finding behaviour have not been as extensively studied and consequently our understanding of the process is not as well developed as for those aspects pertaining to chemical factors (Prokopy & Owens, 1983). The ability of insects to discriminate between different colours was established beyond doubt around 1910. For example, von Hess (1913) (cited in Burkhardt, 1964) claimed that all invertebrates showed responses to different colours on the basis of the relative brightness and not the wavelength and therefore, could not distinguished colour *per se* (Burkhardt, 1964). However, Friedrich (1933) (cited in Burkhardt, 1964) showed that crabs could distinguish between colours of equal brightness. Ilse (1940) demonstrated that for *P. brassicae* egg laying behaviour was elicited in response to cards coloured between green and blue, in preference to other colours offered.

More detailed experiments have been carried out since these pioneering studies, and in general the results indicate that specific colours are involved in eliciting behavioural responses of insects. For almost all phytophagous insects, the preferred colour for oviposition and landing is green or shades thereof (Moericke, 1969). Clearly if plants are all a similar colour (Bernays & Chapman, 1994), then the ability of an insect to discriminate solely using a the colour of a plant will be limited, leaf shape may provide additional information to limit encounters with non-host plants, but the main cue likely to be involved in pre-alighting discrimination is olfaction which when used in conjunction with visual cues could allow host plant recognition.

Before entering into a detailed discussion of the results of previous studies it is appropriate to have a brief explanation of some of the terms involved in the study of colour and insect behaviour. Burkhardt (1964) gives a more thorough review, and the following is a précis of the definitions giving in Burkhardt (1964). A colour is defined by three parameters namely; 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) (Moericke, 1969).

- Intensity of light: This term applies to the physical energy content of light and is usually quantified in terms energy or number of quanta (Burkhardt, 1964).
- Brightness: The brightness of a stimuli can be distinguished from the intensity of a given stimuli and is generally measured in terms of the animal's reaction. The relationship between brightness and response is generally logarithmic and positive (Burkhardt, 1964).
- Wavelength content: The wavelength content of a stimulus describes the physical nature of the stimulus applied and is distinct from colour which is usually judged by response. For example, a pure spectral light at 580 nm appears equally yellow as an appropriate mixture of 520 and 640 nm (Burkhardt, 1964).
- Spectral efficiency or action spectrum: This term is described by plotting the size of response against wavelengths (of equal energy or quanta). The term spectral sensitivity refers to the curve obtained by plotting constant response the reciprocal of the intensity to obtain the given response against the wavelengths used (Burkhardt, 1964).

The visual receptors of insects are able to perceive a broad range of wavelengths extending from ultraviolet (300-400 nm) through blue (400-500 nm), green (500-560 nm), yellow (560-590 nm), orange (590-630 nm) and terminating in red (ca 650 nm). The spectral reflectance curves of foliage are similar over a wide range of plant species due to the absorption of light by chlorophyll which is responsible for the dominant foliar reflectance hue of 500-580 nm. However, other plant visual characteristics (for example size and shape) are far more variable than the diffuse spectral quality of foliage. It follows, therefore, that for many herbivorous insects the principal visual stimulus eliciting landing is the spectral quality particularly hue and intensity, as these characteristics facilitate the discrimination of a plant from other objects (Prokopy & Owens, 1983). Prokopy, Collier & Finch (1983) have demonstrated that once odour cues have brought a female cabbage fly into the vicinity of a host plant (25 cm) the final choice of landing site was based entirely on visual cues.

Similarly, Vaishampayan, Kogan, Waldbauer & Wooley (1975) have shown that the whitefly (*Trialeurodes vaporariorum*) exhibited a strong positive response to colours with maximum reflectance at wavelengths 520-610 nm and a more moderate response to UV light (<400 nm). Blue-violet and the red regions of the spectrum appeared to be inhibitory.

Judd, Borden & Wynne (1988) have demonstrated that the number of *Delia antiqua* captured on traps can be explained by the reflectance of light in the UV (350 nm), blue (450 nm) and green (560 nm) parts of the spectrum. A multiple regression equation using these three variables was able to explain over 90 per cent of the variation in the data (Judd *et al.*, 1988).

The use of a monochromatic light source to study the effects of different wavelengths of light as elicitors of behaviour is a widely used technique (Kolb & Scherer, 1982; Scherer & Kolb 1987a, 1987b; Coombe 1981). The experimental technique involved in these experiments is to expose the insects to a monochromatic light of the same intensity at different wavelengths and observe the responses of the insects. By plotting the resulting behaviour against wavelength, it is possible to establish which wavelengths of light elicit or deter the observed behaviours.

The effect of different wavelengths of monochromatic light has not been studied in *P. rapae* but data on its effects are available in other species of Lepidoptera. The results indicate that species from different families elicit similar behavioural responses to specific wavelengths. For example, the open space reaction is elicited by wavelengths of light in the UV and violet region of the spectrum (ca 320-420 nm) for both *P. brassicae* and *Pararge aegeria* (Lepidoptera: Satyridae). The feeding reaction was stimulated by the blue and orange red regions of the spectrum for *P. brassicae* (ca 420-500 nm and 590-610 nm respectively) and for *P. aegeria* blue and orange-red light (ca 420-500 nm and 570-670 nm). For *Aglais urticae* (Lepidoptera: Nymphalidae) blue and yellow light elicited the feeding reaction (ca 420-500 nm and 550-590 nm) (Scherer & Kolb, 1987a; 1987b). For *P. brassicae* the effect of drumming and oviposition are elicited in response to specific wavelengths of light. The wavelengths of light initiating drumming and oviposition lie in the range 497 to 578 nm, with the most stimulatory wavelength being 548 nm for *P. brassicae*.

For *Pieris rapae* the size and shape of leaves are not deemed important in host plant discrimination (Renwick & Radke, 1988), the colour of the plant is an important cue (Myers, 1985). Unpublished results, referred to in Renwick & Radke (1988), indicate that *P. rapae* showed a preference for landing on artificial leaves with maximal reflectance at 550 nm.

However, Aluja & Prokopy (1993) have argued that the results of many studies have shown the importance of stimuli interactions in the process of finding and acceptance of host plants by phytophagous insects (Aluja & Prokopy 1993 and references therein). During certain stages of the host plant finding process particular stimuli may play a singular role, however, it is apparent that for many herbivorous insects, behaviours are evoked by the gestalt of the plant (Aluja & Prokopy, 1993).

In this study, the effect of different combinations of potential sensory cues:- a wax coating, host plant volatiles and contact stimulants on landing frequencies in a behavioural assays with a choice of six different colours of card (white and five shades of green) was investigated. In contrast to most studies of *P. rapae* behaviour which concentrate on oviposition, I investigated the effect of the various treatments on the frequency of landings. The spectral profiles of the cards used were determined using spectrophotometery in an attempt to establish statistical relationships between observed behaviour and the relative number of quanta of light reflected at different wavelengths.

Methods

Butterflies

All experiments utilised captive bred *P. rapae*. The culture used was started from eggs obtained from a culture maintained at HRI (Wellesbourne). This culture has been continuously reared since the 1960's. The larvae were caged in mesh covered cages (size 50 x 50 x 50 cm). Larvae were fed on whole plants and when a plant had been eaten it was removed from the cage and replaced. Any larvae still present on the plant were carefully removed with either a paintbrush (mostly instars I & II) or with a pair of fine forceps (instars III, IV & V). Any pupae present were removed from the plant and carefully placed onto the floor of the cage. The adult butterflies were raised at a temperature of 22 ^{O}C with a light-dark photoperiod of 18:6 hours respectively. Lighting was provided by full spectrum fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland ltd., Glasgow, UK). The plants used for larval rearing and adult oviposition were glasshouse grown without the use of pesticides. Upon eclosion from the pupae, butterflies were transferred to another cage (usually 95 x 50 x 50 cm) with a host plant. An artificial nectar solution (15 per cent (w/v) sucrose with a dash of egg-yellow food colouring) was provided in a 'feeder'. The 'feeder' comprised a white perspex sheet (15 x 15 cm) with six vials attached by silicon sealer (non-toxic aquarium type). In between generations the cages were washed thoroughly with Decon solution (5 per cent) and wiped with a 70 per cent ethanol solution. This was done to minimise the risk of infection from microbial pathogens (particularly viral and fungal pathogens). The host plant used for rearing and oviposition was Spring Cabbage var. Golden Acre Primo (II).

Optical reflectance of card

The samples were analysed using a computer controlled spectrophotometer, incorporating an integrating sphere to measure diffuse reflectance. Scans were taken from wavelengths of 350 to 800 nm with a resolution of 1 nm, relative to a white tile standard (Spectralon™). The spectrophotometer and associated equipment was supplied by Rees Instruments Ltd (catalogue numbers in brackets) and comprised a system controller (6800) fitted with a monochromatic control module (6810) and a acquisition module ((6811) 1 MHz 12 bit ADC)) and a Helium-neon calibration laser (632.8 nm). A monochromator (6107) was used with a wavelength span of 200-850 nm with a silicon detector (6111(0.45 nm slit resolution 3.4 nm)). The light source used was a Tungsten halogen lamp (FOT 150), connected by a bifurcated cable (BK7 optical glass). The software used was spectral analysis (6850) running on a desk top computer (DX386).

Analysis of spectral profile data

The data from the spectral analysis of the cards was corrected to the relative number of quanta for each card (waxed and unwaxed at 10 nm intervals). The methodology employed to carry out this correction has been described in detail by Kolb & Scherer, 1982; Harris Rose & Malsch, 1993 and Harris *et al.*, 1995).

A brief summary is given here for completeness. The reflectance of the card was corrected at 10 nm intervals, in the range 400-800 nm, with reference to the emission spectrum of the lights (relative energy scale 0-1) (Figure 3.1) used in the experiments. The corrected reflectance curves were used to calculate the relative number of quanta reflected by the cards. In order to account for the differences in the energy of the different wavelengths (the energy of a single quantum is inversely proportional to its wavelength). The scale used was for this conversion was as described in Harris *et al.*, 1993 with the value assigned to 400 nm being 0.67 and to 800 nm 1.33. The resulting value was multiplied by 2.7 for graphing purposes (Harris *et al.*, 1993). To obtain the relative number of quanta (RNQ) in a particular spectral region the values obtained from the above correction was added to the values for that range. The corrected spectral profile of the waxed and unwaxed cards are shown in Figure 3.2.(A & B) respectively.

Figure 3.1 Emission spectrum of the natural daylight fluorescent tubes (Sylvania Activa 172 professional 58 watt) used in this study to illuminate the cards used in the bioassays (data provided by manufacturer).

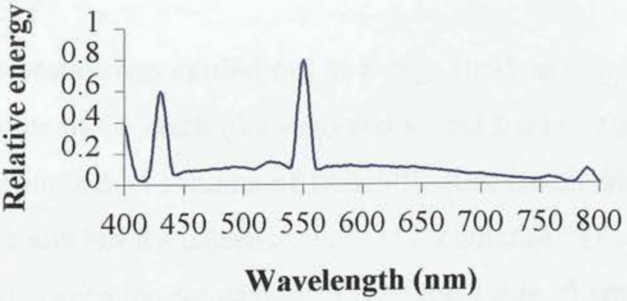


Figure 3.2A. The relative number of quanta reflected in the wavelength band 400-800 nm from each of the waxed cards used in this study.

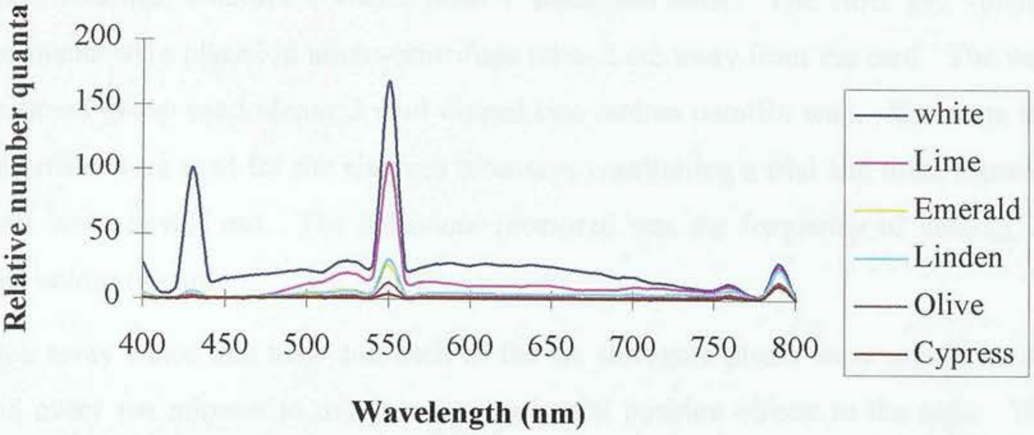
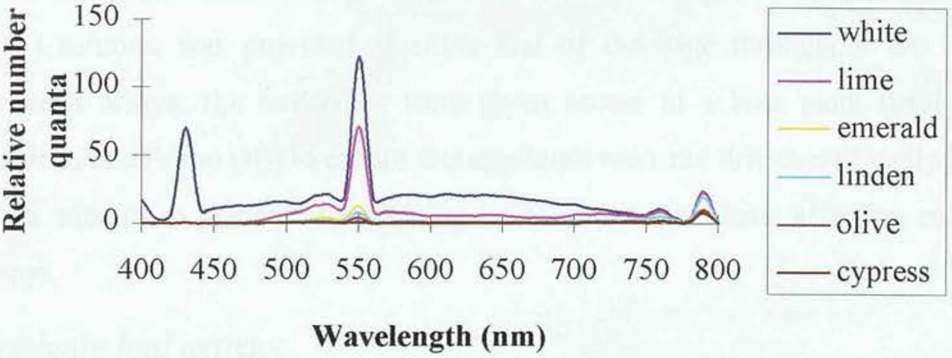


Figure 3.2B. The relative number of quanta reflected in the wavelength band 400-800 nm from each of the unwaxed cards used in this study.



Bioassay

Each bioassay was carried out in a cage (0.95 m long x 0.5 m wide x 0.5 m high) with white nylon mesh (0.3 mm) and a steel frame. The cage was washed after each assay using a 5 % solution of Decon™. Ten female butterflies were introduced into the cage and left for fifteen minutes to acclimatise. They were subsequently offered a choice between the six colours of card (card size 15 cm x 15 cm, total area 225 cm^2) arranged in a 3 x 2 grid configuration 10 cm apart (centre to centre). The cards were supported by an upturned brown plastic 3 inch plant pot and held in an upright position with a fibre glass rod and two paper clips. The colours used were white (Winsor and Newton Art Media Paper; Catalogue number 6437684); lime (6437664); cypress (6437657), emerald (6437658); olive (6437668) and linden green (6437665).

There were nine treatments in all for each group of cards waxed and unwaxed: blank, distilled water, contact stimulants, volatiles + contact stimulants, contact stimulants + ether, volatiles, volatiles + water, ether + water and ether. The ether and volatile treatments were placed in micro-centrifuge tubes 2 cm away from the card. The wax treatment group used identical card dipped into molten paraffin wax. The same ten butterflies were used for the eighteen bioassays constituting a trial and three separate trials were carried out. The behaviour measured was the frequency of landing on each colour of card.

Each assay lasted one hour and each of the six surrogate plants were moved in the grid every ten minutes to minimise any potential position effects in the cage. The cards were moved by hand and rotated around the grid. The initial position of each colour of card was random. The experiments were carried out at 22 ° C and at ambient relative humidity. Lighting was provided by two full spectrum fluorescent tubes to mimic natural daylight suspended above the cage. A 20 per cent sucrose (w/v) solution was provided at either end of the cage throughout the bioassay. Between assays, the butterflies were given access to a host plant (cabbage var. Golden Acre Primo (II)) to ensure that egg-loads were not driven artificially high and in an attempt to prevent any learning with contact stimulants affecting subsequent assays.

Cabbage leaf extract

The cabbage leaf extract used as contact stimulants was prepared as described by Renwick & Radke (1983). For completeness a summary of the method is given here. Freshly excised cabbage leaves (100g/100 ml ethanol) were added to boiling ethanol, and after 5 minutes the mixture was cooled and homogenised in a blender. The homogenate was filtered under vacuum through glass wool. The resulting filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. The residue was first extracted in Di ethyl ether and then distilled water, the Di ethyl ether fraction was discarded and the water fraction was filtered. Prior to use the water fraction was stored at -20 ° C.

The water fraction was used at a concentration of 2.5 gram leaf equivalent and was sprayed onto the card using an airbrush. The plants used for the extractions were cabbage (var. Golden Acre Primo (II)) which were grown in a greenhouse and when 12 weeks old harvested and extracted on the same day. The plants were grown without the use of pesticides under sodium lights with a 14 hour day length.

Collection of host plant volatiles

Cabbage volatiles (var. Golden Acre Primo (II)) were collected by dynamic headspace analysis using a laboratory entrainment system. The equipment used is similar to that described by Robertson *et al.* (1993). The major difference between the two systems is that in the present system air was pulled through the apparatus rather than pushed as in the apparatus of Robertson *et al.* (1993). The construction of the system was glass, stainless steel and PTFE tubing. All joints were wrapped in PTFE tape to provide an airtight seal. Excised leaves were placed in a 2 litre glass container. Air entering the system was filtered through activated charcoal and dried before entering the container with the excised leaves. Air was drawn over the leaves for 20 hours and the volatiles were trapped onto Tenax-Ta. The polymer was contained in a glass tube. All glassware was cleaned in distilled water and methanol and oven baked prior to use.

The Tenax-Ta ((mesh size 60-80) Alltech Associates, Lancashire, UK) was conditioned prior to the collection of volatiles. 3.5 ml of diethyl ether (HPLC grade) were passed through the column of Tenax. The column was then dried by passing a stream of filtered ambient air through the column. The final stage of conditioning was done by passing a stream of helium (BOC Grade A) through the column (flow rate 20 ml/min) while the column was heated to 180 ^{O}C (heating rate 8 ^{O}C per minute) and held at this temperature for three hours. 0.3 g of Tenax-Ta was used in each collection. Volatiles were collected over 20 hours at a temperature of 18 ^{O}C from excised leaves at a flow rate not exceeding 200 ml/min. Leaves used were from 12 week old plants, with between 30 and 50 grams of leaf material per collection. The plants were treated with systemic insecticide granules at sowing (Temik 1 granule per square inch of compost surface Rhone-Poulenc Agriculture, Ongar Essex UK) in an attempt to limit the damage caused by herbivorous insects and any subsequent effect this damage may have had on the volatiles released from the leaves. Elution of volatiles was achieved with 3.5 ml of diethyl ether into a glass sample tube in a bath of ice/methanol. Further dilution to 1.0 gle was undertaken prior to use with diethyl ether.

Statistical Analyses

The data from the assays was analysed using the Glim command in Minitab for Windows (release 11.1), with the colour of the card, waxing, and chemical treatments (volatile, ether, water, contacts as present or absent). The data from the bioassays was tested for normality using a Ryan Joiner test (Shapiro-Wilk) (Minitab for Windows; Release 11.1). The results of this test indicated that the data did not follow a normal distribution (Result of w-test for normality: R-value 0.9037; $P<0.01$). Therefore, the data was subjected to a Log_{10} (data +1) transformation to improve the normality of the distribution. This transformation was carried out to improve the additivity of the model and the normality of the residuals and thus more closely satisfy the assumptions of the analysis of variance with its significance tests. Following the Log_{10} (data +1) transformation the data was again subjected to a Ryan-Joiner test for normality and was found to be normally distributed (Result of W-test for normality R-value 0.9963; $P>0.1$).

Regression analysis was used to investigate the effect of the RNQ from the cards on the number of landings. The RNQ data from the waxed and unwaxed cards were combined to carry out linear regression on the total number of landings (untransformed) made on each colour of card against the RNQ at 550 nm and the ratio of red (675 nm) to infrared (800 nm) RNQ. The rationale behind these choices of wavelengths were the finding of Renwick & Radke (unpubl) (referred to in Renwick & Radke, 1988) that *P. rapae* showed a preference for landing on substrates with maximal reflectance at 550 nm. The ratio of red to infrared reflectance was also used because Myers (1985) showed that a significant linear relationship existed for *P. rapae* between the number of eggs laid on a plant and the ratio of red to infrared reflectance.

Additionally, linear regressions were carried out between the range 400-750 nm at 10 nm intervals to identify areas within the spectral range, which show a statistical relationship between reflectance and behaviour (similar to Judd *et al.*, 1988).

RESULTS

The results of the analysis of the number of landings is summarised in Table 3.1. Colour had a highly significant effect on the number of landings, see Figure 3.3. (ANOVA; DF 5,323; F-value 92.13 $P<0.001$). The presence of contact stimulants significantly increased the number of landings made (ANOVA; DF 1,323; F-value 5.19 $P<0.05$). However, the presence of volatile extracts in ether or ether alone decreased the number of landings made (ANOVA; DF1,323; F-value 4.03 $P<0.05$; ANOVA; DF1,323; F-value 6.57 $P<0.05$ respectively).

For completeness the data for all of the colours tested and chemical treatments are shown in Table 3.2. The effect of colour on the number of landings is illustrated in Figure 3.3. Based on the number of landings, the colours tested fall into three groups with white and lime forming one group and linden, olive and cypress forming another with emerald receiving an intermediate number of landings.

Figure 3.4. shows the effect that the chemical treatments had on the number of landings. As can be seen, the presence of contact stimulants greatly increased the number of landings, whilst for ether and the volatile extract (in ether) the number of landings was decreased.

Figure 3.3. Mean number (Log_{10} (data+1) for each of the six colours tested. Mean of all chemical treatments and waxing. Error bars give standard deviation. (N=108, 36 treatments x 3 trials)

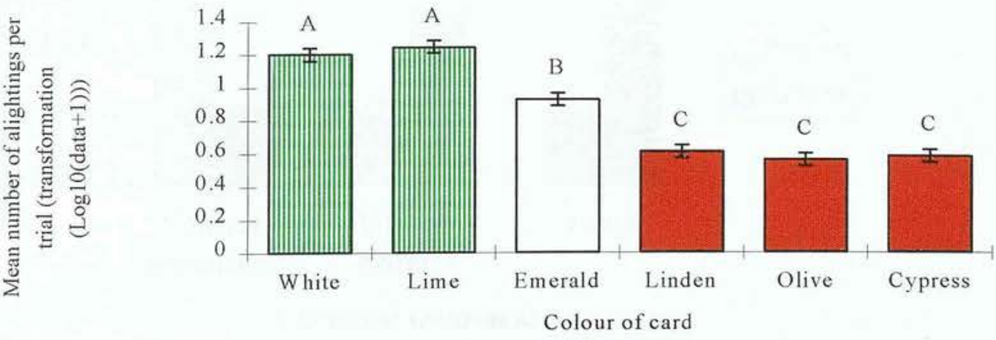


Table 3.1. A summary of the results of the generalised linear modelling analysis carried out on Log_{10} (data +1) number of landings on each colour and chemical treatment.

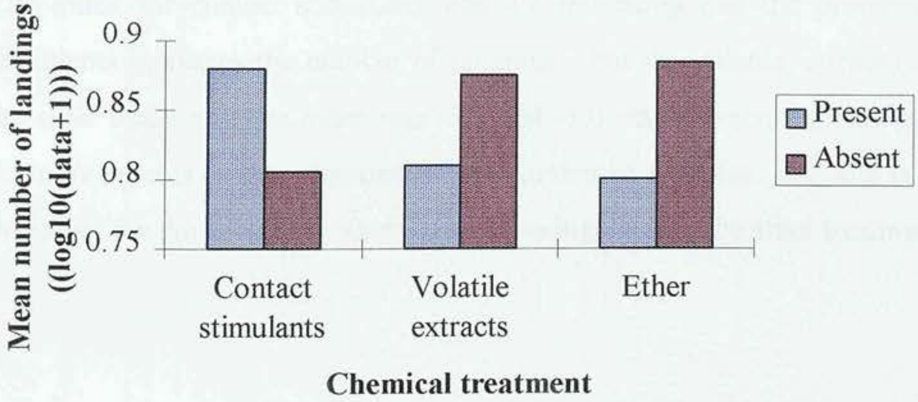
General Linear Model

Factor	Levels	Values
Colour	6	White; Lime; Emerald; Linden; Olive & Cypress
Wax	2	Waxed or Unwaxed
Contacts	2	Present or Absent
Water	2	Present or Absent
Ether	2	Present or Absent
Volatile	2	Present or Absent

Analysis of Variance for Log10 (data +1) number of landings

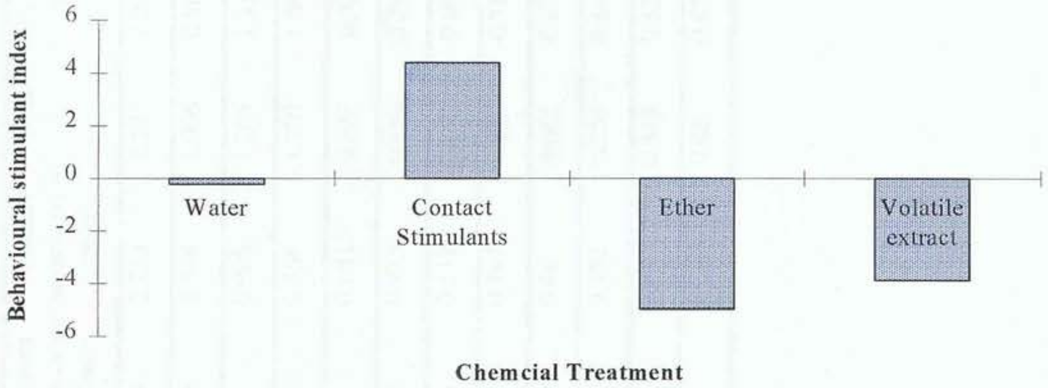
Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Colour	5	26.683	26.683	5.337	92.13	0.001
Wax	1	0.203	0.203	0.203	3.50	0.062
Contacts	1	0.420	0.301	0.301	5.19	0.023
Water	1	0.001	0.001	0.001	0.01	0.916
Ether	1	0.188	0.380	0.380	6.57	0.011
Volatile	1	0.234	0.234	0.234	4.03	0.046
Error	313	18.130	18.130	0.058		
Total	323	45.857				

Figure 3.4. Effect of contact stimulants, volatile extracts and ether on Log_{10} (data+1) mean number of landings by *Pieris rapae* females. Mean of all colours, wax and unwaxed. (N=36 mean of six colours x wax treatment x 3 trials)



Behaviour stimulant indexes (BSI), which is equivalent to the more widely used oviposition stimulant indexes (OSI), as described by Huang & Renwick (1993), were calculated for each of these treatments (Figure 3.5.), using the number of landings made on each treatment. Such indices are a robust and useful measure of the effect of different chemical treatments on the acceptability of an ovipositional substrate (Hern *et al.*, 1996a). The index is calculated by the formula ((T-C/C+T) x 100), where C and T were the number of landings made on the untreated and treated cards respectively. A high positive value indicates that the treatment applied greatly increases the behavioural response, conversely a high negative value indicates that the treatment greatly decreases the behavioural response with a value around zero indicating that the treatment has very little effect on the insect's behavioural responsiveness.

Figure 3.5. Behavioural stimulant indexes for each of the chemical treatments applied in these experiments.



The index for contact stimulants was 4.4 indicating that the presence of contact stimulants increases the number of landings. For the volatile extract (in ether) and the ether treatments the index was -3.9 and -5.0 respectively, indicating that both of these treatments cause a decrease in the number of landings. Of note is the fact that the index for the volatile extract (in ether) is higher than the ether treatment itself.

Table 3.2. The mean number of landings (Log_{10} (data +1)) per trial on each of the colour of cards for each of the chemical treatments. N=3

Colour	Wax treatment	Blank	Water	Contact stimulants	Contact stimulants + volatile	Contact stimulants + ether	Volatile	Volatile + water	Ether	Ether + water
White	Unwaxed	1.187	1.277	1.301	1.219	0.933	1.28	1.24	1.171	1.079
White	Waxed	1.413	1.113	1.317	1.269	1.348	1.006	0.967	1.293	1.19
Lime	Unwaxed	1.162	1.243	1.367	1.214	0.905	1.206	1.188	1.217	1.141
Lime	Waxed	1.308	1.163	1.489	1.454	1.364	1.302	1.084	1.265	1.344
Emerald	Unwaxed	1	0.994	1.072	0.89	0.661	0.937	0.979	0.884	0.784
Emerald	Waxed	1.001	0.878	1.202	0.918	0.975	0.634	0.79	0.981	1.117
Linden	Unwaxed	0.548	0.641	0.534	0.636	0.519	0.693	0.693	0.593	0.36
Linden	Waxed	0.678	0.678	0.866	0.641	0.767	0.36	0.719	0.418	0.667
Olive	Unwaxed	0.551	0.201	0.725	0.766	0.46	0.661	0.674	0.434	0.602
Olive	Waxed	0.615	0.56	0.793	0.693	0.492	0.259	0.641	0.418	0.566
Cypress	Unwaxed	0.46	0.36	0.837	0.46	0.36	0.519	0.625	0.82	0.619
Cypress	Waxed	0.619	0.566	0.854	0.748	0.583	0.46	0.434	0.593	0.651

The relative RNQ at 10 nm intervals were subjected to a linear regression analysis, to compare the 'active spectral region' for the contact stimulants and water treatments and volatile extract and ether treatments. Figures 3.6. & 3.7. show the results of this analysis. The R-squared values from each regression were subjected to a Glim analysis to investigate if the chemical treatment (host plant extract or solvent) or the wavelength of light analysed could be used as explanatory variables in the differences in the R-squared values. A summary of the Glim analyses is provided in Table 3.3.

Figure 3.6. A comparison of the R-squared values obtained from single linear analyses at 10 nm intervals between the RNQ at each wavelength and the mean number of landings for the Contact stimulant and water treatments. Lines for P values are to provide an indication only (analysis of variance 1,11 degrees of freedom).

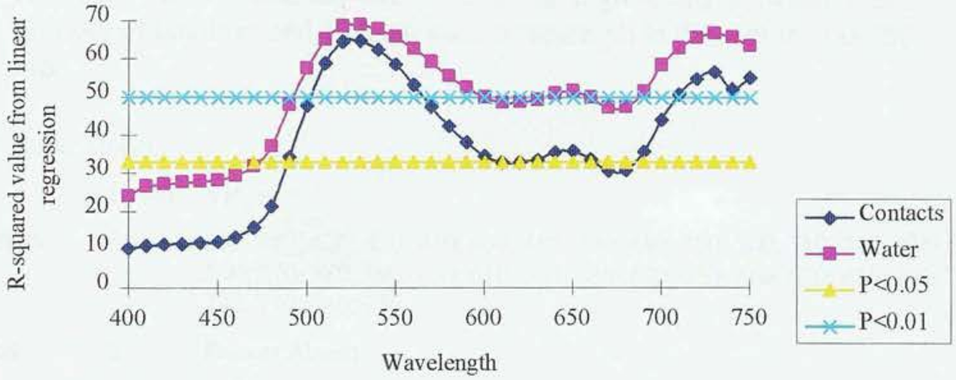
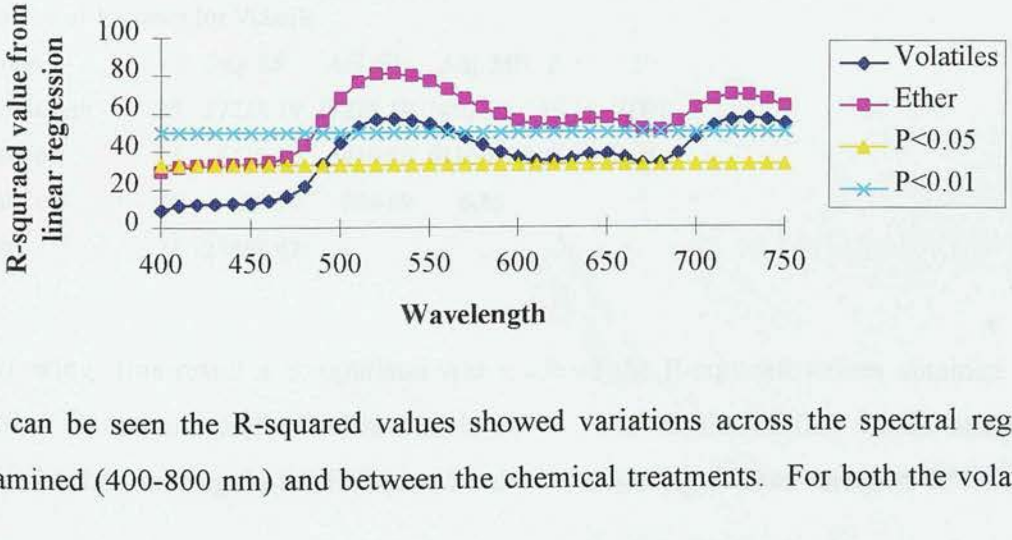


Figure 3.7. A comparison of the R-squared values obtained from single linear analyses at 10 nm intervals between the RNQ at each wavelength and the mean number of landings for the volatile extract and ether treatments. Lines for P values are to provide an indication only (analysis of variance 1,11 degrees of freedom).



As can be seen the R-squared values showed variations across the spectral region examined (400-800 nm) and between the chemical treatments. For both the volatile extract and contact stimulants the R-squared values were significantly lower as compared to the respective solvents (ether and water) (P<0.001 and P<0.001 respectively). This result indicates that host plant chemicals have an effect on the relationship between light reflected and behaviour, which reduces the linearity of the relationship between the amount of light reflected and number of landings. The curves across the spectrum are similarly shaped for the solvent and host plant extracts in both analyses. However, the R-squared values at each wavelengths show significant variations (P<0.001 for contacts stimulants and water and P<0.001 for volatile extract and ether respectively). This indicates that not all regions of the spectrum are equally effective in eliciting landing behaviour.

Table 3.3. Summary output from the Glim analysis performed on the R-square values obtained from the linear regressions between mean number of landings and RNQ at each wavelength in the region 400-750 nm.

```
General Linear Model

Factor        Levels  Values

Wavelength    36      400; 410; 420; 430; 440; 450; 460; 470; 480; 490; 500; 510; 520; 530; 540;
550;                  560; 570; 580; 590; 600; 610; 620; 630; 640; 650; 660; 670; 680; 690; 700;
710;                  720; 730; 740; 750.

Chemical      2       Present Absent

Analysis of Variance for Contacts

Source        DF  Seq. SS   Adj. SS   Adj. MS  F       P

Wavelength    35  17414.15  17414.15  497.55   66.82   0.001

Chemical       1   3036.80   3036.80  3036.80  407.83  0.001

Error         35    260.62    260.62     7.45

Total         71  20711.57

Analysis of Variance for Volatile

Source        DF  Seq. SS   Adj. SS   Adj. MS  F       P

Wavelength    35  17228.19  17228.19  492.23   75.11  0.001

Chemical       1   6410.89   6410.89  6410.89  978.18 0.001

Error         35    229.39    229.39     6.55

Total         71  23868.47
```

Following this result a comparison was made of the R-squared values obtained for contact stimulants and volatiles (Table 3.4.). The results of this GLIM analysis indicate that once again the R-Squared values showed significant variation across the indicate that once again the R-Squared values showed significant variation across the wavelengths tested (P<0.001). However, the differences in the R-squared values for the contact stimulants and volatile extracts were non-significant (P>0.05) indicating that strength of the linearity between behaviour and wavelengths for each of these host plant extracts was the same.

Table 3.4. Summary output from the Glim analysis performed on the R-square values obtained form the linear regressions between mean number of landings and RNQ at each wavelength in the region 400-750 nm.

```
General Linear Model

Factor    Levels  Values

Wavelength  36       400; 410; 420; 430; 440; 450; 460; 470; 480; 490; 500; 510; 520; 530; 540;
550;                 560; 570; 580; 590; 600; 610; 620; 630; 640; 650; 660; 670; 680; 690; 700;
710;                 720; 730; 740; 750.

Chemical     2       Contacts Volatiles

Analysis of Variance for R-squared values

Source       DF  Seq. SS   Adj. SS  Adj. MS  F       P
Wavelength   35  19360.52 19360.52  553.16   113.67  0.001
Chemical      1     1.80      1.80    1.80     0.37  0.546
Error        35   170.32    170.32    4.87
Total        71  19532.64
```

In addition, to the above analyses other parts of the spectrum were examined for the effect that the RNQ had on the observed number of behaviours for the blank treatment and contact stimulants and volatile extract only. These treatments were chosen for the further analyses because of the significant effect the volatile extract and contact stimulants had on the number of landings and the blank card is for comparative purposes.

The RNQ data from the waxed and unwaxed cards were combined to carry out linear regression on the total number of landings (untransformed) made on each colour of card against the RNQ at 550 nm and the ratio of red (675 nm) to infrared (800 nm) RNQ.

A summary of the results of these analyses are shown in Table 3.5. As can be seen, for blank and volatile extract treated cards both measures of reflectance yielded significant linear regressions indicating that the relationship between reflectance and the number of behaviours is linear. However, for contact stimulants only the reflectance at 550 nm yielded a significant linear regression.

Table 3.5. summary of linear regression analyses on the relative number of quanta reflected at 550 nm and the ratio of 775:800 nm for blank, volatile extract and contact stimulant treated card. (data from waxed and unwaxed cards combined). P values refer to analysis of variance of the regression, in all cases degrees of freedom were 1,11.

Treatment	Reflectance at 550 nm		Ratio of reflectance at (675/800 nm)	
	R-sq	p	R-sq	p
Blank	84.00	0.001	55.70	0.05
Contacts	58.60	0.004	10.30	0.309
Volatiles	54.60	0.006	50.00	0.01

Figures 3.8. & 3.9. show line fit plots for the above regressions between the reflectance data and the mean number of landings.

Figure 3.8. Line fit plot for the regression equation between the mean number of landings and the relative number of quanta reflected at 550 nm.

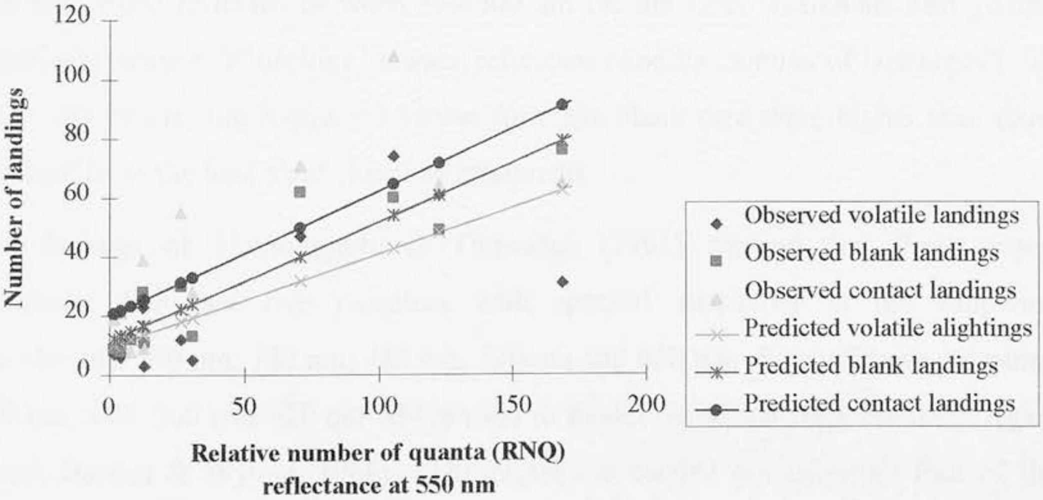
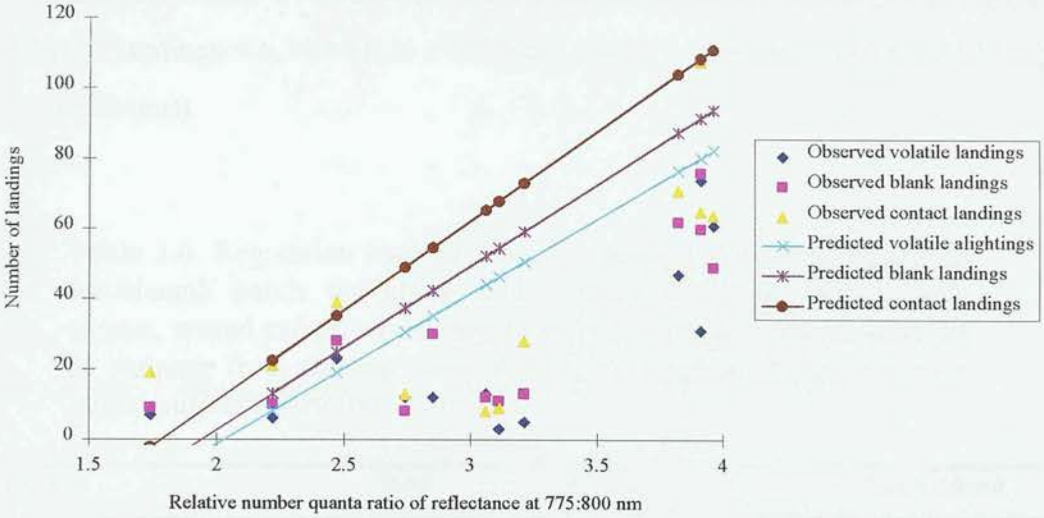


Figure 3.9. Line fit plot for the regression equation between the mean number of landings and the relative number of quanta reflected for the ratio of red to infrared reflectance (775:800 nm).



However, these two variables, the reflectance at 550 nm and the ratio of red to infrared reflectance show a significant correlation (r-squared value 0.822 df= 11 P<0.001).

The total RNQ reflected between 400-800 nm for the three treatments also yielded significant linear relationships between reflectance and the number of landings (Table 3.6.). As before, the R-squared values from the blank card were higher than those obtained from the host plant chemical treatments.

The findings of Shimohigashi & Tominaga (1991) showed that *Pieris rapae crucivora* there are five receptors with spectral sensitivity at the following wavelengths 340 nm; 380 nm; 480 nm; 560 nm and 620 nm. Four of these receptors, 380 nm; 480; 560 and 620 nm where used to model the visual response of *P. rapae* (Judd, Borden & Wynne, 1988). The regression carried out using all four of the wavelengths for the number of landings made on each colour of card treated with contacts stimulants yielded a significant regression: P<0.001; DF 4,11 R-sq. adjusted 91.7 (Regression equation: number of landings = 19.1 - (7.98 x 380 nm) + (17.1 x 480 nm) + (14.2 x 560 nm) - (10.8 x 620 nm)). For cards treated with volatile extracts the four wavelengths yielded a significant regression, Volatile extract: P<0.01; DF=4,11 R-sq. (adjusted) 76.9) (Regression equation: number of landings =

9.89 - (2.7 x 380 nm) - (7.9 x 480 nm) + (1.92 x 560 nm) + (7.05 x 620 nm)). For blank cards the number of landings made on each colour of card also yielded a significant regression: P<0.001; DF 4,11 R-sq. adjusted 93.2 (Regression equation number of landings = 6.54 + (1.06 x 380 nm) + (4.37 x 480 nm) + (10.6 x 560 nm) - (10.8 x 620 nm)).

Table 3.6. Regression analysis between relative number of quanta in wavelength bands for blank card, contact stimulants and volatile extract, waxed and unwaxed card combined. P-values refer to analysis of variance from the regression with 1, 11 degrees of freedom. ns = non-significant (P>0.05)

Treatment		Blank	Contact stimulants	Volatile extract
Total RNQ	R-squared	70.1	39.7	39.5
	P<	0.001	0.05	0.05
400-500	R-squared	46.6	16.3	16.8
	P<	0.05	ns	ns
510-600	R-squared	81.4	54.8	51.8
	P<	0.001	0.01	0.01
610-700	R-squared	63.9	34.4	37.2
	P<	0.01	0.05	0.05
710-800	R-squared	73.1	52.5	52
	P<	0.001	0.01	0.01

DISCUSSION

The results of this assay clearly show that females preferred white and Lime cards when offered in a choice test and, although, the host plant chemical treatments tested had a significant influence on the number of landings made by *P. rapae* females, the overall effect on the choice of card did not change; i.e. the number of landings made by females increased (contact stimulants) or decreased (host plant volatiles) but the colour preferences of the females did not change. Therefore, this study has clearly demonstrated that visual cues are dominant in determining the number of landings made on each card irrespective of chemical treatment.

The regression analysis across the wavelength band 400-750 nm indicates that when host plant chemicals are present a shorter wavelength band exhibits a significant relationship with the number of landings, for contact stimulants this band covers the wavelengths 480-670 nm and 690-750 nm inclusive. For host plant volatile extracts the active region of the spectrum is 490-750 nm inclusive. This result indicates that although, the number of alightings remains the same the regions of the spectrum which are eliciting landing may be different. However, it should be borne in mind that the results are indicative of a statistical relationship and may therefore, not be due to wavelength specific behaviour (Coombe 1981, Kolb & Scherer, 1982 Scherer & Kolb, 1987a; 1987b). However, the fact that these results are similar to those of Kolb & Scherer (1982) and Scherer & Kolb (1987a) for *P. brassicae* may indicate that, in general, for all *Pieris sp.* the wavelength band ca 500-580 nm is most active in eliciting ovipositional behaviours. Additionally, this result may be viewed as an example of Kennedy's (1965) concept in which the cues which are sampled during the catenary process of host plant finding in which the cue just sampled not only allows the insect to proceed to the next cue but the preceding cue may also prepare the insect for the next cue to be sampled. Therefore, host plant chemicals may narrow the responsiveness of the female to those wavelengths which elicit ovipositional behaviours.

The high number of landings on the white card was surprising. For *P. brassicae*, white light failed to elicit a response (Kolb & Scherer, 1982). It would appear, therefore, that the pattern of spectral reflectance emanating from the card and not its colour (as it appears to human vision) *per se* determines the number of landings.

The regression models developed show that an increase in the relative reflectance of either 550 nm or the ratio of red to infrared reflectance would lead to an increase in the number of landings made on that substrate. Furthermore, as the r-square values are higher for the regressions at 550 nm than those of the ratio of red to infrared reflectance, the relative reflectance at 550 nm is a more useful predictor of the number of landings made on each colour of card than the ratio of red to infrared reflectance.

Together these findings corroborate the unpublished results referred to in Renwick & Radke (1988) which found *P. rapae* alighted more often on substrates with maximal reflectance at 550 nm. Similarly, Myers's (1985) results which report a significant relationship between the ratio of red to infrared reflectance and the number of eggs laid on cabbage plants. The index of greenness developed by Myers (1985) also showed a strong relationship with the nitrogen content of the cabbage plant. As the two measures show a high degree of correlation, it would appear that the butterfly gains the same information about the quality of a host. Therefore, the butterflies may actually be selecting the cards on the basis of host plant quality rather than colour *per se*. Clearly then these results would strongly suggest that the colour of a substrate has a strong influence on its attractiveness to *P. rapae*, However, it should be borne in mind that these results were obtained with cards and not plants and the usefulness of the measures for predicting landings in whole plants may not be generally applicable.

However, the results of the multiple regressions would indicate that as the R-squared[6] (adjusted values) values were higher than those of the single wavelength models, the multiple regression models developed for contact stimulants and volatile extracts are more useful in predicting the number of landings made on each of the colour of cards than the single wavelength models of Table 4.5 & 4.6. Therefore, it could be argued that based on these results and the statistical analyses a number of spectral regions are involved in determining the choice of a card for landing in *P. rapae females*.

In the present study, the significant decrease in the number of landings on cards treated with volatile extracts is in stark contrast to the findings of Feeny *et al.* (1989) for *Papilio polyxenes* Stoll. (Lepidoptera: Papillionidae) who found that for female butterflies treating experimental leaves with carrot volatiles increased the frequency of landings as compared to controls. Similarly, the plants (Chapter Two; Section B) treated with volatile extracts caused an increase in the number of landings made on those plants treated with volatiles as compared to the controls. This depression in the

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[6] The R-squared value is a measure of the linearity of the relationship between variables in a regression analysis. However, when more than one variable is used in a regression analysis, the adjusted R-squared value is preferred as this value is adjusted to account for the effect of the number of degrees of freedom in the regression model, in effect the increased number of variables.

number of landings on cards treated with volatile extracts may be due to the depressive effect of ether on the behaviour of *P. rapae*, especially as the BSI of volatile extracts was lower than that of the ether solvent.

The increase in the number of landings made on cards treated with contact stimulants was surprising. This increase may be due to either a post-alighting effect or volatile constituents in the extract attracted the females. The post-alighting effect can be thought of as occurring in the following manner. Once the female has landed and detected the contact stimulant extract, it may lead to a change in behavioural responsiveness which causes the female to land more often, basically the female exhibits area restricted searching. Alternatively, the pre-alighting effect may be due to volatiles present in the extract. The extract was prepared by extracting leaves in ethanol (a moderately polar solvent) and then extracting the residue in ether (very non-polar solvent) and then water (highly polar solvent). Now if the active volatiles which "attract" *P. rapae* are polar or semi-polar then some of these compounds are likely to have been present in the contact stimulant extract, and these chemicals are responsible for the increase in the number of landings. It is impossible to resolve which effect or combination of the effects caused this result.

CONCLUSIONS

The results from this study suggest that for *P. rapae* the dominant cue in host plant finding is probably visual, with chemical cues having no effect on the frequency of landings in this study. The effect of colour on landing was further investigated using a regression analysis of the spectral profiles of the coloured cards. This analysis indicated that the frequency of landings on each colour of card could be predicted, in most cases by the relative reflectance at 550 nm or by the ratio of red to infrared reflectance. Furthermore, it may be that the relative reflectance at 550 nm and the ratio of red to infrared relative reflectance are proxies for the suitability of a host for larval development. However, the multiple regression models developed explain a far greater degree of the variability associated with the data from the choice experiments and as such may show that a number of regions of spectral reflectance are important in determining the selection of substrate based on its pattern of spectral reflectance.

Chapter Four

Influence of pre and post-alighting discrimination of host plants by *Pieris rapae*

INTRODUCTION

The discrimination of host plants by phytophagous insects is conceptualised as having two components, namely pre- and post-alighting discrimination. Visser (1988) has argued that host plant selection can be viewed as a "choice behaviour" with two extremes. The first extreme implies that plants are selected following contact and is usually termed post alighting discrimination. This results in the number of landings on plants being proportional to their abundance in the habitat. The other extreme implies that plants characteristics are perceived at a distance and the choice of plant is affected by these characteristics (pre-alighting discrimination). Pre-alighting discrimination has being defined by MacKay (1985) as "the responses of flying insects to habitat variation resulting in the alighting of the insect on various types of ground cover, with frequencies different from their abundance in the habitat". Pre-alighting discrimination is thought to rely on visual and olfactory cues and post-alighting discrimination relies upon the plants physical and chemical characteristics. Host plant acceptance by insects has been interpreted as a sign of recognition in which the chemistry of the plant is encoded by gustatory receptors into a neural message. Acceptance occurs when the message's code matches a hypothetical template in the brain of the insect (van Loon, 1996). However, a complete description of host plant finding by phytophagous insects should include both plant characteristics perceived by insects and the insects subsequent behavioural response to those characteristics (Visser, 1988). That is, the behavioural response of the insect to a sensory cue is not only dependant upon the nature of the cue but also on the insects internal state (Miller & Strickler, 1984).

When searching in a habitat for a potential host plant phytophagous insects are confronted by an array of sensory cues both positive and negative which may influence their acceptance or rejection of a potential host plant. The effect of sensory information emanating from a plant is thought to influence a insect's behaviour via a catenary chain (Kennedy, 1965), stimuli evoke responses which lead on to subsequent cues in the chain (Courtney, 1986a) and a view of this process is shown in Figure 4.1.

Figure 4.1. An example of the catenary chain of events and sensory information utilised by a female *Pieris rapae* in finding and accepting a host plant for oviposition (Courtney, 1986a; Renwick & Huang 1994).

Behavioural events	**Receptors used**	**Examples of cues**
Habitat selection	Visual	Shade
↓		
Host vicinity cue	Olfactory	Volatile compounds
↓		
Host location	Visual	Leaf shape, Colour
↓		
Host identification	Contact Chemoreception	Surface chemistry
↓		
Post contact discrimination	Visual Olfactory Ovipositor contact	Colour Water content Deterrents Stimulants
↓		
Oviposition site selection	ovipositor contact	Mechanical Surface chemistry Presence of other eggs
↓		
Oviposition		

The effect of colour on landing preference has been investigated and is known to be an important sensory cue for *P. rapae* (Myers, 1985; Renwick & Radke, 1988; Traynier, 1979). The results of Chapter Three would also provide evidence for a strong effect of visual stimuli on the host plant finding process. Volatile odours also influence the process of host plant selection in *P. rapae* (Hern, McKinlay & Edwards-Jones, 1996b).

The mechanisms insects use to orientate to a source of odour have received much attention (Bell, Kipp & Collins, 1996; David, 1986; Kennedy, 1983; 1986; Murlis, Elkingotn & Carde, 1992; Visser, 1986; 1988). Chemo-orientation requires the integration of external and internal factors to generate a search strategy. Searching strategies comprise a series of tactics which may be summarised as (Bell *et al.*, 1996):

- Initiation.
- Orientation.
- Arrestment (stopping or landing).
- Closure (achieving the final approach to a resource after arrestment).

Many insects have been shown to respond to wind borne odours by approaching the point source by making a succession of alternate left and right turns; this is termed counter-turning. Counter-turning can be further refined into two categories (Kennedy, 1983):

- Casting which is movement across wind.
- Zigzagging which is movement upwind.

The control of orientation is thought to be due to ideothetic (internal) and alleleothetic (external) information sources. Clearly the resultant orientation pattern is due to the interaction of these two sources of information (Visser, 1988). The functional significance of counter-turning is to regain contact with the wind borne odour (Kennedy, 1983).

Host plant chemistry has been investigated as a factor mediating ovipositional decisions by *P. rapae*. Host plants contain different compounds which either stimulate or deter oviposition, with the decision to accept or reject a host being based on the relative degree of stimulation and deterrence by these chemicals (Huang & Renwick, 1993; Huang, Renwick & Chew, 1995; Renwick & Chew, 1996; Renwick & Huang, 1994).

The aim of the present study was to investigate host plant choice by *P. rapae* in two varieties of cabbage (vars. Golden Acre Primo (II) and Greyhound) and nasturtium (*Tropaeolum majus*, var. Salmon Pink) using whole plants. The effects of sensory information emanating from these hosts was investigated in an attempt to establish which points in the catenary chain supply information utilised in the processes of host plant finding and discrimination by *P. rapae*. I also investigated the effect that these volatile extracts had on the counter-turning behaviour of *P. rapae*.

METHODS

Butterflies

All experiments utilised captive bred *P. rapae*. The culture used was started from eggs obtained from a culture maintained at HRI (Wellesbourne, UK). This culture has been continuously reared since the 1960's. The larvae were caged in mesh covered cages (size 50 x 50 x 50 cm). Larvae were fed on whole plants and when a plant had been eaten it was removed from the cage and replaced. Any larvae still present on the plant were carefully removed with either a paintbrush (mostly instars I & II) or with a pair of fine forceps (instars III, IV & V). Any pupae present were removed from the plant and carefully placed onto the floor of the cage. The adult butterflies were raised at a temperature of 22 ^{O}C with a light-dark photoperiod of 18:6 hours respectively. Lighting was provided by full spectrum fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland ltd., Glasgow, UK). The plants used for larval rearing and adult oviposition were glasshouse grown without the use of pesticides. Upon eclosion from the pupae, butterflies were transferred to another cage (usually 95 x 50 x 50 cm) with a host plant. An artificial nectar solution (15 per cent (w/v) sucrose with a dash of egg-yellow food colouring) was provided in a 'feeder'. The 'feeder' comprised a white perspex sheet (15 x 15 cm) with six vials attached by silicon sealer (non-toxic aquarium type). In between generations the cages were washed thoroughly with Decon solution (5 per cent) and wiped with a 70 per cent ethanol solution. This was done to minimise the risk of infection from microbial pathogens (particularly viral and fungal pathogens). The host plants used for rearing and oviposition was Spring Cabbage var. April.

Choice test using whole plants

Assay procedure

These experiments were carried out to determine the response of female *P. rapae* to three different host plants, two cabbage varieties and nasturtium were used.

A single butterfly was released into an enclosure 2.0 Metres wide 1.75 in length and 1.0 Metres high. Illumination was provided by eight evenly spaced fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland Ltd., Glasgow, UK) providing full spectrum light with an emission spectrum close to that of daylight but with additional reflectance in the ultra violet. Three plants of each host plant type (Golden Acre Primo (II), Greyhound and Nasturtium) were randomly arranged in the arena in a 3 x 3 grid configuration, which was evenly spaced. Individual females were introduced and their behaviours (landing and oviposition) recorded on each host type for 30 minutes. 24 Butterflies were tested in total. The individual butterflies were tested in groups of eight on three separate occasions.

The plants used in this experiment were grown in a glasshouse under natural lighting conditions in 6 inch pots of compost. To minimise the impact of herbivorous insects on the plants, the plants were treated with a systemic insecticide (Temik granules 1 granule per square inch of compost surface Rhone-Poulenc Agriculture, Ongar Essex UK). Experiments were carried out when plants were 10 weeks old. The treatment with insecticide was carried out to minimise the effect of herbivore feeding which induces changes in the profiles of volatile chemicals released by the plants (Mattiacci *et al.*, 1994).

Spectral analysis of the host plants used.

Four plants (grown as described above) from each variety were analysed as detailed below. The samples taken, from the adaxial surface of a leaf, were analysed using a computer controlled spectrophotometer (as described in Chapter Three)

The data from the spectral analysis of the plants was corrected to the relative number of quanta for each plant. The methodology employed to carry out this correction was that described by Kolb & Scherer, 1982; Harris *et al.*, 1993 and Harris *et al.*, 1995) and is described in detail in Chapter Three.

Effect of host plant volatiles on flight behaviour and landing on model plants in a windtunnel

The effect of volatiles on *P. rapae's* behaviour was assayed for volatile extracts from each of the three host plants used (Cabbage var. Golden Acre Primo (II) and var.

Greyhound; Nasturtium var. Salmon Pink). The assays investigated the effect of the volatiles on the behavioural response of *P. rapae* females to model plants treated with volatile extracts, and on their flight behaviour.

Wind tunnel description

The wind tunnel was comprised of four main sections; the transition section, the settling chamber and the test section which also incorporated the experimental area (Figure 4.2.). The transition section consisted of a wide angle diffuser fitted with a combination of adjustable air splitters and guide vanes to control the boundary layer. The settling chamber, was fed by a non-overloading centrifugal blower fan, with minimum disturbance to airflow. The settling chamber contained a combination of screens and honeycombs to provide a laminar air flow to the entrance of the test section (P. J. Bowden, unpublished).

The wind tunnel experimental area was 2.0 metres wide, 1.75 metres in length and 1 metre high. The airflow within this section of the windtunnel has previously shown to be laminar (P.J. Bowden unpublished). Lighting was provided by eight evenly spaced fluorescent tubes (Sylvania Activa 172 professional 58 watt; supplier Lightbox Scotland ltd., Glasgow, UK) providing full spectrum light with an emission spectrum close to that of daylight with additional reflectance in the ultra violet. The wind tunnel showed variation in the levels of light reaching the floor and for completeness this is illustrated in Figure 4.3. Ambient air is drawn from outside and electric heaters provide heating to 22 °C for experiments (P. J. Bowden unpublished).

Figure 4.2. Diagrammatic representation of the wind tunnel used in these experiments.

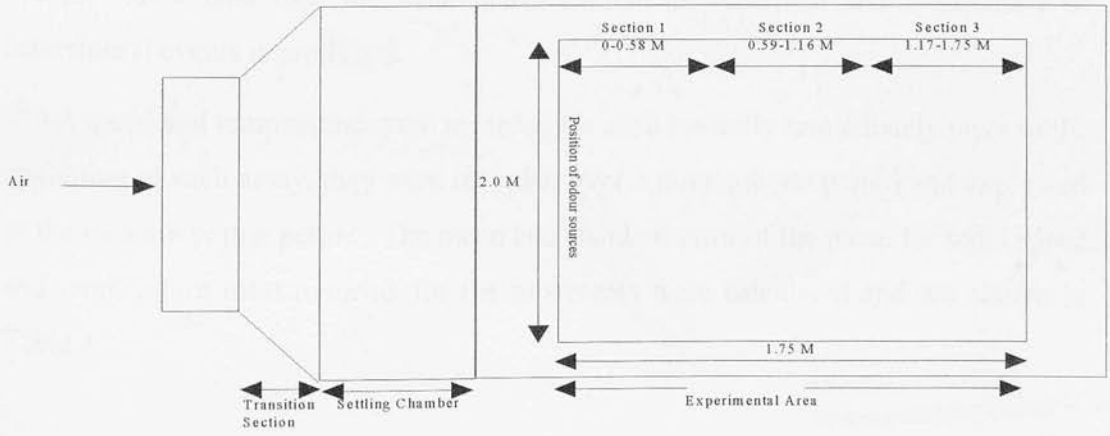
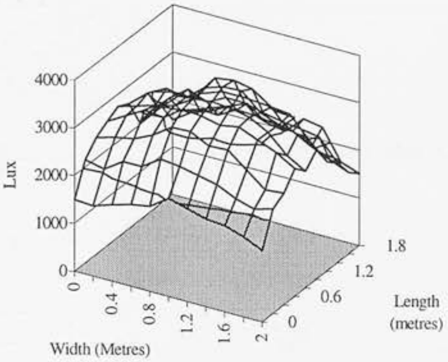


Figure 4.3. Variation in light intensity within the wind tunnel experimental area.



Flight Behaviour

In this assay, individual butterflies were exposed to a single vial of test chemical of either mineral oil or the test volatiles at a concentration of 2.5 gram leaf equivalents (gle), see Figure 4.4. The number of times females exhibited counter-turning flights was recorded, as was the duration of such flights. The female (5-6 days old) was given a 10 minute acclimatisation period and behaviours were recorded for 15 minutes. Five females were used in each trial and three trials were carried out. Behaviours were recorded using the Observer software package, version 3.0 (Noldus Information Technology). This software package allows the recording and analysis of behaviours in experiments. The user defines behaviours to be recorded and general assay conditions, duration of the assay etc. The user observes the behaviour occurring and records what is seen, the software records the behaviour and the time at which it occurs. At a later date the behavioural records are analysed and a summary of behavioural events is produced.

Wind speed and temperature were recorded for each butterfly immediately prior to the beginning of each assay, they were recorded over a three minute period and expressed as the mean over that period. The mean and standard error of the mean for wind speed and temperature measurements for the treatments were calculated and are shown in Table 4.1.

Figure 4.4. Schematic representation of the wind tunnel layout in these experiments.

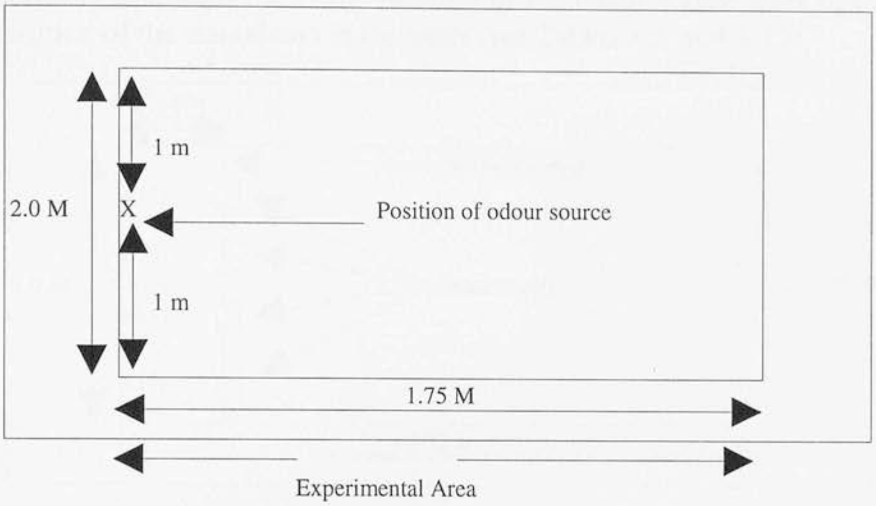


Table 4.1. Mean wind speed (m/s) and Temperature (^{O} C) for each of the three volatile extracts tested and the mineral oil control. N=15

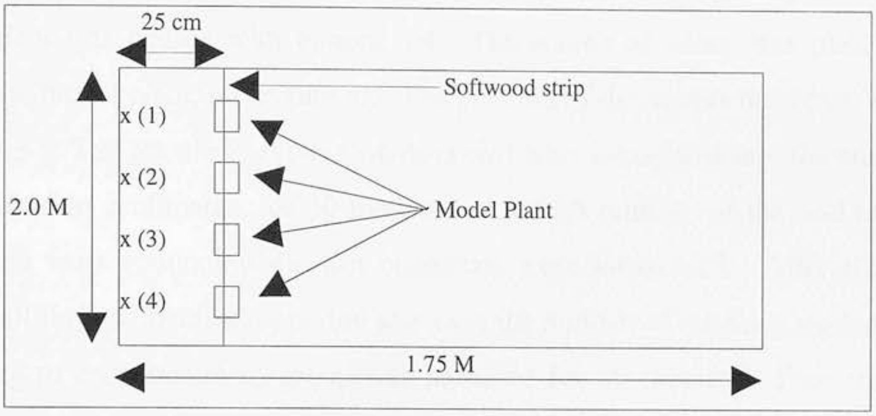
Treatment	Wind speed	Temperature
Golden Acre Primo (II)	1.03 ± 0.005	23.78 ± 0.119
Greyhound	1.10 ± 0.012	24.04 ± 0.097
Salmon Pink	1.03 ± 0.002	23.88 ± 0.107
Mineral Oil	1.04 ± 0.004	23.91 ± 0.108

Pre-alighting discrimination Assay procedures

Choice of volatile and oil

In this assay, females were offered a choice between four model plants, two of which were treated with a volatile extract (2.5 gle) from one of the three host plants tested and two of which were treated with mineral oil. The source of odour was placed 25 cm behind the model plant (See Figure 4.5.). The position of the treatments was randomised, the positions are shown in Table 4.2.

Figure 4.5. Schematic representation of the windtunnel layout in the investigations of pre-alighting discrimination by volatiles. The numbers in brackets after the position of the odour source refer to the position of the test odours in the trials (see Tables 4.2. and 4.3.)



Experimental Area
x denotes position of odour source.

Table 4.2. Position of volatile and mineral oil odour sources for each trial and volatile extracts. (See Figure 4.5. for details of position within the windtunnel).

Odour	Golden Acre Primo (II)			Greyhound			Nasturtium		
position	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	v	o	v	o	v	v	o	o	o
2	v	v	o	v	o	v	v	o	v
3	o	o	o	v	v	o	o	v	v
4	o	v	v	o	o	o	v	v	o

Ten female *P. rapae* (5-6 days old) were introduced into the wind tunnel and allowed to acclimatise for 30 minutes. After 25 minutes of the acclimatisation period the vials containing the test chemicals were introduced. After the full 30 minutes of the acclimatisation period was over the number of landings made on plants belonging to each treatment group was recorded for 30 minutes. Three trials were carried out. The mean wind speed and temperature was recorded as for the flight behaviour experiment. The mean wind speed for Golden Acre Primo (II) was 0.54 m/s ± 0.01 and the mean temperature was 23.4 ° C ± 0.16. The mean wind speed for Greyhound was 0.51 m/s ± 0.01 and the mean temperature was 23.3 ° C ± 0.09. The mean wind speed for Nasturtium was 0.53 m/s ± 0.01 and the mean temperature was 22.9 ° C ± 0.13.

Choice between the three volatile extracts and oil

In this assay, females were offered a choice between four model plants. Each model plant was treated with a volatile extract from one of the host plants in question and the fourth plant was treated with mineral oil. The source of odour was placed 25 cm behind the model plant, see Figure 4.5. The position of the treatments was randomised (Table 4.3.). Ten female *P. rapae* (5-6 days old) were introduced into the wind tunnel and allowed to acclimatise for 30 minutes. After 25 minutes of the acclimatisation period the vials containing the test chemicals were introduced. After the full 30 minutes of the acclimatisation period was over the number of landings made on plants belonging to each treatment group was recorded for 30 minutes. Four trials were carried out. The mean wind speed during the trials was 0.53 m/s ± 0.01 and the mean temperature was 23.1 ° C ± 0.13.

Table 4.3. Position of volatile and mineral oil odour sources for each trial. (See Figure 4.5. for details of position within the windtunnel).

Odour position	Trial 1	Trial 2	Trial 3	Trial 4
1	Greyhound	Greyhound	Mineral oil	Golden Acre Primo (II)
2	Mineral oil	Golden Acre Primo (II)	Nasturtium	Mineral oil
3	Nasturtium	Nasturtium	Greyhound	Greyhound
4	Golden Acre Primo (II)	Mineral oil	Golden Acre Primo (II)	Nasturtium

Construction of model plants

The models were constructed using four plastic ‘leaves’ per ‘stem’. The leaves were made from a corrugated plastic sheet (Plasboard plastics Ltd, Montrose, UK. Colour green grade 2/350). Each leaf was made from an isosceles triangle 10 cm high and 20 cm across the base. The ‘Plants’ were place 40 cm apart across the width of the wind tunnel. Each plant was constructed as follows. A 40 cm glass fibre rod was used as a stem and the four leaves were positioned at right angles to each other around the stem. The leaves were positioned with a vertical distance of 10 cm from the base. The position of the leaves around the stem was identical for each plant. The four plants were then held in position using a strip of softwood (2.5 cm x 2.5 cm) fastened to the floor of the wind tunnel.

Extraction of volatiles

The extraction of volatiles was carried out as described by Feeny *et al.* (1989). Volatiles were extracted by dipping bunches of freshly excised leaves into dichloromethane (250 g per 750 ml of dichloromethane), twice for 60 seconds. The extract was then filtered and concentrated under reduced pressure in a rotary evaporator. The residue was dissolved in mineral oil (Sigma Chemical Company) to a concentration of 2.5 gle. The plants extracted were grown as described for the whole plant choice assay.

Effect of contact stimulants on host plant choice

French Bean plants

French bean plants (*Phaseolus vulgaris* var. Tendergreen) were grown in a glasshouse as described above, with the exception that no insecticide treatment was applied and were presented in three inch plastic pots at the two leaf stage (Huang & Renwick, 1993).

Assay Procedure

Assays were carried out in screen cages (50 x 50 x 50 cm) in a controlled environment room (22 ^{O} C). Each cage was provided with a 20 per cent sucrose (w/v) solution to allow nectar feeding. 10 female *P. rapae* (aged 5-6 days old) were grouped as a trial. The females were released into a cage containing four French bean plants. Each plant was painted with either 5 gram leaf equivalents of one of the host plant extracts from the three host plant varieties or distilled water (control) and left to dry prior to the release of the butterflies into the cage. Lighting was provided by two full spectrum fluorescent lights suspended above the cage (lights are as described for the whole plant choice assay). Each assay lasted six hours. After which the number of eggs laid on each plant was counted. Three trials were carried out.

Extraction of contact stimulants

The extraction of the leaf surface contact stimulants was as described in Huang & Renwick (1993). Freshly excised leaves from each variety were extracted separately, by boiling in ethanol for five minutes. The mixture was then cooled and homogenised in a blender. The homogenate was then filtered under vacuum and the filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. The residue was defatted with n-hexane, and the then dissolved in water. The defatted residue was extracted three times in *n*-butanol. This butanol fraction was discarded and the water fraction was diluted to 5 gle and stored at -20 ° C until use. The plants extracted were grown as described in the whole plant choice assay.

RESULTS

Whole plant assay

The data from this assay was tested for normality using a Ryan-Joiner test (Shapiro Wilk) using Minitab for Windows (Release 11.1). The results of this test indicate that the data for the number and duration of landings and the number of eggs laid were normally distributed, W-test for normality, (R 0.9530, $P>0.05$; R 0.9133 $P>0.05$; R 0.9852 $P>0.05$ respectively). Following the results of these tests, the data was analysed using the Glim command in Minitab for Windows (Release 11.1) with the plant variety as a factor. For the number of eggs, the data from each of the eight females tested together was combined and the statistical analysis was carried out on the basis of per trial. This was done because of the low number of eggs laid per female. A summary of this analysis is included in Table 4.4.

Figure 4.6. indicates that significant differences were found in the number of landings made on the three plant species ($P<0.05$ Table 4.4), with females landing more frequently on the Golden Acre Primo (II) variety of cabbage varieties as compared to greyhound or nasturtium. There were no significant differences in the duration of landings on the plant species tested ($P>0.05$, Table 4.4.). Females also showed significant differences in the number of eggs laid in each of the plant species ($P<0.05$, Table 4.4.) with the two cabbage varieties receiving more eggs than nasturtium (Figure 4.7.).

Figure 4.6. Mean number of landings made by *Pieris rapae* females on each of the three variety of plants tested in a choice test. Error bars show standard deviation of the mean. Columns with different letters are significantly different from each other. N=24.

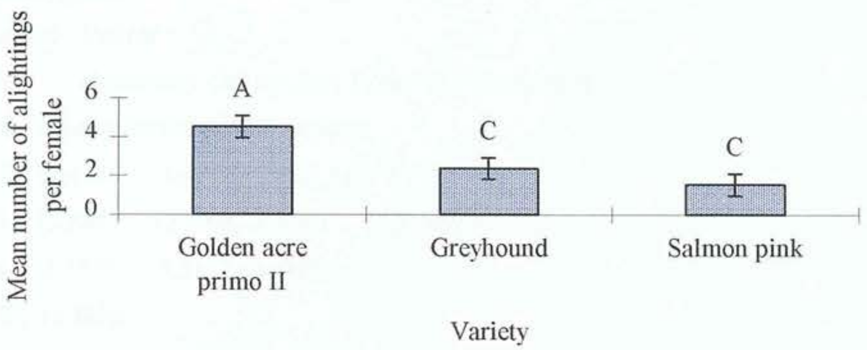


Figure 4.7. Mean number of eggs laid by eight *Pieris rapae* females on each of the three variety of plants tested in a choice test. Error bars show standard deviation of the mean. Columns with different letters are significantly different from each other. N=3

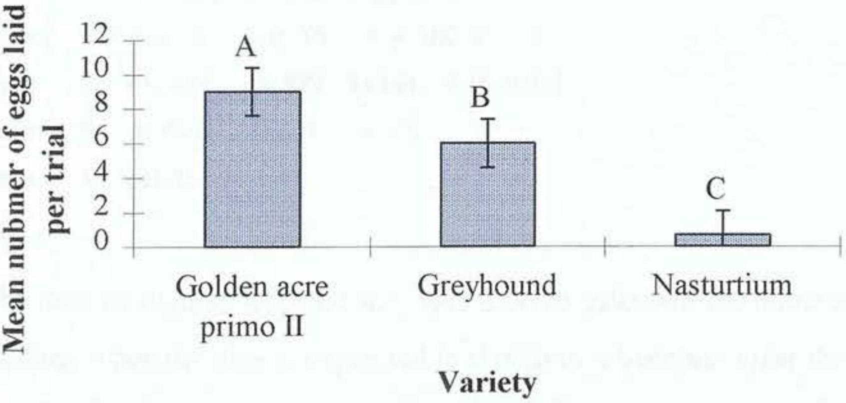
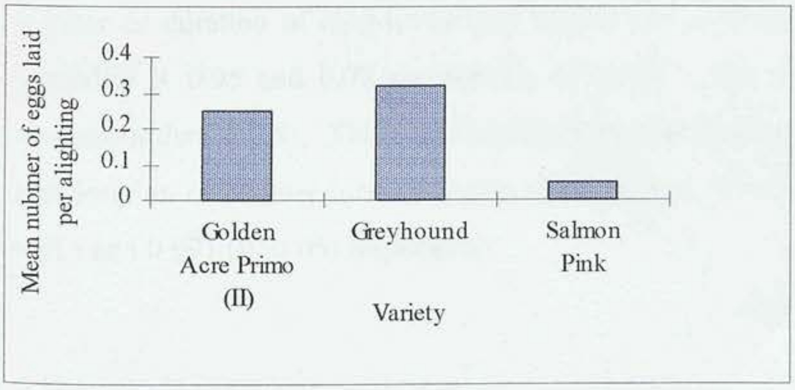


Table 4.4. Summary of Glim analysis of the data on the number and duration of landings and eggs laid by *Pieris rapae* females on each of the three host plants tested.

```
General Linear Model
Factor   Levels  Values
Plant    3       Nasturtium Golden Acre Primo (II)  Greyhound
Analysis of Variance for number of landings
Source   DF Seq. SS   Adj. SS   Adj. MS  F    P
Plant    2  13.8941   13.8941  6.9471   7.50 0.023
Error    6   5.5557    5.5557  0.9259
Total    8  19.4498
Analysis of Variance for duration of landings
Source   DF  Seq. SS   Adj. SS   Adj. MS  F     P
Plant    2   425178    425178   212589   4.03 0.078
Error    6   316312    316312    52719
Total    8   741490
Analysis of Variance for number of eggs laid
Source   DF Seq. SS   Adj. SS   Adj. MS  F    P
Plant    2  106.889   106.889  53.444   9.25 0.015
Error    6   34.667    34.667   5.778
Total    8  141.556
```

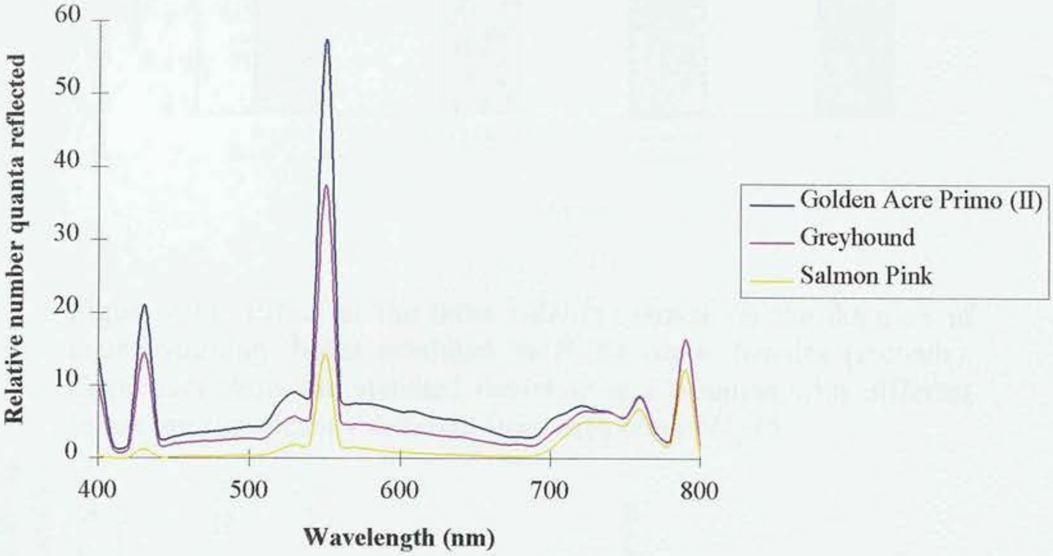
The data in Figures 4.6. and 4.7. was used to calculate the number of eggs laid per landing, when the data is expressed in this form it becomes clear that more eggs were laid on Greyhound per landing than either Golden Acre Primo (II) or Salmon Pink (Figure 4.8.).

Figure 4.8. Mean number of eggs laid per landing on each of the three host plant varieties tested by *Pieris rapae* females. N=24



Spectral analysis of the hosts plants used

Figure 4.9. Relative number of quanta reflected from each plant variety. N=4



The relative spectral reflectance of each of the three plant species is shown in figure 4.9. The pattern of reflectance is similar for each species. Peaks occur at c.a. 420 (blue) and 550 nm (green) and another peak is evident at 750 and 790 nm (far red). The highest values for all of the reflectance are found for Golden acre Primo (II), followed by Greyhound with Nasturtium having the lowest.

The effect of volatile emissions on pre-oviposition behaviour

Flight behaviour

Prior to analysis the data from this assay were tested for normality as previously described. The results of the Ryan-Joiner tests showed that neither the data for the number or duration of counter-turning flights was normally distributed, W-test for normality R 0.95 and 0.97 respectively ($P<0.01$). The data was transformed by squareroot(data + 0.5). This transformation resulted in the data for both the number and duration of counter-turning flights being normal, W-test for normality R-values 0.995 and 0.991 ($P>0.05$) respectively.

Figure 4.10. Effect of the three volatile extracts on the mean number of counter-turning flights exhibited by *Pieris rapae* females. Error bars show the standard deviation. N=15

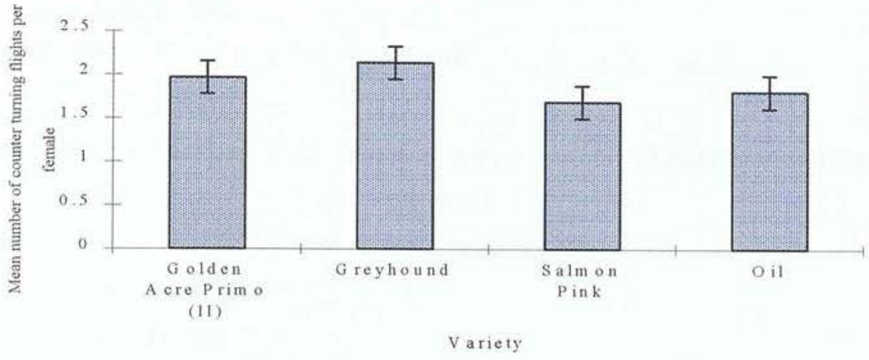
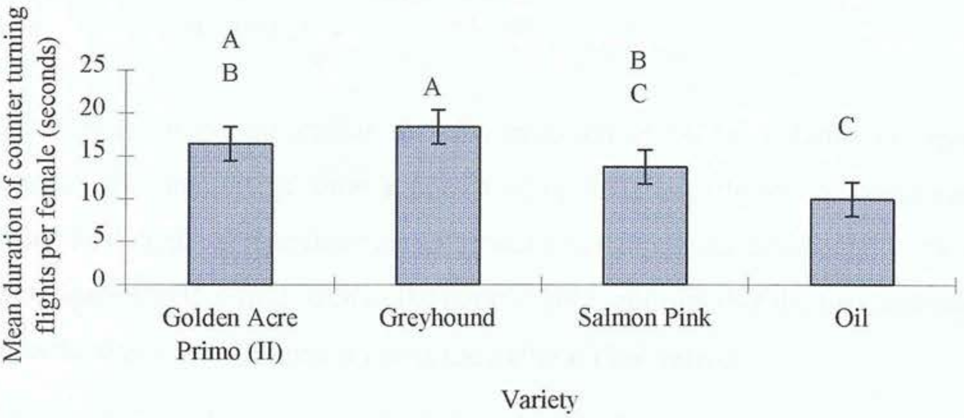


Figure 4.11. Effect of the three volatile extracts on the duration of counter-turning flights exhibited by *Pieris rapae* females (seconds). Error bars show the standard deviation and columns with different letters are significantly different from each other. N=15



The summary from this Glim analysis is shown in Table 4.5. The volatile extracts tested did not exhibit a significant effect on the number of counter-turning flights made by the females (P>0.05; Table 4.5) Figure 4.10. However, a significant effect on the duration of counter-turning flights was recorded (P<0.05, Table 4.5). The volatile extracts from the two cabbage varieties resulted in the longest time spent in counter-turning flights by the females (Figure 5.11).

Table 4.5. Summary of GLIM analysis of the effect of the three volatile extracts on the flight behaviour of *Pieris rapae* females.

General Linear Model

Factor	Levels	Values
Treatment	4	Oil; Golden Acre Primo (II); Greyhound; Nasturtium

Analysis of Variance for number of counter-turning flights (data transformed Squareroot (data + 0.5)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	3	1.7093	1.7093	0.5698	1.06	0.375
Error	56	30.1829	30.1829	0.5390		
Total	59	31.8922				

Analysis of Variance for duration of counter-turning flights (data transformed Squareroot (data + 0.5)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	3	593.95	593.95	197.98	3.34	0.026
Error	56	3324.34	3324.34	59.36		
Total	59	3918.29				

In summary, it would appear that the main effect of the volatile extracts was to increase the amount of time spent in counter-turning flights as compared to the mineral oil control. The differences between the three volatile extracts were not large as compared to the difference to the control but it appears that the two cabbage variety extracts were more stimulatory than the Salmon Pink extract.

Effect of volatiles on pre-alighting discrimination

Choice of volatile and oil

The data from this assay were tested for normality using a Ryan-joiner test as previously described, the results of which indicate that the data from all three volatile extracts followed a normal distribution (W-test for normality Golden Acre Primo (II) R:0.98; P>0.05; Greyhound R:0.97, P>0.05; Salmon Pink R:0.97, P>0.05). The results of this assay are shown in Figure 5.12., 5.13. & 5.14. There was a significant increase in the number of landings made on the model plants treated with volatiles as compared to mineral oil for all three of the volatile extracts (Table 4.6.).

Figure 4.12. Mean number of landings per trial made by 10 Female *Pieris rapae* on model plants treated with greyhound volatile extract or mineral oil in a choice test. Error bars give standard deviation columns with different letters are significantly different from each other. N=3

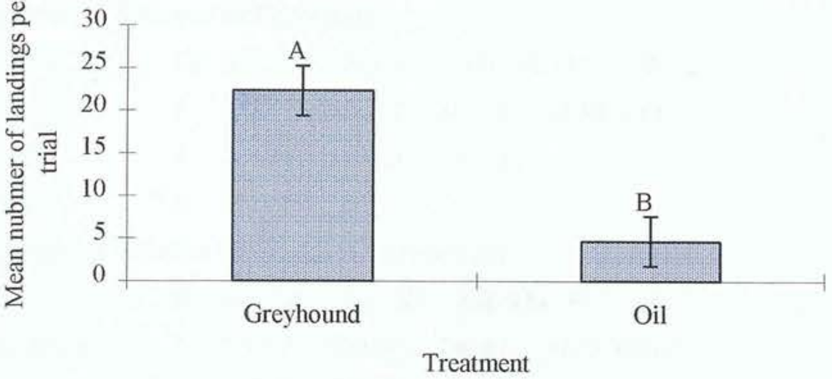


Figure 4.13. Mean number of landings per trial made by 10 Female *Pieris rapae* on model plants treated with Golden Acre Primo (II) volatile extract or mineral oil in a choice test. Error bars give standard deviation columns with different letters are significantly different from each other. N=3

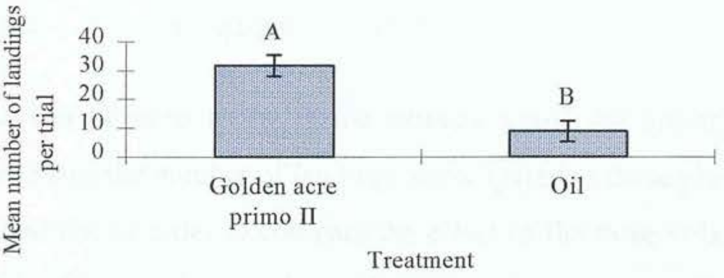


Figure 4.14. Mean number of landings per trial made by 10 Female *Pieris rapae* on model plants treated with Salmon Pink volatile extract or mineral oil in a choice test. Error bars give standard deviation columns with different letters are significantly different from each other. N=3

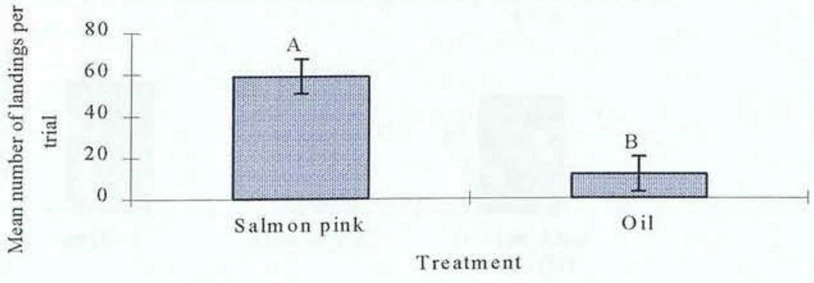


Table 5.4 Summary of Glim analysis for the choice of volatile extract or mineral oil for each of the three volatile extracts.

General Linear Model

Factor	Levels	Values
Treatment	2	Volatile Oil

Analysis of Variance for Greyhound

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	468.17	468.17	468.17	18.12	0.013
Error	4	103.33	103.33	25.83		
Total	5	571.50				

Analysis of Variance for Golden Acre Primo (II)

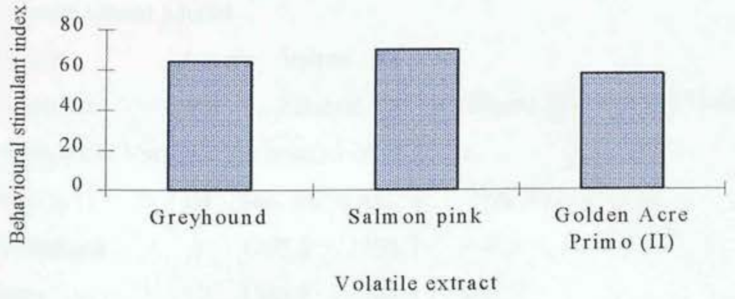
Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	770.67	770.67	770.67	19.43	0.012
Error	4	158.67	158.67	39.67		
Total	5	929.33				

Analysis of Variance for Salmon Pink

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	1	3408.2	3408.2	3408.2	15.84	0.016
Error	4	860.7	860.7	215.2		
Total	5	4268.8				

As can be seen for all three extracts tested the presence of volatiles on a plant increased the number of landings as compared to those plants treated with mineral oil. However, in order to compare the effect of the three volatile extracts on the number of landings made an index of behavioural stimulation (BSI) was calculated for each volatile extract (using means presented in Figures 4.12., 4.13. and 4.14.; as described in Chapter Three). The results of these calculations are shown in Figure 4.15.

Figure 4.15. Mean behavioural stimulation index for each of the three volatile extracts when *Pieris rapae* females were offered a choice between each volatile extract separately or mineral oil.



As can be seen when each volatile was offered separately with mineral oil, the volatile extracts were all equally preferred in comparison to the mineral oil.

Choice between the three volatile extracts and oil

The data from this assay were tested for normality using a Ryan-joiner test as previously described, the results of which indicate that the data followed a normal distribution, W-test for normality (R:0.96, P>0.05). The results of this assay are shown in Figure 4.16. When females were offered a choice between the three volatiles and mineral oil simultaneously there was a significant increase in the frequency of landings on the volatile extract treatments as compared to the oil control (P<0.05; Table 4.7.).

Figure 4.16. Mean number of landings per trial made on each of the model plants treated with volatile extracts or mineral oil in a choice test by *Pieris rapae* females. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=4

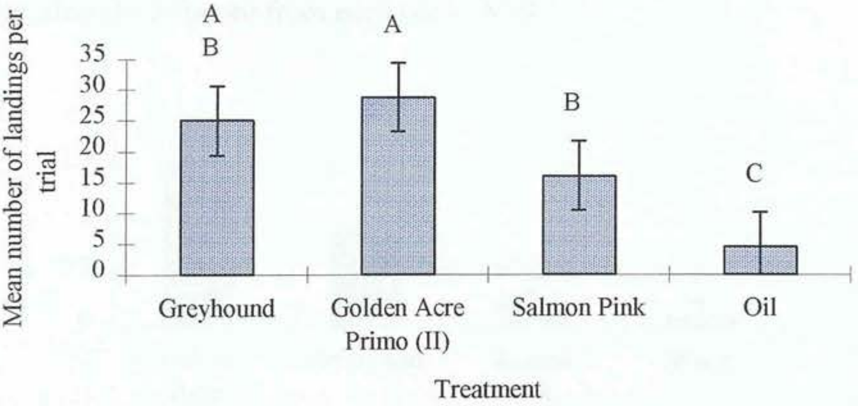


Table 4.7. Summary of Glim analysis for the number of landings made on model plants treated with three different volatile extracts or mineral oil in a choice test by *Pieris rapae* females.

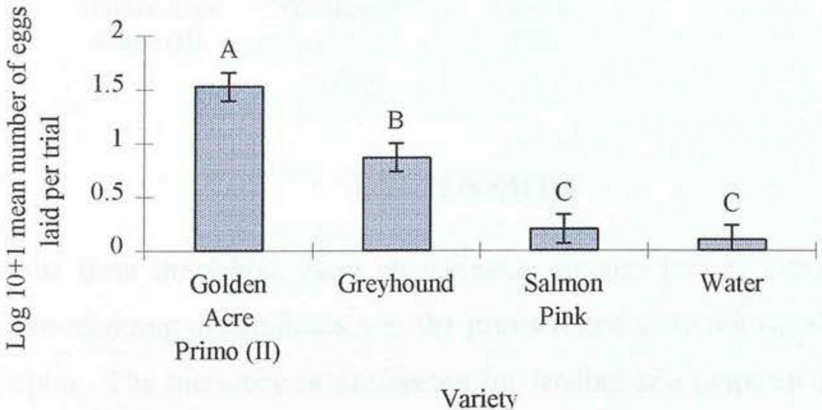
```
General Linear Model
Factor      Levels  Values
Treatment    4      Mineral Oil  Greyhound Salmon Pink Golden Acre Primo (II)
Analysis of Variance for number of landings
Source      DF  Seq. SS   Adj. SS   Adj. MS  F     P
Treatment    3  1398.2    1398.2    466.1    3.71  0.042
Error       12  1505.8    1505.8    125.5
Total       15  2903.9
```

As can be seen, for all three extracts tested the presence of volatiles on a plant increased the number of landings as compared to those plants treated with mineral oil.

Effect of contact stimulants on host plant choice

The data from this assay were tested for normality prior to analysis as described previously. The data were found not to be normally distributed W-test for normality (R:0.7803; $P<0.01$). Prior to analysis the data was subjected to a Log_{10} (data+1) transformation before the GLIM analysis was carried out. This transformation resulted in a normal distribution of the data (R0.986; $P>0.05$). The data were then analysed using the GLIM command Minitab for Windows (Release 11.1) with the leaf surface extract as a factor. A summary of this analysis is shown in Table 4.8.

Figure 4.17. Mean Log10 (data+1) number of eggs laid by *Pieris rapae* on bean plants treated with either a leaf surface extract from each of the three host plant varieties or distilled water. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=3



As can be seen (Figure 4.17.), significant differences were found between the number of eggs laid on each of the treatments ($P<0.001$). Most eggs were laid on Golden Acre Primo (II) followed by Greyhound with Salmon Pink not being significantly different from the water control (Figure 4.17.). Oviposition stimulant indexes were calculated as described in Huang & Renwick (1993) and these are shown in Figure 4.18. Clearly the two cabbage extracts were more stimulatory as compared to the Salmon Pink extract.

Table 4.8. Summary of GLIM analysis for the number of eggs laid by *Pieris rapae* females on bean plants treated with the leaf surface extracts or distilled water.

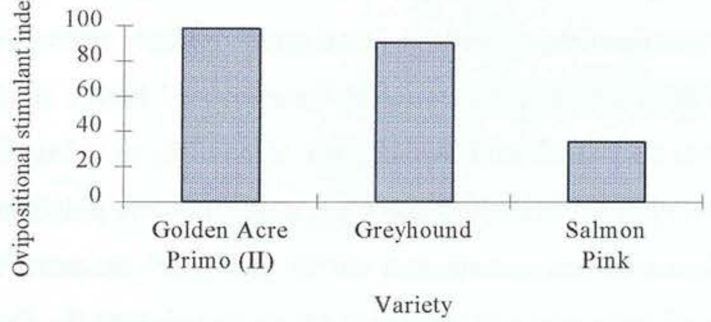
General Linear Model

Factor	Levels	Values
Treatment	4	Golden Acre Primo (II) Greyhound Salmon Pink Water

Analysis of Variance for Log_{10}(data+1) number of eggs laid.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Treatment	3	3.9532	3.9532	1.3177	24.74	0.001
Error	8	0.4261	0.4261	0.0533		
Total	11	4.3793				

Figure 4.18. Ovipositional stimulant index for each of the three leaf surface extracts tested.



DISCUSSION

The results from the whole plant choice assay suggest that *P. rapae* females did exhibit pre-alighting discrimination as the plants tested were not equally likely to be landed upon. The hierarchy of preference for landing and oviposition were similar i.e. Golden Acre Primo (II) received the most landing and eggs; greyhound was intermediate and nasturtium was the least preferred for landing and oviposition. However, when the number of eggs per landing was calculated this pattern changed and on this basis, Greyhound received the most eggs. Therefore, these results indicate that as well as exhibiting pre-alighting discrimination of potential host plants, *P. rapae* females also exhibit post-alighting discrimination, as once landed, a female was not equally likely to oviposit on each of the host plant varieties tested.

The pre-alighting discrimination of host plants by butterflies is a common occurrence and has been documented in a number of species including; *Euphydryas editha* (MacKay, 1985; Rausher *et al.*, 1981); *Colias p. eriphyle* (Stanton, 1982; 1984) and *P. rapae* (Ives 1978). In addition, to the pre-alighting discrimination exhibited by *E. editha*, Rausher *et al.* (1981) argued that a female also actively discriminated between plants after alighting and did not oviposit on any plant when motivation to oviposit crossed some single threshold.

The demonstration of pre-alighting discrimination is fairly straightforward in comparison to showing which factors are responsible for the observed pattern of landing. Rausher *et al.* (1981) argued that the differences in the number of landings made on plants which differ in size may be due to either differences in the apparency[7] of the plants (Feeny, 1976) or a preference for larger plants (Rausher *et al.*, 1981). However, distinguishing between these experimentally would be extremely difficult as it would be necessary to know what a butterfly does and does not perceive (Rausher *et al.*, 1981). By analogy, therefore, the exact cues which lead to the pre-alighting discrimination observed can never be fully elucidated. In the rest of this Discussion the results of the experiments are discussed in terms of how they may provide information on the suitability of a host plant for oviposition.

The spectral profiles of the three hosts clearly show that the Golden Acre Primo (II) reflected the highest number of quanta. The results of Chapter Three, indicate that the higher the reflected number of quanta the more attractive a substrate is for landing to *P. rapae* females within a suitable wavelength band. Similarly, Myers (1985) showed that fertilised cabbage plants receive significantly more eggs than unfertilised plants, but the application of fertiliser affects several physiological parameters of the plant. A significant correlation between % nitrogen of the foliage and the colour of the plant was found, plants receiving more fertiliser were greener (as measured by the ratio of red to infra red reflectance). The greenness of the plants was also significantly correlated with the number of eggs a plant received. There was also a

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[7] Apparency is defined by Feeny (1976) as "visible, plainly seen, conspicuous, palpable, obvious". Therefore, the vulnerability of a plant to its herbivores may then be referred to as its apparency, which is used to denote its susceptibility to discovery.

consistent trend for fertilised plants to have higher transpiration rates. Higher transpiration rates also tended to increase the number of eggs a plant received. Langan Dunleavy & Wheater (1996) have shown that the number of eggs laid by *P. rapae* females is significantly correlated with several physiological parameters of the host plant including photosynthetic rate, rate of transpiration and stomatal conductivity. It is likely that these correlations are all measuring the physiological activity of a host plant, especially its photosynthetic rate which is obviously related to the rate of transpiration which in turn is related to the stomatal conductivity. This occurs because the observed photosynthetic rate of a plant is related to gas exchange via the plants stomata.

Between 50 per cent (Jensen & Bahr, 1977) and 65 per cent (Ellis, 1979) of the total soluble protein in a leaf is in the form of Ribulose 1,5-bisphosphate carboxylase oxygenase (the enzyme responsible for carbon dioxide fixation in photosynthesis), assuming that leaf nitrogen concentration is positively related to the concentration of this enzyme, the photosynthetic ability of a plant is clearly related to its nutrient status. Therefore, it may be that although the primary cue used to assess a plant is colour, this is a proxy for the underlying physiological "health" of a host plant or its suitability for larval growth, particularly its nitrogen content.

Similarly, Jansson *et al.* (1991) reported that the abundance of lepidopterous pests on crucifers was significantly and positively correlated with the rate of nitrogen applied to the plant as fertiliser. In a study of the effect of the application of nitrogen fertiliser on oviposition by *P. rapae* using potted kale, the mean number of eggs laid was higher on fertilised plants by two orders of magnitude reflecting differences in fertiliser treatments; leaf nitrogen concentration explained over 90 % of the variation in oviposition rate. However, the effect of nitrogen on oviposition rate was not repeated when a larger scale experimental design was employed (Letourneau & Fox, 1989).

The importance of nitrogen to *P. rapae* is related to the caterpillar, in an elaborate study of this insect's feeding habitats (Slansky & Feeny, 1977) found no significant correlation between the nitrogen content of the plant and the larval growth rate. However, as plant nitrogen content increased the consumption rate of the larvae declined and the efficiency of conversion of ingested food increased. The increase in the efficiency of conversion of ingested food was due to largely to the assimilation efficiency which increased as less food was ingested (Slansky & Feeny, 1977). Overall, it appeared that the larvae actively stabilised their accumulation of nitrogen into larval biomass and the nitrogen accumulation rate is homeostatically maintained via changes in the consumption rate of the food offered (Slansky & Feeny, 1977).

Loader & Damman (1991) have shown that when *P. rapae* caterpillars are fed on low nitrogen hosts they spend a greater time feeding and develop more slowly than did those on higher nitrogen plants. This increase in the amount of time spent feeding may increase the rate of predation on the caterpillars. The importance of growth to *P. rapae* larvae lies in the fact that a longer period of time spent as a larva maybe commensurate with a higher risk of mortality particularly from parasitoids and predators.

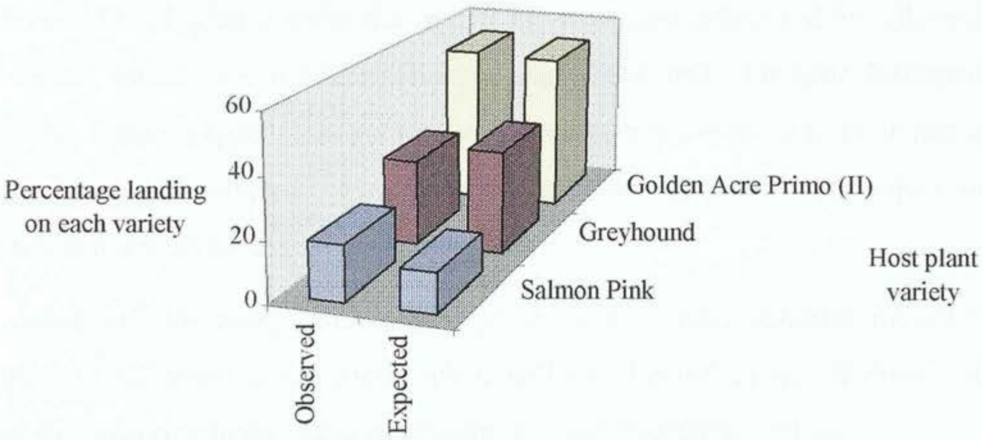
However, irrespective of what the female is using as a cue, the colour of the plant is considered to be important. To investigate whether colour alone was responsible for the observed number of landings in the whole plant choice assay, data from Chapter Three was used to build a regression model for the number of landings made on each colour of card in the presence of volatile and contact stimulant extracts. The regression was found to be statistically significant ($P<0.001$; R-sq. 0.72; ANOVA df =1,11, F-Value 25.2 (Table 4.9.). The regression equation was: the number of landings = 13.49 + 0.727 x Reflectance at 550 nm. This regression was used as the plants in the experiments described in this Chapter contained both volatile and contact stimulants which the data in Chapter Three shows to be important in the visual behaviour of *P. rapae*.

Table 4.9. The result of a regression analysis on the total number of landings and the reflectance at 550 nm for 12 colours of cards (Data from Chapter Three).

ANOVA	df	SS	MS	F	P
Regression	1	4770.082	4770.082	25.15974	0.001
Residual	10	1895.918	189.5918		
Total	11	6666			

The expected number of landings calculated, from the regression equation above using the reflectance at 550 nm from the three host plant varieties (expected values in Figure 4.19.), were converted to percentages of the total number of landings and compared to the percentage of the total number of landings obtained in the whole plant assay (observed values in Figure 4.19.). The results of these calculations are shown in Figure 4.19.

Figure 4.19. The observed and expected (as determined by the regression equation between reflectance at 550 nm) percentage of the total number of landings made by female *Pieris rapae* in the experiments described in the whole plant choice experiments of this Chapter.



For all three host plants varieties there is a very good agreement between the observed and expected values. This indicates that the colour of a plant, more specifically, its reflectance is the most important factor in determining the number of landings made and thereby is responsible for the observed pattern of pre-alighting discrimination of host plants by *P. rapae*. However, there are a number of weaknesses of this analysis; the most obvious being that the data used in determining the regression equation was derived from a study in which model plants were used and not real plants. I would suggest therefore, that the conclusion that colour alone, in particular the reflectance at 500 nm, is solely responsible for the pre-alighting discrimination exhibited by *P. rapae* should be treated with caution.

The results of the assay investigating the flight behaviour of *P. rapae* in the presence of host plant volatile extracts showed that there was no significant differences in the number of flights made in which counter-turning was exhibited between the four treatments (three volatile extracts and the solvent control). However, the duration of counter-turning flights was significantly different. Therefore, it may be assumed that the initiation of such flights is predominately under ideothetic control. However, a possibility which cannot be excluded is that the flights were initiated in response to plant volatiles carried into the wind tunnel with the air stream. As the wind tunnel used was very large (volume of experimental chamber alone 3.5 m^3) it was not practical to purify air entering the wind tunnel and such air can be expected to be permeated with a blend of volatile odours from many plants. However, as the duration of such flights showed significant differences then we may assume that the duration of such flights is under the control of, or at least influenced by, alleleothetic information sources (or a combination of allelethetic and idoethetic information). Therefore, it would appear that volatile extracts may be considered to be important in the initiation of searching behaviour, even if they do not actively participate in the orientation phase of the search for a host plant.

The results of the assays with model plants treated with volatiles indicate that volatiles do influence which model plant will be alighted upon, all three volatile treatments receiving higher rates of landing than the respective control.

Therefore, volatiles would appear to have an effect in pre-alighting discrimination; however, since there was no evidence that the source of volatiles affected the number of landings in the no-choice test the volatile extracts may be thought of as acting more as a cue for host plant recognition rather than as a cue for host plant discrimination. This distinction is important and may allow the avoidance of non-host plants whilst engaging in pre-alighting search.

In summary, I believe that I have demonstrated that volatile odour cues are potentially involved in the process of finding a host plant and in the pre-alighting discrimination of plants by *P. rapae*. However, as in two of the three experiments the cabbage variety Greyhound was more "attractive" than Golden Acre Primo (II), host plant volatiles may not be directly involved in the pre-alighting discrimination of a potential host plant by an ovipositing female. It should be borne in mind that all of these tests concerned only one sensory modality: olfaction, and demonstrating a potential effect of olfaction in a laboratory is not the same as demonstrating that olfaction is an important sensory modality in the pattern of utilisation of host plants by *P. rapae* in the field.

The results of this assay clearly show that the three plants contained differing amounts of ovipositional stimulants. The most stimulatory plant was Golden Acre Primo (II), Greyhound was intermediate and the least stimulatory plant was Nasturtium, which was not significantly different from the control.

When the data from the whole plant choice assay was expressed in terms of the number of eggs per landing (Figure 4.8.), then the most acceptable plant was Greyhound. This difference may be due to an additional factor being involved in post-alighting discrimination. Additionally, the assays conducted did not take into account the role of ovipositional deterrents, which are known to be important in mediating oviposition (Huang & Renwick, 1993; Huang *et al.*, 1995). However, as the pattern of oviposition, in terms of number of eggs laid, followed the OSI indexes it may be concluded that the most important factor in mediating oviposition in *P. rapae* are the contact stimulants present in each plant. However, as well as containing ovipositional stimulants Nasturtium is known to contain ovipositional deterrents (Huang & Renwick, 1993).

These results are in general agreement with those of Giamoustaris & Mithen (1995) who tested 28 lines of oilseed rape plants (*Brassica napus*) with varying aliphatic glucosinolate concentrations. The lines were tested in field experiments to investigate how the glucosinolates present mediated the interaction of herbivores with the plants. They found that the presence of *P. rapae* larvae showed a statistically significant positive correlation with the concentration of total leaf glucosinolates.

CONCLUSIONS

These experiments have clearly shown that *P. rapae* females exhibited both pre-and post alighting discrimination of the plants tested. The sensory cues were evaluated as possible elicitors of this discrimination, the results for pre-alighting discrimination would suggest that it is possible that both visual and olfactory cues are involved in pre-alighting discrimination. However, the relative contribution of both of these two sensory modalities and the effects of interactions between them are unknown. However, the results of previous studies would suggest that visual cues are dominant, particularly colour; with volatile odours acting as host recognition cues; that is, they define a plant as a potential host. Similarly, the post alighting discrimination of the plants would appear to be due to the relative degree of stimulation from these plants, the contribution of deterrents to this process was not quantified but have been shown to be important in mediating host plant selection.

Chapter Five
The effect of prior experience on the ovipositional behaviours of *Pieris rapae*.

INTRODUCTION

Insect behaviour has previously been considered to be determined by genetic programmes, leaving little room for plasticity or any role of learning (Szentesi & Jermy, 1991). However, more recently learning in phytophagous insects has received much attention and has been shown to occur in a variety of insect orders (reviews in Papaj & Prokopy, 1989; Papaj & Rausher, 1983; Szentesi & Jermy, 1991). In the Lepidoptera, studies of the effects of learning fall into two broad categories, namely, with respect to nectar resources (Lewis, 1986; Lewis, 1993; Lewis & Lapani, 1991; Swihart & Swihart, 1970; Weiss, 1995) or ovipositional resources (Papaj 1986; Parmesan, Singer & Harris, 1995; Rausher, 1978; Stanton, 1984; Traynier, 1979). Studies on learned ovipositional preference can be classified as either sensitisation or associative learning. The adult evaluates a resource and then associates visual cues with chemical cues (Szentesi & Jermy, 1991).

The effect of learning has been studied under field conditions in butterfly species including *Battus philenor* (pipevine swallowtail) (Rausher, 1978) and *Colias p. eriphyle* (Stanton, 1984). In a study of *B. philenor* searching behaviour, there were two species, *Aristolochia reticulata* and *A. serpentaria* (Aristolochiaceae), of host plant in the herbaceous vegetation. These two hosts differ in leaf shape: *A. reticulata* has broad ovate leaves whereas *A. serpentaria* has long narrow parallel-sided leaves (Rausher, 1978). It was observed that *B. philenor* females appeared to be highly selective in their response to leaf shape, with females searching and alighting preferentially on either narrow-leaved or broad-leaved hosts but not both concomitantly. Females did not change their search mode during a 30 minute observation period. Females alighting and ovipositing on a host plant with a leaf shape different to that being searched for then adopted that leaf shape as their 'search mode' (Rausher, 1978).

In addition, *B. philenor* females responded preferentially to a particular leaf shape during search and had a higher rate of encounter with that shape. Females with a stronger preference for a particular leaf shape discovered a greater proportion of host plants with that leaf shape than did females with a weaker preference (Rausher, 1978).

The conditioning of *B. philenor* to leaf shape through association with host plant phytochemical cues in laboratory studies was investigated by Papaj (1986). It was found that the host leaf shape to which a gravid female was exposed had a marked effect on the leaf shapes on which she alighted. These differences in alighting preference were significant and contact only was necessary to train the butterflies.

The training of *B. philenor* to leaf shape was easily reversed when a female exposed to host plants of a particular leaf shape was exposed to host plants with a different leaf shape. With a single exception, all females exposed first to one host plant species adopted a search mode for the second host plant to which they were exposed. The association between the final search mode and re-training of the insect to the host plant is significant (Papaj, 1986). The results of Papaj (1986) and Rausher (1978) would seem to show that *B. philenor* can learn to search preferentially for a particular leaf shape by conditioning of perception, defined by Papaj & Rausher (1983) "as any effect of experience that improves the probability of detecting a stimulus".

Stanton (1984) has studied the foraging behaviour of three *Colias* spp. (Pieridae), namely, *C. p. eriphyle*, *C. meadii* and *C. alexandra*. She found that the activity of females of all three species was split into two well defined phases of oviposition search and nectar search. For females of all three species, a host plant landing was more likely to be followed by another host plant landing than by a nectar landing. Similarly, nectar landings were predominantly followed by visits to flowers rather than to host plants. This segregation of activity had been noted previously in butterflies by Jones (1977) for *P. rapae*, and in that case was attributed to the segregation of *P. rapae's* habitat into areas with plants for oviposition and different areas with plants for nectaring. In the study area utilised by Stanton (1984), there was considerable overlap between the distribution of these two resources.

The proportion of alightings on host as opposed to non-host plant foliage in an oviposition bout of at least 15 uninterrupted foliage alightings was compared. It was found that females of all three *Colias* spp. showed a significant decrease in the number of error alightings (non-host foliage) in the first half of the same oviposition bout as compared to the second half, implying that short-term learning can modify the searching behaviour of these species under field conditions and that experience of hosts leads to an increase in the accuracy of a female's searching behaviour. Similarly, the proportion of error alightings made immediately after flower and legume visits showed females made significantly more error alightings when switching from nectaring to oviposition than those in the middle of an oviposition bout (Stanton, 1984).

Therefore, if the increased accuracy in alighting seen at the end of *Colias* spp. oviposition bouts results from conditioning of perception then females making fewer error alightings should also perceive a smaller set of legume species. In an analysis of the behaviour of *C. p. eriphyle*, three insect behaviour/host plant interactions affecting the relationship between alighting diversity and alighting accuracy were identified. These were: the proportion of alightings on host plants made by a female was significantly positively correlated with the abundance of legumes along her flight path, mean host abundance sampled during a flight sequence is significantly negatively related to legume species diversity, and the diversity of legume species visited by a female showed a significant correlation with the diversity of species growing in her flight path (Stanton, 1984).

When host plant abundance was held constant, females landing on a narrow range of legumes made fewer errors than those visiting a broad range of species. Although these results do not show that this decreased landing diversity resulted from a narrowing of perception rather than preference, the patterns are very reminiscent of experimental studies on search image formation in other animals (Stanton, 1984).

Such field studies have not been carried out using *P. rapae*, yet associative learning has been shown to occur. Indeed, Lewis (1986) has shown that *P. rapae* uses previous experience to increase its efficiency when nectar feeding and it would not be particularly surprising to find *P. rapae* using some form of learning to locate host plants for oviposition. For *P. rapae* laboratory studies have demonstrated that associative learning of colour with host plant phytochemicals does occur (Traynier, 1984; 1986; 1987 and Traynier & Truscott, 1991). However, there appears to be differences between the learned stimuli. *P. rapae* has been shown to associate colour with host plant phytochemistry but an association with leaf shape or size has not been demonstrated (Traynier, 1987), unlike *Battus philenor*. Furthermore, the learning response does not occur with respect to negative stimuli in the form of non-host phytochemicals (Traynier, 1987). These results contrast with studies involving *B. philenor* which found that the butterfly learnt to associate leaf shape with host plant phytochemistry (Allard & Papaj, 1996; Papaj, 1986; Rausher, 1978) while Stanton (1984) did not attempt to investigate the sensory information on which the apparent learning was based.

As noted previously leaf shape is not considered an important cue in the host plant finding behaviours of *P. rapae* (Renwick & Radke, 1988); colour is deemed to be important to *P. rapae*. Judd & Borden (1991) suggest that, in general, the most important cue in host plant finding by phytophagous insects is leaf shape, they note two exceptions to this generalisation; namely, *Hylapterous pruni* (an aphid) (Moericke, 1969) and *Delia Radicum* (Finch *et al.*, 1983). Therefore, we may assume that in general terms the shape of a brassica plant is less important than its colour which may be considered to be more distinctive than is normally the case.

Gould (1993) has suggested that a description of classical conditioning (in behavioural psychology terms) is as follows "an unconditioned stimulus (US) triggers an unconditional response (UR); pairing of the US with a conditioning stimulus (CS) leads to an association between the two, and, eventually, the ability of the CS to trigger the UR". The relationship can be expressed as:

US →UR; CS + UR → UR; then CS → UR. (Gould, 1993).

The terms used in this description may not be familiar and to state them in ethological terms can be readily achieved. In this notation "unconditioned becomes innate", the US is a sign stimulus (which is a key, innately recognised feature of an object that helps to trigger a response), the UR is a is a fixed reaction pattern; the CS is a learned gestalt (the collection of cues an animal learns to use in identifying a meaningful stimulus) (Gould, 1993).

From the studies of Traynier (1984; 1986), in terms of *P. rapae's* learning in oviposition behaviour the US is the glucosinolates which elicit oviposition, the UR is oviposition itself and the CS is the learning of colour on which the glucosinolates were placed. This may be represented diagramitically as:

> US (glucosinolates) → UR (oviposition) → CS (colour) + US (glucosinolates) → oviposition; then colour → oviposition.

In this chapter, two sets of experiments are reported. In the first (section A), preliminary studies were carried out in which the effect of prior experience on the subsequent ovipositional behaviour of *P. rapae* was quantified in terms of oviposition. In section B a more detailed analysis of the behavioural decisions prior to oviposition was made in order to investigate which aspects of the pre-ovipositional behaviour in *P. rapae* engages in were affected by the prior experience.

The rationale behind this approach is briefly outlined. Sequential analyses offer advantages in so far as important information can be retrieved from behavioural sequences of individual insects, and standard statistical techniques can be applied to such sequences to provide answers to ethological or ecological questions (Hopkins *et al.*, 1996). Therefore, the aim of this study was to investigate the behavioural sequence of oviposition displayed by *P. rapae* and to compare sequences from butterflies with differing prior experience in order to elucidate more clearly how associative learning affects ovipositional decision making by analysing sequences of behaviour.

The behavioural sequence of oviposition displayed by *P. rapae* has been described. The process by which a female *P. rapae* searches, locates and accepts a host plant is a catenary chain (Courtney; 1986a, Kennedy, 1965, Renwick & Huang, 1994), stimuli evoke responses which lead on to subsequent cues in the chain. A chain of behaviour prior to oviposition by *P. rapae* occurs. The approach flight is hardly discernible from normal flight behaviour. During drumming there is an alternate tapping of the fore-legs on the leaf surface. Curving of the abdomen often begins during drumming and both are usually accompanied by wing fluttering. The touching of the leaf surface with the extended ovipositor occurs only after drumming and wing fluttering have stopped (Courtney, 1986a; Klijnstra, 1985). Therefore, the behaviours which I have chosen to study in more detail are the number of landings made by females, the number of times females drum on the leaf surface with their fore legs, the number of flutter bouts and the number of times females engage in curving of their abdomens and the number of eggs laid.

SECTION A

METHODS

Butterflies

Captive bred *P. rapae* were housed at 22 ^{O}C with a light-dark photoperiod of 18:6 respectively. Adults had constant access to a solution of 20 per cent (w/v) sucrose and to host plants of each variety, for training, for oviposition. Larvae were reared on Spring Cabbage var. April. Upon pupation pupae were removed from the rearing cage and placed into a clean cage. Upon emergence both males and females were transferred into a cage containing the appropriate host plant (either Golden Acre Primo (II) or Greyhound or Nasturtium) for training[8]. Experiments were conducted when females were five days old.

The plants used in this experiment were Nasturtium, var. Salmon Pink; Spring Cabbage vars. Golden Acre Primo (II) and Greyhound.

Bioassay

Five Females were released into a cage (98 x 50 x 50 cm), containing Greyhound, Golden Acre Primo (II) and Salmon Pink. The position of the plants within the cage was randomised, using random number tables. The plants were spaced evenly in a row along the centre of the cage. After one hour the number of eggs laid on each host plant type were recorded. The assay was conducted in a controlled environment room

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[8] Training in these experiments was simply allowing the females to oviposit on the host plant offered. The training period lasted five days, from the emergence of the butterfly until immediately prior to the experiments taking place.

at 22 ° C and ambient relative humidity. Three trials of this experiment were carried out for each post plant variety to which the females were trained.

RESULTS

Effect of prior experience on host plant selection

The data from these experiments were analysed using the GLIM command in Minitab for windows (release 11.1) with the variety of plant the butterflies had prior experience of and the variety on which eggs laid were used as factors, interactions between these factors were also used in the model. Prior to analysis with the Glim command the data was tested for normality using a Ryan-Joiner test and was found to be normally distributed (W-test for normality R: 0.97; $P>0.05$). A summary of the analysis is given in Table 5.1.

As can be seen from Figure 5.1. and Table 5.1., the prior experience itself did not alter the number of eggs laid by the females in the subsequent experimental assays (F-value 0.97; DF 2,26 $P>0.05$). However, the host plant variety in the assays did show significant differences in the number of eggs laid on them (F-value 12.98; DF 2,26, $P<0.001$) (Table 5.1.). As can be seen from Figure 5.2., the two cabbage varieties received significantly more eggs than the nasturtium plants.

Additionally, there was a significant interaction between the number of eggs laid on each host plant variety and the prior experience which those butterflies had received (F-value 4.19; DF 3,26 $P<0.05$ Table 6.1). Fig 5.3 a., b. & c. illustrates the effect of the interaction between the number of eggs laid on each host plant variety and the prior experience host that the females were given access to.

As can be seen butterflies with prior experience of Golden Acre Primo (II) laid significantly more eggs on this variety than either Greyhound or Nasturtium.

Figure 5.1. The effect of the prior experience host variety on the mean number of eggs laid per trial by *P. rapae* females. (Mean of the three host plant varieties, five females per trial). Error bars indicate standard deviation of the mean. N=9

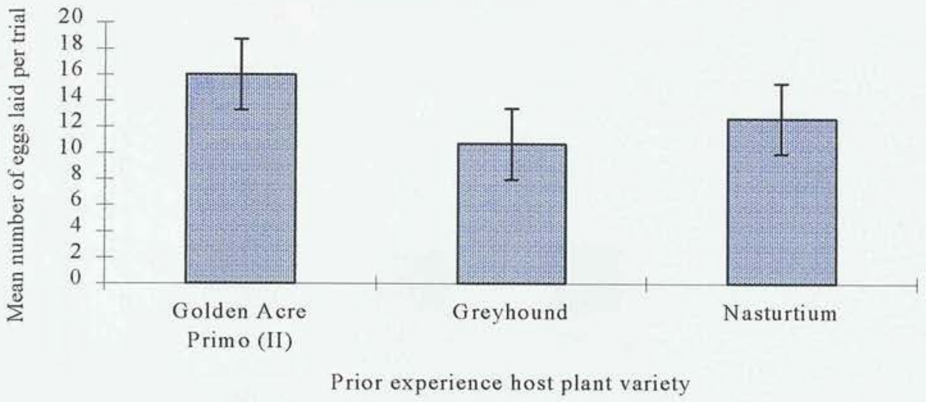


Figure 5.2. Mean number of eggs laid on each host variety by *P. rapae* females, mean of the three prior experience host plant varieties. Error bars indicate the standard deviation. Columns with different letters are significantly different from one another. N=9

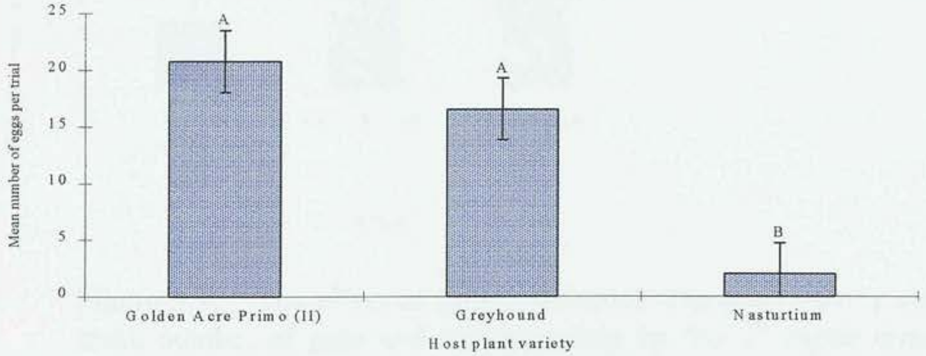


Figure 5.3a. The effect of prior experience host plant variety on the mean number of eggs laid on Golden Acre Primo (II) by five *P. rapae* females. Error bars indicate standard deviation. Columns with different letters are significantly different from one another. N=3

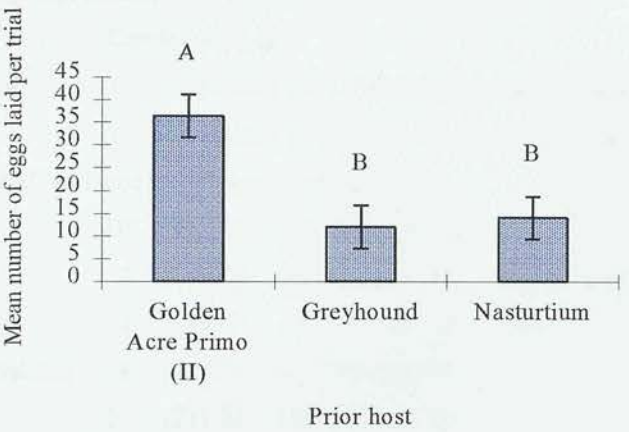


Figure 5.3b. The effect of prior experience host plant variety on the mean number of eggs laid on Greyhound by five *P. rapae* females. Error bars indicate standard deviation. N=3

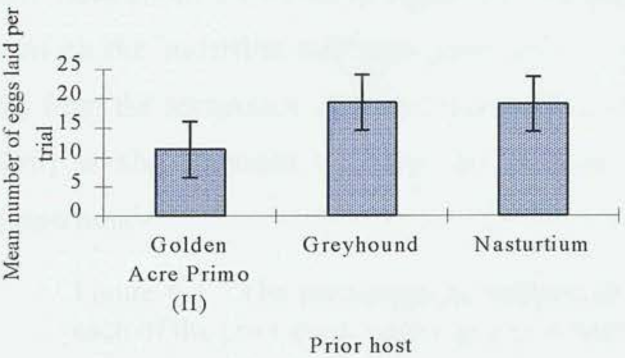


Figure 5.3c. The effect of prior experience host plant variety on the mean number of eggs laid on Nasturtium by five *P. rapae* females. Error bars indicate standard deviation. N=3

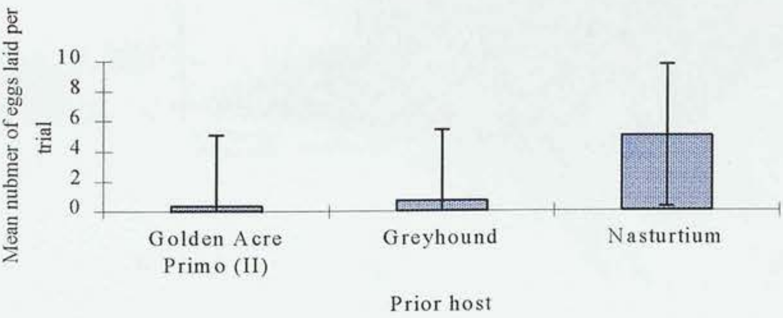


Table 5.1. Summary of the Glim analysis for the number of eggs laid by *Pieris rapae* females on each type of host plant and the effect of the female's prior ovipositional experience.

General Linear Model

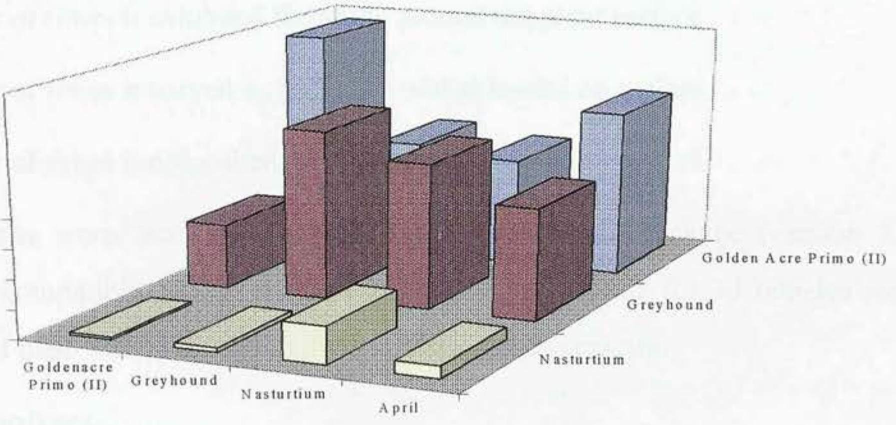
Factor	Levels	Values
Prior host	3	Golden Acre Primo (II) Greyhound Nasturtium
Plant	3	Golden Acre Primo (II) Greyhound Nasturtium

Analysis of Variance for the number of eggs laid

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Plant	2	1746.89	1746.89	873.44	12.98	0.001
Training	2	130.67	130.67	65.33	0.97	0.398
Plant*Training	4	1127.78	1127.78	281.94	4.19	0.014
Error	18	1211.33	1211.33	67.30		
Total	26	4216.67				

The percentage acceptance of each host plant variety was calculated[9]. The results of these calculations are shown in Figure 5.4. As can be seen on average, the host plant to which the butterflies had been given prior experience received more eggs. The data from the acceptance of these hosts in Chapter Four (prior experience host plant April) is also included to show the percentage acceptance of these hosts for comparison.

Figure 6.4. The percentage acceptance of each host plant variety for each of the prior experience regimes to which *P. rapae* was exposed.



SECTION B

METHODS

Butterflies

See Section A, except that butterflies were not trained to Nasturtium which was not sued in these experiments.

Plants

The plants used in this experiment were cabbage vars. Golden Acre Primo (II) and Greyhound. Plants were grown in a green house with additional lighting (18 hours light 6 hours dark) and were 12 weeks old when the experiments were carried out.

Bioassay

A cage (98 x 50 x 50 cm), containing Greyhound and Golden Acre Primo (II) was set up. Lighting was provided by two full spectrum fluorescent tubes to mimic natural daylight, these were suspended above the cage. The position of the plants within the cage was randomised. A female was released into the cage and its behaviour observed and was recorded for 20 minutes. The behaviours recorded were:

- The number of times it landed on a plant.
- The number of times it drummed on the leaf surface.
- The number of times it exhibited fluttering around the plant surface
- The number of times it curved its abdomen whilst landed on a plant
- The number of times it oviposited on a plant.

These behaviours were recorded using the observer software package (version 3, using an IBM compatible computer). Measurements were made for 20 females for each of the host plant varieties used in the prior experience regimes.

Statistical Analyses

The data collected was analysed using the Glim command with Minitab for Windows (Release 11.2), with the factors prior experience regime (either Golden Acre Primo (II) or Greyhound) and the host plant variety on which the behaviours were carried out (either Golden Acre Primo (II) or Greyhound); the interaction between these two factors was also considered. Prior to analysis the data (from both varieties combined) were analysed using a Ryan-Joiner test to test for normality. The results of these tests are shown in Table 5.2. The data for the number of ovipositions was found to be normally distributed and this data was analysed untransformed. For the other behaviours the data was not normally distributed and was transformed prior to analysis using the following transformation Log_{10} (data+1). Following this transformation in all cases the data for the number of behaviours exhibited a normal distribution (Table 5.2.).

Table 5.2. The results of Ryan-Joiner tests for normality for the number of behaviours exhibited by *P. rapae* females.

Behavioural category	Raw data		Transformed data	
	W-Test for normality		W-Test for normality	
	R-value	P	R-value	P
Alighting	0.97	<0.01	0.99	>0.05
Drumming	0.98	<0.01	0.99	>0.05
Flutter Bouts	0.93	<0.01	0.99	>0.05
Curling	0.97	<0.01	0.99	>0.05
Ovipostion	0.99	>0.05		

RESULTS

Table 5.3. shows the results of the statistical analysis carried out on this data. For all behaviours, the host plant variety on which the behaviour occurred was not a significant variable on its own (Table 5.3. P>0.05). Similarly, the prior experience regime was also not a significant explanatory variable of the observed variation in the data set (Table 5.3. P>0.05). However, the interaction term between these two explanatory variables was highly significant (P<0.001) for all behavioural categories (Table 5.3.). For all of the behavioural categories recorded a higher number of behaviours were recorded on the host plant variety to which the butterflies had been given prior experience of than the alternative host (Figure 5.5.-5.9. inclusive) (P>0.001).

Figure 5.5. Effect of prior experience on the mean number of landings (Log $_{10}$ (data+1) made by female *P. rapae* butterflies. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=40

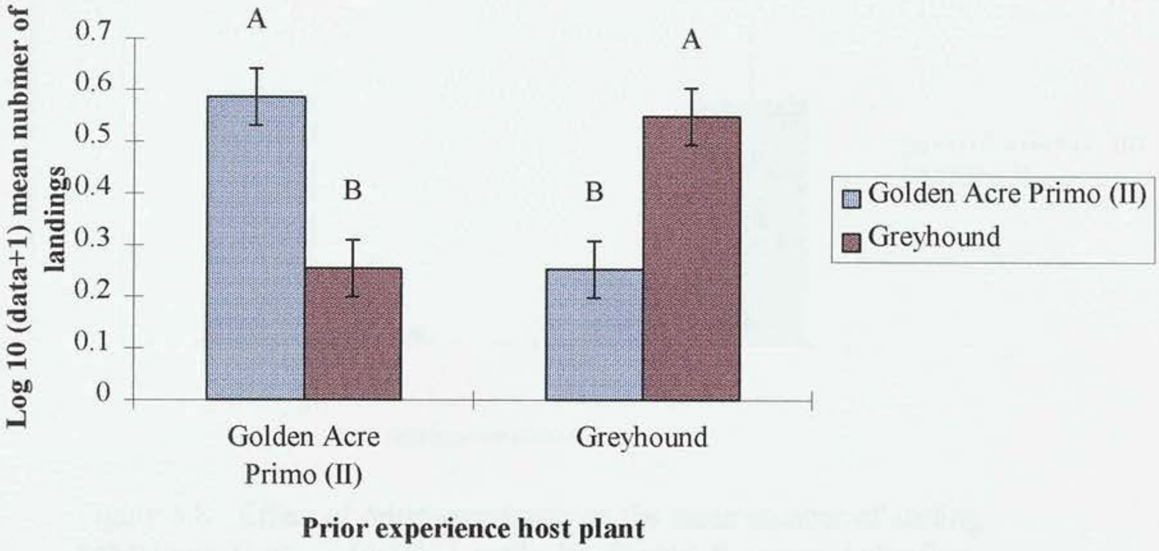


Figure 5.6. Effect of prior experience on the mean number of drumming behaviours observed (Log $_{10}$ (data+1) made by female *P. rapae* butterflies. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=40

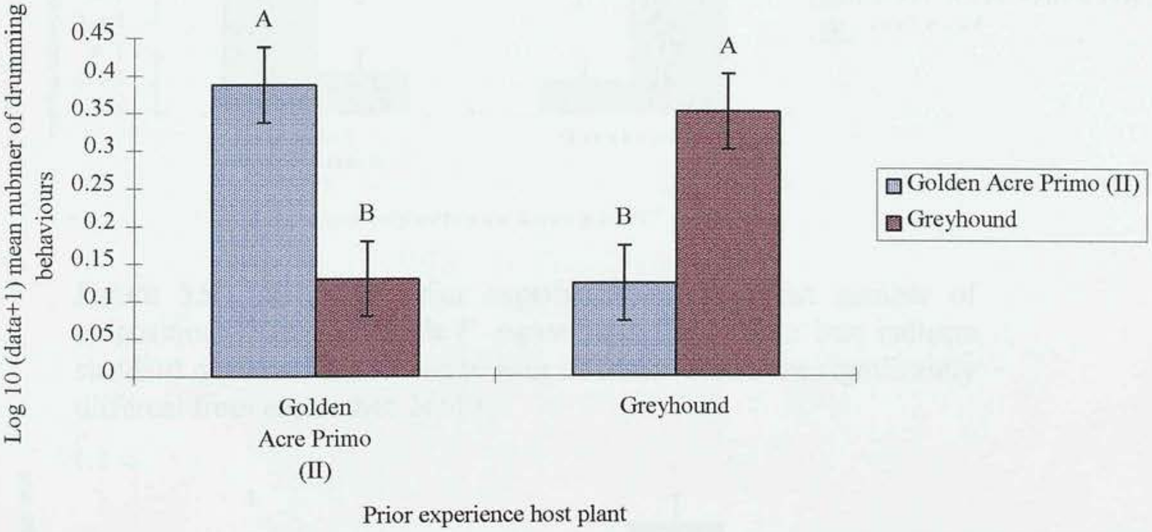


Figure 5.7. Effect of prior experience on the mean number of flutter bouts (Log $_{10}$ (data+1) made by female *P. rapae* butterflies. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=40

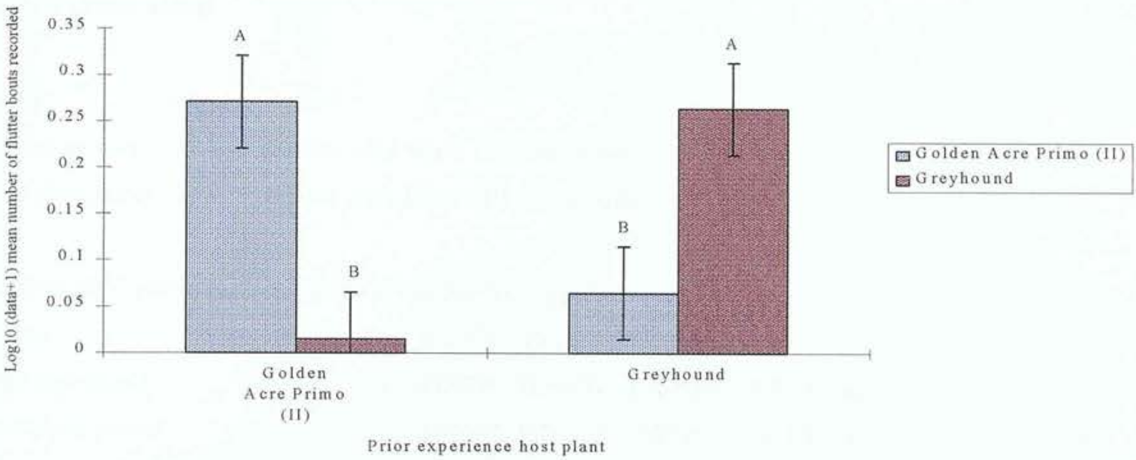


Figure 5.8. Effect of prior experience on the mean number of curling behaviours (Log $_{10}$ (data+1) made by female *P. rapae* butterflies. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=40

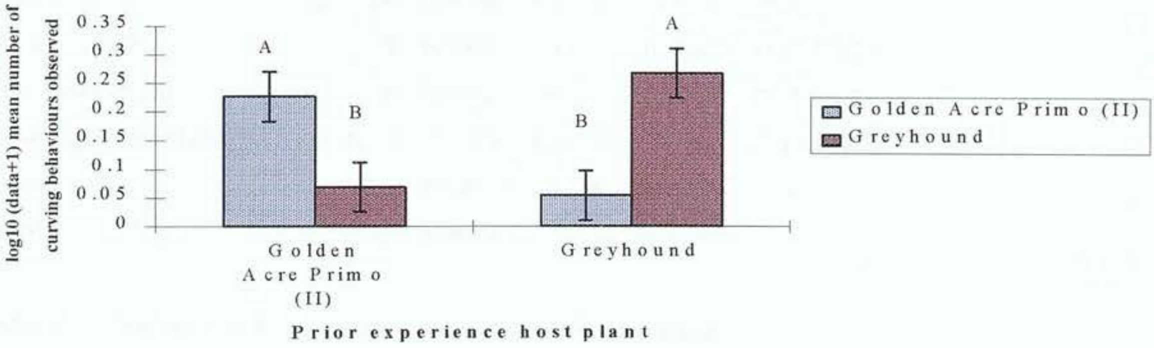


Figure 5.9. Effect of prior experience on the mean number of ovipositions made by female *P. rapae* butterflies. Error bars indicate standard deviation and columns with different letters are significantly different from each other. N=40

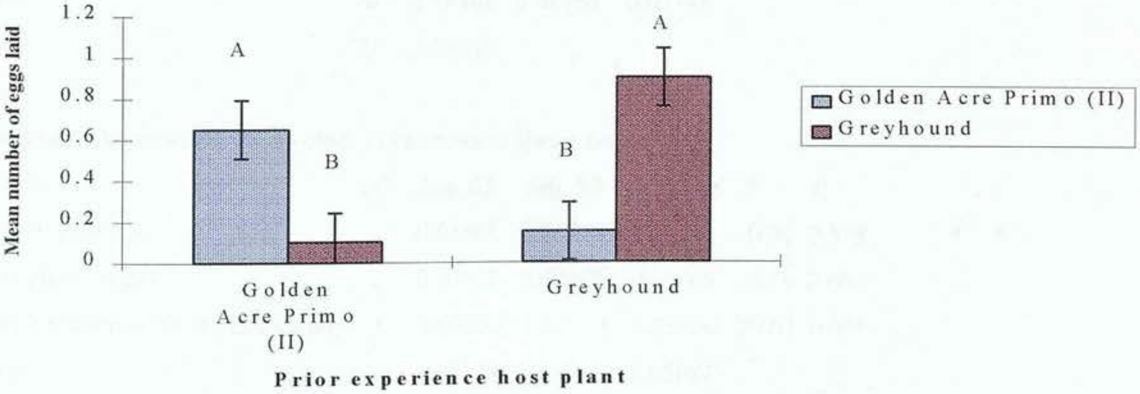


Table 5.3. Summary of Glim analysis for the effect of prior experience and host plant variety on the number of behaviours carried out be *P. rapae* females.

General Linear Model

Factor	Levels	Values
Prior experience	2	Golden Acre Primo (II) Greyhound
Host plant variety	2	Golden Acre Primo (II) Greyhound

Analysis of Variance for Log$_{10}$ (data +1) number of alightings

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Prior experience	1	0.00559	0.00559	0.00559	0.09	0.762
Host plant variety	1	0.00657	0.00657	0.00657	0.11	0.743
Prior experience*host plant variety	1	1.97063	1.97063	1.97063	32.54	0.001
Error	76	4.60267	4.60267	0.06056		
Total	79	6.58546				

Analysis of Variance for Log$_{10}$ (data +1) number of drummings

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Prior experience	1	0.00412	0.00412	0.00412	0.08	0.776
Host plant variety	1	0.00849	0.00849	0.00849	0.17	0.683
Prior experience*Host plant variety	1	1.18650	1.18650	1.18650	23.45	0.001
Error	76	3.84500	3.84500	0.05059		
Total	79	5.04411				

Analysis of Variance for Log$_{10}$ (data +1) number of curving behaviours

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Prior experience	1	0.01582	0.01582	0.01582	0.42	0.521
Host plant variety	1	0.00342	0.00342	0.00342	0.09	0.765
Prior experience*Host plant variety	1	0.69223	0.69223	0.69223	18.24	0.001
Error	76	2.88468	2.88468	0.03796		
Total	79	3.59615				

Analysis of Variance for Log$_{10}$ (data +1) number of flutter bouts

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Prior experience	1	0.01568	0.01568	0.01568	0.31	0.578
Host plant variety	1	0.00968	0.00968	0.00968	0.19	0.662
Prior experience*Host plant variety	1	1.03630	1.03630	1.03630	20.64	0.001
Error	76	3.81673	3.81673	0.05022		
Total	79	4.87839				

Analysis of Variance for number of ovipositions

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Prior experience	1	0.2000	0.2000	0.2000	0.50	0.484
Host plant variety	1	0.4500	0.4500	0.4500	1.11	0.295
Prior experience*Host plant variety	1	8.4500	8.4500	8.4500	20.92	0.001
Error	76	30.7000	30.7000	0.4039		
Total	79	39.8000				

The behaviours after landing in the sequence can, by definition, only occur once a female has landed. Therefore, it is possible that for behaviours exhibited after landing, the results of the statistical analysis are an artefact. If, for example, the tendency to land more often upon the host plant to which the females had been given prior experience than the alternate host occurs, then the resulting other behaviours could well follow this trend. That is, the proportion of subsequent behaviours would merely reflect the pattern in the data for landing, there being no real difference in the frequency of the other behaviours on the two hosts. In order to test this hypothesis that prior experience affects only the choice of initial landing site and the significant differences in the number of subsequent behaviours are due to this effect alone, the analysis was repeated with the number of landings as a covariate. The results of this Glim analysis are shown in Table 5.4. The effect of the number of landings was a significant covariate for all of the subsequent behaviours ($P<0.001$, Table 5.4). In addition, once the effect of the number of landings was considered (as a covariate), the interaction between the host plant variety and the prior experience regime was not found to be significant for any of the subsequent behaviours exhibited by *P. rapae*. Therefore, it would appear that the effects of prior experience in these experiments was limited to the choice of initial landing site.

Table 5.4. Summary of Glim analysis for the effect of prior experience and host plant variety on the number of behaviours carried out be *P. rapae* females with log $_{10}$ (data+1) number of landings used as a covariate in the analysis.

General Linear Model

Factor	Levels	Values
Prior experience	2	Golden Acre Primo (II) Greyhound
Host plant variety	2	Golden Acre Primo (II) Greyhound

Analysis of Variance for log $_{10}$ (data+1) drumming

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Log $_{10}$ (data+1) landing	1	3.12925	1.95302	1.95302	77.42	0.001
Prior experience	1	0.00016	0.00024	0.00024	0.01	0.923
Host plant variety	1	0.00132	0.00154	0.00154	0.06	0.805
Prior experience*Host plant variety	1	0.02140	0.02140	0.02140	0.85	0.360
Error	75	1.89198	1.89198	0.02523		
Total	79	5.04411				

Term	Coef	StDev	T	P
Constant	-0.01984	0.03523	-0.56	0.575
Log $_{10}$ (data+1) landing	0.65140	0.07403	8.80	0.001

Analysis of Variance for log $_{10}$ (data+1) curving behaviours

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Log $_{10}$ (data+1) landing	1	1.64791	0.99542	0.99542	39.52	0.001
Prior experience	1	0.02665	0.02574	0.02574	1.02	0.315
Host plant variety	1	0.00984	0.00923	0.00923	0.37	0.547
Prior experience*Host plant variety	1	0.02248	0.02248	0.02248	0.89	0.348
Error	75	1.88926	1.88926	0.02519		
Total	79	3.59614				

Term	Coef	StDev	T	P
Constant	-0.03663	0.03520	-1.04	0.302
Log $_{10}$ (data+1) landing	0.46505	0.07398	6.29	0.001

Analysis of Variance for log $_{10}$(data+1) flutter bouts

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Log $_{10}$(data+1) landing	1	1.92324	0.98425	0.98425	26.06	0.001
Prior experience	1	0.00720	0.00821	0.00821	0.22	0.642
Host plant variety	1	0.02021	0.01843	0.01843	0.49	0.487
Prior experience*Host plant variety	1	0.09525	0.09525	0.09525	2.52	0.116
Error	75	2.83248	2.83248	0.03777		
Total	79	4.87839				

Term	Coef	StDev	T	P
Constant	-0.03619	0.04311	-0.84	0.404
Log $_{10}$(data+1) landing	0.46243	0.09058	5.11	0.001

Analysis of Variance for oviposit

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Log $_{10}$(data+1) landing	1	14.6116	7.3505	7.3505	23.61	0.001
Prior experience	1	0.3123	0.2931	0.2931	0.94	0.335
Host plant variety	1	0.6280	0.5970	0.5970	1.92	0.170
Prior experience*Host plant variety	1	0.8986	0.8986	0.8986	2.89	0.093
Error	75	23.3495	23.3495	0.3113		
Total	79	39.8000				

Term	Coef	StDev	T	P
Constant	-0.0694	0.1238	-0.56	0.577
Log $_{10}$(data+1) landing	1.2637	0.2601	4.86	0.001

DISCUSSION

Rosenheim (1993) has argued that learning has been applied as a proximate explanation for behavioural variability within and among individuals of an insect population. Apart from learning there are many other explanations of this observed variability including:

- Genetic effects.
- Exogenous environmental effects.
    - Host-contact.
    - Availability of key resources.
    - Abiotic factors.

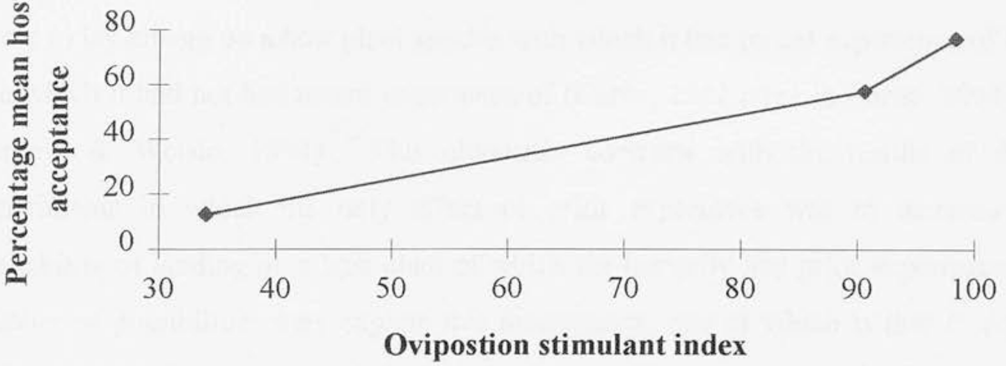
  - Density of conspecifics.
- Endogenous Environmental effects.
  - Age.
  - Egg-load.
  - Size.
  - Maternal effects.
  - Migration and diapause.
  - Nutritional status.
  - Other physiological factors.
- Mixed evolutionary stable strategies.

The random assignment of treatments to experimental units in manipulative experiments controls for variation in many of these variables and prevents them confounding the analysis (Rosenheim, 1993). Genetic effects are unlikely to confound the analysis of these particular experiments as the population used has a high degree of inbreeding. The culture used in these experiments was first started in the 1960's and has been continuously reared since then. Under these conditions I would expect that genetic variability will be reduced greatly, of course as Rosenheim (1993) points out inbreeding reduces genetic variability but does not eliminate it completely.

However, perhaps the most important factor which may confound learning effects in subsequent analysis are egg-load effects (discussed by Rosenheim, 1993). A prerequisite of the learning experimental design is that females are given differing ovipositional histories and then their subsequent ovipositional choices are monitored. Changes in oviposition are deemed to be due to learning effects. However, Rosenheim (1993) points out that there are at least three ways in which individuals with different ovipositional histories may differ from one another. These include; learning-based changes to the nervous system, perception of the density and quality of hosts and egg-load effects. Rosenheim (1993) asserts that "perceived host availability and egg-load may have profound influences on virtually all aspects of insect foraging decisions". The importance of these factors is "likely to depend upon the degree to which different conditioning treatments generate different levels of ovipositional activity". The importance of host quality on oviposition activity is known to be important (Hopkins & Ekbom, 1996). These authors showed that the female pollen beetle (*Meligethes aeneus*) exposed to high quality hosts had a higher egg-load than females exposed to lower quality hosts. Clearly if such an effect occurred in these experiments then attributing the results obtained to learning induced changes would be misleading and the analysis of the data confounded. However, the fact that the training regime itself did not exert a significant effect on the number of eggs laid in the choice tests (Section A) and was also not a significant factor in the number of behaviours recorded (Section B) lessens this risk; i.e. it is more likely that the observed differences in behaviour are due to learning induced changes. Furthermore, Hopkins & Ekbom (1996) argue that the life history of the pollen beetle allows this particular strategy (to allow fewer eggs to develop in the presence of lesser quality hosts) to be beneficial. Specifically, they argue that this species has a relatively low risk of mortality in the reproductive female as well as a long oviposition period. Consequently, the benefits of delaying a commitment to reproduce may be marked. However, for *P. rapae* such arguments are reversed, the average longevity of females is 20 days (Richards, 1940) and the two major factors limiting butterfly populations are predation and the failure of the female to lay her full complement of eggs (Dempster, 1983). Therefore, it appears likely that in these experiments that the influence of egg-load on these results may be minimal. However, this is not to state categorically that such an effect is not at work. I have perhaps laboured the point that learning experiments can be confounded and would suggest that although the experimental design employed here was not perfect (for example, the number of eggs laid during conditioning were not counted and the size of the female was ignored) the interpretation that the changes in host acceptance between the treatments are due to effects of the female's prior experience are valid. Therefore, it appears that changes in the host plant acceptance behaviour may be attributed to changes induced by associative learning.

The results from section A would indicate that the degree to which host acceptance was altered by prior experience is influenced by the oviposition stimulant index of the host plants used (data from Chapter Four), the relationship between host acceptance and oviposition stimulant index is illustrated in Figure 5.10. Traynier & Truscott (1991) found that the degree of associative learning was higher when glucobrassicin was used as compared to sinigrin for the unconditioned stimuli. Work by Renwick *et al.* (1992) has shown that the activity of sinigrin was lower than glucobrassicin, even at concentrations higher than those found in plants (Renwick *et al.*, 1992). Therefore, the findings of Traynier & Truscott (1991) that the strength of the association between the learned stimuli (colour) and the unconditioned stimuli (glucosinolate) may be confirmed in plants.

Figure 5.10. The relationship between the ovipositional stimulant index of the three host plants used in the learning experiments and the percentage host acceptance of those plants.



The relationship appears to be non-linear. A regression was carried out using the OSI data from Chapter Four and the percentage host acceptance data (Log_{10} transformed); the resulting regression was statistically significant (ANOVA F-value 705.7; DF=1,2 P>0.05; R-sq. 0.998) (Table 6.5) and the regression equation was Log $_{10}$ (% Host acceptance)= 0.12*OSI + 0.7

Table 6.5 Summary of regression between oviposition stimulant index and Log_{10} percentage host acceptance.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.353	0.353	705.671	0.024
Residual	1	0.001	0.001		
Total	2	0.354			

These results are indicative of the notion that the strength of the learned relationship is adaptive, in that it allows the female to search for higher quality hosts.

Previous work with *P. rapae* has shown that associative learning occurs with colour and host plant chemicals, specifically glucosinolates (Traynier, 1979; Traynier, 1984; Traynier, 1986; Traynier & Truscott, 1991). However, the present study did not attempt to elucidate which factors were learnt and therefore, has not advanced our knowledge of which cues can be learnt in association with host plant chemicals by *P. rapae*. It should be borne in mind that in the Lepidoptera reports exist of associative learning of odour (Landolt & Molina, 1996) and that the learning of odour cues are common in parasitoids (Lewis & Tumlinson, 1988; see Turlings *et al.*, 1993 for a review).

In a study of the blue butterfly (*Glauchopsyche lygdamus*, Lycaenidae) it was reported that in field experiments once the butterfly had landed it would be more likely to lay an egg on a host plant species with which it had recent experience of than one which it had not had recent experience of (Carey, 1992 cited in Carey, 1994 and Bernays & Wcislo, 1994). This obviously contrasts with the results of these experiments in which the only effect of prior experience was to increase the probability of landing on a host plant of which the butterfly had prior experience. A number of possibilities may explain this discrepancy, one of which is that *P. rapae* differs in its behaviour from that of *G. lygdamus*. However, it may be that because the two cabbage varieties were similar in their acceptabilities the butterflies did not discriminate after landing. In the repeated analysis in which the number of landings was used as a covariate the effect of the interaction between prior experience and host plant variety on oviposition was low (although not significant $P<0.1$), and if more insects were used, this effect may become significant statistically. However, it is also important to remember that a lack of significance is best interpreted as an absence of evidence rather than as absolute proof that the observed effect does not occur. Similarly, there are significant differences in the studies which are important. The present study was carried out in the laboratory which may have significant repercussions for the conclusions if they are transferred to the "field" without caution.

In my opinion, the complexity of vegetational communities and the time limitations facing insects for oviposition (Dempster, 1983), conditioning of perception allows an insect to filter the stimuli emanating from a host, for example colour (Traynier, 1984), shape (Papaj, 1986; Rausher, 1978) and olfactory cues (Lewis & Tumlinson, 1988), to improve future searching efficiency leading to a higher rate of host encounters. The lack of field studies for *P. rapae* in this area not only limits our understanding of the host plant location behaviour of *P. rapae* but also our understanding of the role of an individual's history in influencing its 'decisions' to accept or reject host plants encountered.

CONCLUSIONS

The results of these experiments have clearly shown that the prior experience of a female does indeed affect subsequent ovipositional behaviour. Furthermore, the quality of the host (in terms of its stimulatory chemicals) of which the butterflies have had prior experience would appear to have a large influence on the subsequent acceptability of the host.

Moreover, the primary behaviour affected appears to be the choice of landing site and from what is known of the cues learned in *P. rapae* it appears that prior experience of a host allows the females to search preferentially for that host in subsequent search bouts using plant colour.

Chapter Six
Development of an artificial life model of insect ovipositional behaviour based on *P. rapae*

INTRODUCTION

The behavioural mechanisms of host plant selection in phytophagous insects have received much attention, theoretical models (either verbal or mathematical) and the simulation of specific species host plant utilisation patterns have been developed, for example, *Anthonomus grandis* (the boll weevil) (Cate, Curry & Feldman, 1979; Jones *et al.*, 1975). However, the actual behavioural chain of events prior to host plant acceptance has not been simulated. Therefore, the aim of this work is to develop a model simulating the behavioural decisions made by *P. rapae*, in the process of finding and accepting host plants, with the net result of producing egg distributions.

In this chapter, the modelling approach is outlined and the rationale behind the approach explained. As some of the techniques may not be familiar, brief reviews and an introduction of the use of Individual based modelling (IBM), Object orientated programming (OOP) and the use of artificial intelligence (AI) and Artificial life (Alife) in an ecological context are included (Section A). Following this outline of the techniques to be employed, the model is outlined in Section B.

SECTION A

INTRODUCTION

Models of host plant utilisation have been developed previously, including the conceptualised models of Jaenike (1978) and Courtney, Chen & Gardner (1989) which address theoretical aspects of host plant acceptance. Theoretical simulations have addressed the optimal strategies of patch use (Ives, 1989); clutch size (Parker & Courtney, 1984) and search behaviour (Cain, 1985). The egg laying pattern of specific species has also been simulated; for example *P. rapae* Jones (1977).

The ovipositional behaviour of *P. rapae* is reasonably well understood, information is available on its general biology and ecology, ovipositional preferences for plants, and factors controlling egg distributions. The process by which a female *P. rapae* searches, locates and accepts, i.e. oviposits on a host plant is a catenary chain (Courtney, 1986a; Kennedy, 1965; Thorstein, 1960), stimuli evoke responses which lead on to subsequent cues in the chain.

PREVIOUS MODELS OF INSECT BEHAVIOUR

This section briefly outlines previous simulations of insect behaviour. The discussion of previous models here makes little distinction between insect herbivores and parasitoids, since the models discussed are theoretical and as such the actual decision making processes may be thought of as being analogous. This analogy arises as a consequence of the fact that females of both type of insects are utilising similar cues to locate hosts and the survival of the offspring are dependant upon the same type of factors.

Patterns of insect oviposition comprise three components: where, when and how many eggs are laid in a single bout (Skinner, 1985). The number of eggs laid by an individual on a host depends upon two factors, reproductive effort and how resources are divided between offspring. Lack (1947) (cited in Lessells, 1991) proposed a hypothesis for the evolution of clutch size in birds. He proposed that as brood size increased, each of the offspring would receive less food and therefore, would survive less well. As a result, an intermediate number of offspring may produce the highest number of survivors, with the optimal clutch size being determined by the trade off which exists between the number and fitness of offspring (Lessells, 1991).

The Lack Clutch Size (the clutch size producing the highest number of surviving offspring) can measure the value of a host, as larger clutches should be laid on better host's. The Lack Clutch Size is determined by the relationship between the fitness a female derives from laying a number of eggs on a host with a resource value (measured in terms of the hosts quality in terms of the number of 'fit' progeny the host can support). Competition between the progeny affects the Lack Clutch Size. Less intense competition leads to larger clutches (for equivalent hosts) (Skinner, 1985). For example, when larvae compete in scramble competition, those which do not complete development because the host is depleted will either be forced to migrate to an alternative host or die. Therefore, when no alternate host is available or the risk of predation during the migration of the larva is high, the mother will benefit by not laying more eggs than the host can support.

Patterns of resource use by phytophagous insects vary, some species lay many eggs on a single host, while others distribute their eggs more evenly. A clumped distribution can cause food competition among the offspring. In order for the larvae to avoid starvation, many of them will leave the original host and migrate. It is generally accepted that this migration is dangerous to the insect, due to the risk of predation and the possibility that no other suitable host plants are available (Sjerps, Haccou, Meelis & van der Meijden, 1993). Much of the theory associated with patterns of resource use has been developed using optimal foraging theory as a framework either explicitly in the case of most foraging models here or with the underlying concept that foraging behaviour is adaptive. The rational behind this approach relies on the assumption that foraging behaviour is optimised over evolutionary time. That is, the costs and benefits associated with alternative patterns of resource exploitation affect fitness. Therefore, animals may perceive and act upon cues correlated with the energy content of a food and an animal will forage in such a way as to maximise fitness (Mitchell, 1981). However, the suitability of a plant for larval development is a function of many variables, including its chemical and physical properties, micro habitat and degree of infestation. In addition, host plant suitability is subject to intra and inter-specific variations (Jaenike, 1978).

Jaenike (1978) formulated a model specifically aimed at investigating the optimal strategy for a phytophagous insect's ovipositional behaviour. He argues that plants should be accepted in order of decreasing suitability, with the specific time being determined by the suitability of alternative hosts and the probability of there being encountered. A given plant species should be accepted sooner if the probability per unit time of finding that host is small. When the time available for search is short the discrimination phase before a host is accepted should also show a commensurate decrease. However, the abundance of a plant species does affect when other species should be accepted; the more common it is, the greater the time for finding that host must be before less suitable plants are accepted by the insect (Jaenike, 1978).

Predictions from models of the optimal clutch size of an insect suggest that for a constant time spent searching, more eggs should be laid on better hosts. For hosts of equivalent value, the number of eggs laid by a female should increase with increasing time between hosts and even if search time is infinite, there is a maximum number of eggs a female should lay on a host; this is the Lack Clutch Size. For a super-parasite, clutch size declines with increasing time between the oviposition events; the Lack Clutch Size of a super-parasite is lower than that of the primary-parasite. However, the clutch size of the secondary parasite is affected by the relative search times of each parasite, the first parasites clutch size and on the time between the two oviposition events (Skinner, 1985).

The effect of the probability of encountering a host plant on the insect's clutch size has been investigated (Mangel, 1987). A high probability of encounter with a host plant which has a large optimum clutch size, leads to decreases in the frequency of large clutches. This can be interpreted as a 'risk spreading mechanism'. Therefore, when hosts which can support a large clutch size are plentiful it is advantageous to deposit fewer eggs per host. Similarly, host deprivation increases the number of large clutches and older insects should be less selective about where they lay their eggs (Mangel, 1987).

The influence of mortality and limited egg number showed the range of host species utilised for oviposition becomes narrower in richer environments with high host frequency, and the decision to utilise a host is independent of the abundance of the host concerned and is instead determined by the abundance of the host with higher profitability. Host range is also expected to widen as egg-load increases. Conversely, when the egg-load is very small only the most profitable hosts are utilised (Iwasa, Suziki & Matsuda, 1984).

A large host plant should favour larger clutches and clutch size should decline throughout the insect's life, especially as egg-load decreases. In addition, clutch size should increase with increased search time for new host plants i.e. females should dedicate a higher number of eggs to a resource when it is harder to find. When the probability of two separate females utilising the same host for oviposition is low, the first female should lay her normal clutch size. However, as the frequency of double oviposition events increase. The first female to oviposit can gain an advantage by lowering her clutch size because of the adverse affects of the second clutch so that the total number of eggs per host stays virtually constant. However, when the frequency of double ovipositions is low, there is a slight increase in the total number of eggs (Parker & Courtney, 1984).

The effect of the risk of adult mortality on clutch size was also investigated by Parker & Courtney (1984) and they suggested that if the probability of surviving to lay a second clutch is low, more eggs should be laid at clutch one, even though these eggs may do less well due to sibling competition. Furthermore, when egg-load is low and the risk is high all available eggs should be laid in the first clutch (Parker & Courtney, 1984).

The models considered so far have dealt with the situation in which a forager utilises only one patch and when more than one patch is visited, the insect has to make two decisions. How many eggs to lay in each patch and how to distribute these eggs within a patch (Sjerps *et al.*, 1993). Weisser & Houston (1993) investigated the optimal behaviours of a solitary parasitoid wasp foraging in a patchy environment that has been previously exploited by either conspecific or heterospecific females. The model predicted the number of patches visited and the number of eggs laid decrease as travel time increases between patches, and the number of previously parasitised hosts accepted increases with increasing travel time. Similarly, as the number of unparasitised hosts in a patch decreases, the wasps accept more parasitised hosts for oviposition (Weisser & Houston, 1993).

The effect of age was also considered by these authors and for a young wasp the optimal behaviour is to parasitise unparasitised hosts until their numbers drop to one and then move to a new patch. However, as the end of the insect's life approaches parasitoids adjust their policy to the time constraint and exploit the last patch visited in a different way. When the time left is too short to parasitise unparasitised hosts in a further patch, parasitoids stay in the last patch, but also accept non-self-parasitised hosts if there are only one or two unparasitised hosts left in the patch. Therefore, towards the end of their life parasitoids expand their host range during a patch visit. A similar observation is made with respect to travel mortality. As travel mortality increased, wasps spend more time in the patch and accept more non-self-parasitised hosts for ovipositions (Weisser & Houston, 1993).

Sjerps & Haccou (1993) have investigated the ovipositional Evolutionary Stable Strategy (ESS) of two females laying eggs on the same host and found the ESS is dependent on the 'knowledge' of females. They found that when a female is constrained (i.e. cannot detect the presence of other clutches) the optimal clutch size for both females is equal and can be larger or smaller depending upon the fitness gain per egg. For knowledgeable females, (i.e. those which can detect the presence of other eggs, but cannot assess the clutch size), there are two optimal clutch sizes, one for the first female to oviposit and another for the second female. Depending on the probability of the two females utilising the same host plant the optimal clutch size may increase or decrease and the second female may lay more or less eggs than the first female. If the probability of the double oviposition is one, then the ESS is the same as for a constrained female. For an omniscient female, (i.e. a female which can detect the presence of other eggs and assess the size of the clutch), the first female, knowing that the second female can assess her clutch size, can use this information to manipulate oviposition by the second female. In this situation, an omniscient female will either increase or decrease her clutch size depending on the fitness curve. Similarly, for an omniscient female laying her eggs second she may either decrease or increase her clutch size depending on the fitness curve (Sjerps & Haccou, 1993).

Similarly, Ives (1989) has developed an optimality model of clutch size for when more than one female utilises a patch. In this model, females which are knowledgeable that is can assess accurately whether eggs have been previously laid on a patch and adjust their clutch sizes accordingly, omniscient in Sjerps & Haccou, 1993 terminology. The evolutionary stable clutch sizes for both the first and second female increase as the average number of females ovipositing per patch increases. However, the decision of the second female to lay more or less eggs than the first depends on the magnitudes of the first clutch advantage and searching time. When the searching time is short in comparison to handling time, patches are easy to find and consequently a second female is likely to benefit from withholding some of her eggs, because she can easily find an empty patch on which to lay them. Therefore, the clutch size of the second female decreases relative to the first when search time is short. Similarly, when there is a first clutch advantage the second female ovipositing on a patch incurs a greater cost and withholding eggs for an empty patch becomes more profitable. In addition, it is always optimal for the second female to lay at least one egg, provided that the fitness she gains through laying that egg is greater than the expected rate of fitness she would gain by searching for another patch (Ives, 1989).

Rosenheim & Mangel (1994) have developed models which simulate the effect of imperfect host discrimination on patch leaving rules. In the simplest general model developed, they show that parasitoids will leave a patch when the cost of leaving is less than the product of the level of parasitism in the current patch and the host discrimination error rate. Therefore, leaving is favoured by low costs of travelling to a new patch, high error rates (frequent oviposition "mistakes") when discriminating previously self-parasitised hosts, and high levels of parasitism in the currently occupied patch and leaving is never favoured when the error rate is zero, perfect host discrimination (Rosenheim & Mangel, 1994).

The preceding section has put forward the theory associated with the behavioural strategies employed by parasitoids or herbivorous insects. Many of the models developed in order to provide useful insights must by definition remain general and this leads to problems in so far as the models cannot be tested. However, Driesson, Bernstein, van Alphen & Kacelnik (1995) have experimentally tested the model of parasitoid foraging developed by Waage (1979). In this model the foraging behaviour of the parasitoid *Nemeritis canescens* was investigated. Driesson *et al.* (1995) found that two of the components proposed by Waage (1979) are confirmed:-

- The responsiveness to the patch edge increases with the concentration of contact kairomone, which is a function of host density.
- The probability per unit time of ending the patch visit increases as time in the patch proceeds.

- The third of Waage's (1979) conclusions was not supported in this experiment as each successive oviposition caused a decrease in patch residence time. Whereas, Waage (1979) reported that oviposition caused an increase in patch residence time.

In an experimental study, Volkl & Mackauer (1990) investigated the ovipositional strategy of the aphid parasitoid *Ephedrus californicus*. They found that females tend to super-parasitise at high parasitoid-to-host ratios or if the parasitoid lacked prior experience, i.e. have been deprived of hosts. In addition, after eclosion, searching activity increases with egg-load and peaked in females at 4-5 days old and decreased in older females. In *E. californicus* conspecific super-parasitism varied with egg-load, which is dependent on female age. When unparasitised 'high quality' hosts were not available, wasps with a high egg-load readily accepted conspecific-parasitised 'low quality' hosts, provided the interval between the first and second oviposition was short. However, when egg-load was low or when unparasitised hosts were available, or the interval between the first and second ovipositions was long, wasps avoided super-parasitism. These results are clearly in line with the theoretical concepts outlined above.

MODELLING TECHNIQUES

Individual based modelling

Ecology deals with three levels of the biological hierarchy, the organism, population of organisms and the communities of populations. Ecology is confronted with uniqueness via the number of species and the countless numbers of genetically unique individuals all of which live and interact in a varied and ever changing world (Begon, Harper & Townsend, 1996). One of the advantages of individual based models (IBM) are that they may integrate different levels of the traditional hierarchy of ecological organisation (Huston, DeAngelis & Post, 1988). In order to try and better understand the interactions of species and to accommodate the biological realities of ecosystems the study of systems at the individual level has been developed. The individual approach attempts to derive the properties of an ecological system using the properties of the individuals constituting that system and as such is essentially reductionism (Lomnicki, 1992).

The use of the individual approach in ecology seems obvious as individual organisms are the fundamental units of ecosystems and underpin all aspects of ecology, including ecosystem function and all species interactions. Additionally, individuals are easier to define than a species or ecosystem and individuals are the units of selection (Judson, 1994).

When building models in the ecological domain, the 'real world' has been drastically simplified to allow mathematical analysis. There is some justification to searching for simple explanations and including every component of a system in a model would lead to obscurity (Laval, 1995). However, the application of mathematical modelling requires simplifying assumptions which are not compatible with the reality of an ecological system (DeAngelis & Gross, 1992).

A major conceptual shift in ecological simulation is currently occurring. Many Ecologists are moving away from models governed by general equations and towards those which 'create' each individual in an ecological system in order to generate the dynamics of that system as a whole. The use of IBMs in recent years has increased dramatically, but Judson (1994) cites an example showing that this type of model has appeared sporadically for at least 40 years in the ecological literature. This paradigm shift has two main causal agents one of which is increase in the power of computers and software enabling meaningful IBMs to be developed and run (Judson, 1994), the other is the acknowledgement by ecologists that the two assumptions underlying traditional models are flawed:

- Mathematical models often combine many individual organisms and assume that they can be described by a single variable.
- Each individual is assumed to have an equal effect on every other individual.

These two assumptions deny each individual is different, with behaviours and physiology resulting from a unique combination of genetic and environmental influences. Additionally, interactions between individuals are inherently local. An organism is affected primarily by the organisms with which it comes into contact (Huston *et al.*, 1988).

Uchmanski & Grimm (1996) have argued that there are four appropriate criteria for classifying ecological models, namely:

- The degree to which the complexity of the individual's life cycle is reflected in the model.
- Whether the dynamics of the resources (for example, food, space or habitat quality) are explicitly taken into account.
- The use of real or natural numbers in representing the size of a population.
- The extent to which the variability of individuals of the same age is considered.

Therefore, genuine IBMs are those models which "describe a population made up of individuals that may differ from one another and take into account the complexity of the individual's life cycle, describe changes in the numbers of individuals rather than in the population density and also take resource dynamics explicitly into account" (Unchmanski & Grimm, 1996).

There are a number of advantages of IBMs over traditional models the biggest of which may simply be the conceptual shift from mathematical descriptions of ecological processes and ecosystems to rule based specifications of the behaviour of individuals. Despite their apparent complexity IBMs are often conceptually simpler than more traditional models as they require fewer unreasonable assumptions. Their simplicity lies in the way behaviour is controlled; typically a few rules govern the interactions of individuals. These rules depend upon the physiological and behavioural characteristics of individual organisms and cannot be easily incorporated into large-scale models (Huston *et al.*, 1988).

Artificial intelligence in ecology

A primary focus of Artificial Intelligence (AI) research has been to develop ways to store and utilise information in a computer which mimics the processes in the human mind. A relatively small step is required to apply these techniques to the problems of animal reasoning and behaviour (Stone, 1992). AI in recent years has moved from the laboratory to the marketplace (Loehle, 1987) and the prodigious leaps in the development of hardware and software in computing have made AI methodologies available to non-specialists. This is clearly seen in the agricultural and ecological spheres as expert or knowledge based systems. The utilisation of expert systems has grown and since the first report in the early 1980's, over 300 expert systems have been developed, virtually all of which are decision support tools (Edwards-Jones, 1993). Despite the success of expert systems as decision support aids, little research has been published on the application of AI techniques to ecological modelling. One area in which AI techniques have been successfully applied to ecological modelling is to simplify and improve the model building stage (Muetzelfeldt Robertson, Bundy & Uschold, 1989; Robertson *et al.*, 1991).

Artificial Life

Artificial Life (Alife) is a relatively new field which employs a synthetic approach to study life as it could be. Life is viewed not as a property of the matter which is organised in a particular way but rather as a property of the organisation of matter (Langton, 1989).

Biology has largely concerned itself with the material basis of life starting at the top and treating a living organism as a complex biochemical machine. The study of such life has then worked its way down through the traditional biological hierarchy of organism, organ, cell etc. Alife on the other hand treats an organism as a large population of simple machines and as such starts from the bottom, synthetically working its way up constructing aggregates of simple rule-governed objects which interact with another non-linearly in the support of life-like global dynamics (Langton, 1989). Synthesis has been defined as "rather than start with the behaviour of interest and attempting to analyse it into its constituent parts, we start with the constituent parts and put them together in the attempt to synthesise the behaviour of interest" (Langton, 1989).

Alife is something of an umbrella term and covers three principal approaches to studying artificial ecologies (Ray, 1994):

- Hardware, for example, robotics and nanotechnology.
- Software, for example replicating and evolving programs. The software approach can be further divided into:

- Alife simulations representing an advance in biological modelling.
- Alife instantiations of life processes. These are applications in which the data in the computer are not representative of anything, but instead are considered to be "alive" and self-replicating in the computers memory, for example, Tierra (Ray, 1991; 1994).
- Wetware, for example replicating and evolving molecules.

In this chapter I will only consider software approaches of Alife.

In the traditional approach to modelling ecological phenomena, systems of differential equations express the relationships between entities (for example, genes, individuals or species) in populations or communities. In Alife the bottom-up approach creates a population of data structures where each instance of the data structure corresponds to an entity. Rules define how individual structures interact with one another and their environment. Populations of these structures interact according to the local rules, and the global behaviour of the system emerges from these interactions (Ray, 1994). In effect, the entities created are autonomous, in so far as, interactions between them are dependent upon the state of the entity and the simple rules specified. The medium of the interactions between entities are messages, which are passed between them allowing them to identify themselves and pass data. The information received is then processed according to the rules previously specified.

The Alife approach, therefore, tries to create entities in the simulation which are direct representations of the entities in the real system. This approach relies heavily on the concepts of Object Orientated Programming techniques (see page 149, for a brief review). This process of representation allows the simulation to be developed more easily as the degree of abstraction is lowered, since the entities used are analogous to their counterparts in the physical world. This type of representation in simulation may be of immense importance in allowing biologists, to understand more easily what is occurring during a simulation.

Artificial organisms which exhibit life-like processes may serve as explanatory models of corresponding processes in naturally arising biological systems.

Consideration of the capabilities and characteristics of artificial organisms can also to the development of a broad theoretical biology (Laing, 1989).

- Approaches to Alife can be classified as either strong or weak (Kawata & Toquenaga, 1994).
- Strong Alife attempts to create life and search for the nature of life which may or may not be found on earth.
- Weak Alife attempts to simulate the life of known existing organisms in order to better understand the processes of real organisms.

The contrast between instantiation and simulation, lies in the fact that in simulations the data structures are representations of biological organisms. In instantiation the data structures are not a representation of an entity, they are considered to be 'living' forms in their own right and not a model of any natural life form. These data structures may be considered to form the basis of a comparative biology. Therefore, the aim of the instantiation approach is to introduce the natural form and processes of life into an artificial medium resulting in an Alife form which is not carbon based (Ray, 1994). The instantiation approach is an example of strong Alife, examples include Tierra (Ray, 1991; 1994) and the Computer Zoo (Skipper, 1992).

Alife attempts to study natural life by capturing the essence of the components of a living system, by endowing a collection of artificial components with similar behavioural repertoires to real organisms. The aggregated artificial components should exhibit the same dynamic behaviour as the natural system. This is achieved by identifying behavioural primitives, rules governing their behaviour in response to local conditions are specified and the primitive behaviours are organised in a similar manner to their natural counterparts (Langton, 1989).

Therefore, the essential features of an Alife model according to Langton (1989) are:

- They consist of simple programs or specifications.
- No single program directs all of the other programs.
- Each program details the way in which a simple entity reacts to local situations in its environment, including other entities.

- No rules anywhere in the system dictate global behaviour.
- Therefore, any behaviour at levels higher than the individual programs is emergent.

The best examples of Alife programmes are those which simulate some aspects of group behaviour such as flocking birds (Toquenaga, Kajitani & Hoshino, 1994); social insects (ants, Collins & Jefferson, 1991); fish (Terzopoulos, Tu & Grzeszczuk, 1994) and for a general approach to group behaviour (Werner & Dyer, 1993; Mataric, 1996). However, they have also been used to study food webs (Lindgren & Nordahl, 1994), which suggests that they are not limited to applications involving animal behaviour.

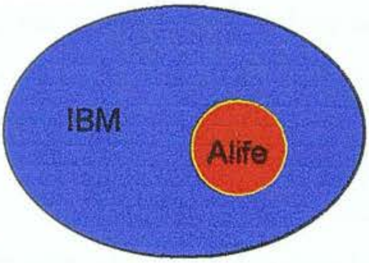
It is likely in the short term at least that weak Alife will become of greater interest to biologists. A particular area of interest may be in the development of simulation tools for studying animal behaviour. These tools include Petworld (Coderre, 1989); RAM (Taylor *et al.*, 1989); PolyWorld (Yaeger, 1994); Brainworks (Travers, 1989) and LAGER (Olson & Sequeira, 1995). The most sophisticated and flexible of these simulation tools is the SWARM simulation system which has been developed at the Santa Fe Institute (USA). It was developed as a general purpose simulation tool for implementing Alife models and IBMs.

The relationship between IBM and Alife simulations.

In a broad sense some IBMs can be regarded as Alife as they adopt the same bottom up approach. However, an IBM can only be regarded as an Alife implementation if it does not assume any global rules (Kawata & Toquenaga, 1994).

IBM and Alife are closely related, however, Alife researchers consider the essence of life to be emergent and is, therefore, required to achieve Alife. However, Kawata & Toquenaga (1994) have suggested that the search for life by artificial systems including IBM can be regarded as Alife. Therefore, there is a close relationship between Alife and IBMs, and as by definition an Alife simulation must be an IBM it is appropriate to consider Alife implementations in ecology as a subgroup of the wider IBM implementation as shown in Figure 6.1.

Figure 6.1. A Venn diagram illustrating the inter-relationship between Alife and IBM in biology.



As Alife and IBM are closely related they also share some of the same limitations. These limitations include the following:

- Mathematical analysis of a system is a more rigorous approach than Alife or IBM, and assuming the model can be described by formulae and solved analytically. The predictions arising are likely to be more accurate. However, the limitations of such mathematical analysis ignores the fact that the promise of Alife is that simulations can be used to test 'our understanding' (that is the collective understanding of the scientific community) of the process of interest.
- The validity of the program controlling the computer simulation can be difficult to check. Similarly, it can be difficult to tell why the observed pattern appeared.
- For Alife simulation especially, the results obtained are difficult to predict from the programs and so it may not be easy to determine whether the simulation results are really emergent or are caused by unknown or irrelevant factors. For ecological applications this is a crucial point since the search for causality is important as ecologists are interested in whether global patterns are determined by local rules alone, or whether global rules exist which are responsible for the appearance of global patterns (Kawata & Toquenaga, 1994).

The use of Alife, however, does have a very large advantage in modelling ecological systems and arises as a consequence of the way the system is developed. One of the major drawbacks in using ecological simulations for predictive purposes is the fact that the model's predictions can only be considered valid over the range for which the variables used to construct and the test the model were considered. Once, extrapolation beyond this range occurs the model predictions are untested. This problem does not arise in Alife simulations since the model's behaviour is caused by fundamental rules specifying the organism's behaviour and so the observed behaviour within the model is a result of underlying mechanisms and not limited to observed effects in experimental situations. This difference is crucial to the usefulness of Alife.

The way the model is constructed and conceptualised within an Alife project is such that the model can be thought of as being directly analogous to the mental conceptualisation of the scientist. The model, therefore, becomes a basis for testing the theoretical constructs underlying the conceptualisation. This in itself represents an advance in ecological modelling in which the model specification is usually distorted to fit mathematical assumptions.

Object Orientated Programming

The immediate origin of Object Orientated Programming (OOP) can be traced to the development of Smalltalk or Simula, however, Sequeira *et al.*, (1991) have suggested that the conceptual bases for such a paradigm can be traced to the notion of the 'universal' from early Greek philosophy. Very briefly, Aristotle formulated his theory of universals in which a universal was defined as "that which is of such a nature as to be predicated of many subjects, by individual that which is not thus"; i.e. in language there are proper names and adjectives, while proper names apply to only one thing universals (adjectives) apply to many different things (Russell, 1961).

OOP is gaining momentum and popularity in computer science. OOP is based around objects. An object is a group of data and procedures for manipulating that data within the programme. This differs from the traditional approach in that the data and the methods for manipulating the data are separate. As the data and procedures are collected into a single structure the style of programming is different from traditional programming which is based on data structures and control structures which manipulate the data structure (Maley & Caswell, 1993).

The use of OOP in simulation is relatively new, the model of a system is viewed as a collection of components which are treated as objects. These objects have knowledge programmed within them about their function and are activated by messages from other objects. The other objects do not have to know anything about the knowledge contained within the object to which messages are passed. The programmer in OOP is not concerned about how messages are passed, the OOP environment deals with this (McKinion, 1992). In OOP, individuals are the level of interest. Population equations are inappropriate, with the characteristics of individuals being modelled and the net effects of the individuals producing effects at the population level (Stone, 1992).

OOP languages are characterised by three properties; encapsulation, inheritance and polymorphism (Thomson, 1995). Encapsulation allows data and methods to be grouped leading to protection. Object orientated simulations require objects of many kinds, and an object type can declare a previously defined object as its ancestor. The descendant object "inherits" the data fields and methods of its ancestor. It can then add data fields and methods specific to itself. Polymorphism permits efficient programming as a procedure declared as manipulating a certain type of object can also manipulate any of that type's descendants. This contrasts with traditional programming as separate procedures are required for the manipulation of each type of data structure (Maley & Caswell, 1993).

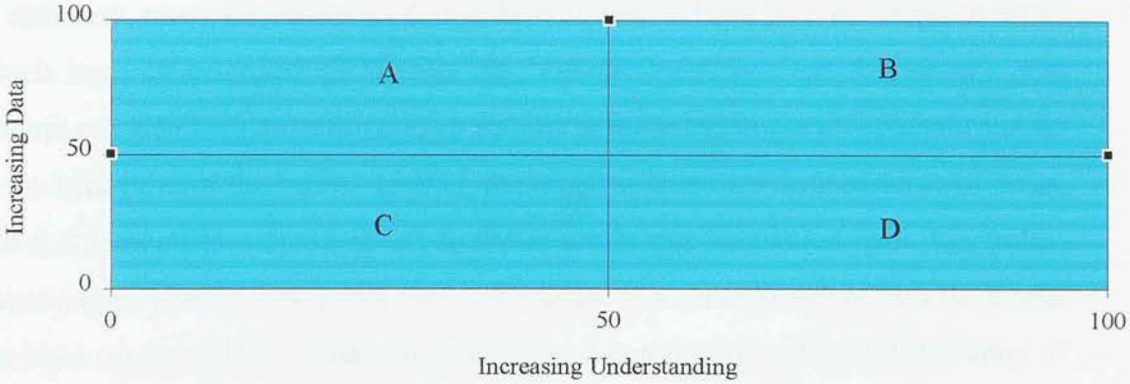
Rationale for the Alife approach in insect behaviour

Stone (1992) has argued that the modelling strategy adopted is affected by the amount of data and understanding available in the domain of interest. The relationship between data and understanding lead to four basic strategies for simulation (Figure 6.2.). Category A is suited to statistical approaches, category B to quantitative analysis. However, most insect problems fall into categories C & D (Stone, 1992).

Figure 6.2. suggests that in general the best approach to adopt for a particular application is affected by the amount of data and understanding. When the amount of data available for developing a simulation is large and the process of interest is well understood then the easiest approach is a mathematical analysis. Conversely, when the amount of data is high but the process of interest is poorly understood then a statistical approach may be most profitable. The statistical approach to simulation relies on the extraction of relationships between causes and effects without implying the mechanism underlying the effect. Whereas, the mathematical approach implies

that their is a relationship between two variables and the mechanism of the interaction is understood and can be expressed in mathematical terms. For problems where data is scarce, but the process of interest is well understood then using AI or Alife techniques the simulation can be developed from "first principles". In effect the relationship between two variables is deduced by the mechanism which affects there interaction.

Figure 6.2. The relationship between the amount of data and understanding in selecting an approach for modelling an ecological system (After Stone, 1992).



For *P. rapae* there is a considerable degree of conceptual understanding of the processes of host plant selection but the database for developing a mathematical approach is less well developed. Therefore, this domain lies in category D which may be amenable to an AI approach (Stone, 1992).

Alternate advantages centre around the concepts of IBMs, virtually all of the simulation models developed so far in biology have treated an individual as the basis of the model and then multiplied up the interactions of that individual to obtain the results for a population, thereby treating populations as a group of organisms with the same properties. This approach ignores the fact that populations comprise individuals which have differing life histories, for example, age, number of mature eggs, nutritional status etc., and experiences. In addition, biological interactions between these individuals occur locally.

This type of model is seen as increasingly untenable in light of biological realities. This is certainly true of host plant finding behaviour, research on host plant utilisation in phytophagous insects has subtly shifted from a deterministic approach to the acceptance of a more dynamic view including motivational effects and the role of learning in influencing ovipositional decisions. This is seen in the conceptual models of host plant acceptance in which the final decision is affected by the insect's motivational state (Miller & Strickler, 1984). For example, this model has been built around the assumption that some aspects of the butterfly's behaviour are traits which are species specific, namely hierarchy of host preference and perceptual abilities. Whereas, other aspects of the butterfly's behaviour are considered to be driven by factors which are not common to all individuals, namely, age and egg-load.

In summary, many theoretical models of host utilisation have been developed most of which have concentrated on finding the 'optimal behaviour' of the insect. The attempt reported here differs in that no attempt is made to impose an optimal solution to the behaviour of the insect. Instead, the insect is presented with an array of hosts, and the decision to oviposit is left to the insect in this simulation, with the 'rules' governing oviposition being followed. This arises as a consequence of how the model has been conceptualised. The initial task is to deconstruct the overall distribution of eggs found in the field into discrete steps, identifying rules which when followed act in the same way as the a female responds to cues in the 'real world'. The 'rule set' actuates the female into behaving on perception of 'cues' from the artificial world. The basic rules specified are 'weighted' by the female to explicitly take into account her motivational state and any prior experience she may have had.

As such, this research represents a shift away from the traditional implementation of simulations in ecology to a newer approach. This new approach promises to be of considerable value. However, the usefulness of Alife has yet to be demonstrated to be applicable to ecological problems outside of the Alife community. I aim to show that these techniques are applicable to real ecological problems and that the approach is of value in giving insights into the observed patterns of host use by phytophagous insects.

The model was not developed beyond its conceptualisation as it was necessary to devote time to experimentation to fill knowledge gaps in the areas of pre-alighting discrimination identified in the literature review (Chapter One). This experimental work has allowed the model to be conceptualised fully without resorting to the use of data from other species.

Section B
Model Structure

INTRODUCTION

A major influence on the conceptualisation of the model developed in this Section is the hierarchy threshold model of host plant selection developed by Courtney *et al.* (1989). In this model, when a potential host has been encountered, a decision is made whether to accept or reject that host for oviposition. The probability that the insect will accept a particular host will then depend upon on the acceptability of that host to the insect. A host with a higher intrinsic acceptability will result in it being accepted more readily; leading to a hierarchy of acceptability, with potential hosts ranked in sequence. The hierarchy threshold model assumes that if an insect will accept a low ranking host it will also accept all hosts higher in the hierarchy. The current egg-load of a female is known to influence host acceptance thresholds; a high egg-load should act centrally to increase the tendency to accept any host. The transition between the hosts may be abrupt and higher ranking hosts will still be acceptable (Courtney *et al.*, 1989).

In conceptualised models of host plant acceptance, the decision to accept a plant at each stage of the catenary process is mediated by the balance of sensory inputs, some of which are positive and others negative. The overall sensory input is then compared to the insect's internal state and a decision is made (Dethier, 1982; Miller & Strickler, 1984).

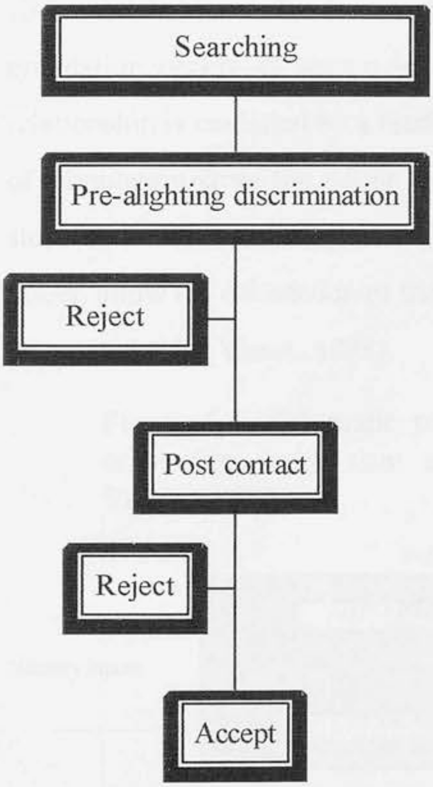
MODEL STRUCTURE

For the purpose of this discussion the behaviour of the butterfly has been split into discrete units. Pre-alighting behaviours are defined as all behaviours up to contact with a plant, and post-alighting behaviours are all behaviours exhibited after contact with a plant. For the purpose of explaining the model pre-alighting behaviours have also been categorised into:-

- Searching Behaviour.
- Volatile Discrimination.
- Visual Discrimination.

The behavioural decisions of the female are considered to be hierarchical and are shown in Figure 6.3.

Figure 6.3. The decisions made by *Pieris rapae* females engaging in ovipositional behaviours for the purpose of this model

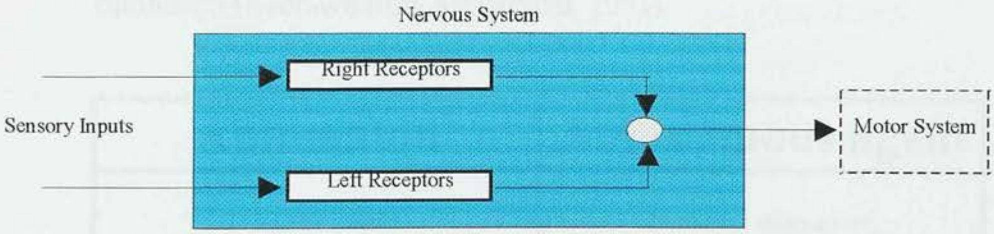


Searching Behaviour

Animals often exhibit random search paths. However, this intrinsic randomness does not prevent the animals from orientating towards specific goals or aggregating in suitable areas of the environment. To understand space use mechanisms (those which regulate the time an animal spends in a particular area of its environment) and orientation mechanisms (those which regulate how an animal moves towards a specified goal) it is necessary to model the search path taken. Application of the simple random walk model used in physics is inappropriate to represent the movement of an animal as most animals have a tendency to move forwards. A first order correlated random walk model is able to take into account this property. Therefore, a search path is represented as a sequence of steps of varying length and changes of direction (Benhamou & Bovet, 1991).

Visser (1988) has suggested that the orientation mechanism of phytophagous insects is under the influence of two control systems. These are information originating external to the insect (allothetic control) and internal information (ideothetic control). For the purposes of this model the influence of ideothetic control during the orientation of the insect to a host is abandoned for simplicity. However, ideothetic control is retained for initiating search. Therefore, the control of the insect's orientation occurs as shown in Figure 6.4. In allothetic control the input-output relationship is mediated by a feedback loop. The input is the asymmetry in the degree of stimulation from the odour source as measured by the receptors located on both sides of the insect's longitudinal axis. The commands to the motor system of the insect, allow the orientation of the insect to the stimulus source by turning to the more stimulated side (Visser, 1988).

Figure 6.4. Schematic representation of the control of an insect's orientation mechanism using external sensory information (After Visser, 1988).



Degreee of stimulation	Motor command
Higher left	Move left
Higher right	Move right
No difference	Forward

The butterfly's sensory input is used to find a host plant. i.e. there are no rules specified for movement such as specifying a random walk with a fixed move length. Instead, movement occurs as a result of a combination of sensory input and motivational state which activates searching behaviour.

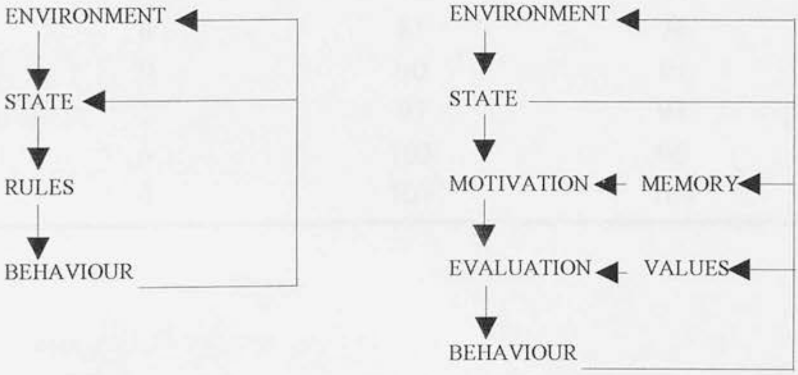
Motivational State

Animals can be classified according to behavioural criteria as either autonomous agents or as automatons (Figure 6.5.). When an animal is considered to be an automaton, the motivational state of the animal means that for a given state the animal follows a particular rule where some variables may have do to with physiological factors, the perception of environmental cues etc. So that although the animal may appear to have various options with regard to behaviour the actual behaviour performed is entirely controlled by the state of the animal (McFarland, 1991).

Assuming *a priori*, that it is notoriously difficult and somewhat anthropomorphic to consider such questions as to whether the observed behaviour of an animal is due to it being an autonomous agent or an automaton. I would suggest that by McFarland's (1991) criteria *P. rapae* is best considered an automaton. This classification also simplifies the model as the evaluation step is removed and the motivation to perform a specified behaviour is simplified to the animal's state. Therefore, animals which are automatons still have a motivational state in the sense that the state influences its behaviour, however, the animal is not self motivating (McFarland, 1991).

Figure 6.5. The relationship between an autonomous agent and an automaton (Redrawn from McFarland, 1991).

Automaton	**Autonomous agent**
Follow rules	Evaluates alternatives
to control must know the state and the rules	to control must know the state, the history and the evaluation criteria



Under these conditions, motivational processes are simply those reversible processes which move the animal at any particular time. They depend upon physiological status, environmental cues and the consequences of current behaviour. The consequences of current behaviour can influence the animal's motivational state in five main ways (McFarland, 1991):-

- By altering the external perceived stimuli of the animal and its cue state.
- By direct feedback into motivational state.
- By altering the animal's physiological state.
- Preventing the animal engaging in some alternative behaviour.
- Energy expenditure.

Table 6.1. The effect of a females age on the number of eggs laid per day by *Pieris rapae* females (data from Renwick and Radke, 1983). (Cumulative percentage of total was calculated by the following formula: Cumulative percentage of total =(cumulative number of eggs laid/total number of eggs laid) x 100).

Age	**Number of eggs laid per female**	**Cumalitve number of eggs laid**	**Cumalitive percentage of total**
1	7	7	7
2	5	12	11
3	9	21	20
4	9	30	28
5	10	40	37
6	21	61	57
7	12	73	68
8	8	81	76
9	9	90	84
10	7	97	91
11	6	103	96
12	4	107	100

The motivational state of the insect is defined by the number of eggs left to lay by that individual (Equation 1). The number of eggs left to lay is derived from the data in Table 6.1, and the number laid is derived from the number of eggs laid by the butterfly in the simulation. This point of view of motivational state is derived from egg-load and comes from the findings of a number of studies in which ovipositional behaviour is altered, the major factor in these studies appears to be egg-load but these effects can be confounded by other variables (reviewed by Minkenberg, Tatar & Rosenheim, 1992).

$$\text{Motivational State} = \frac{\text{Number to lay}}{\text{Eggs laid}} \qquad \text{Equation 1.}$$

Renwick & Radke (1983) have shown that the number of eggs laid by a female is a function of female age (Table 6.1.). There is an increase in the number of eggs laid as the female ages until the maximum is reached then there is a steady decline (Renwick & Radke, 1983; Gossard & Jones, 1977). Therefore, motivational state in the model will be defined by the number of eggs a butterfly has to lay on a given day for its age in comparison to the number actually laid that day. Any carry over from previous days will also be included to create the effect of inclement weather as found by Gossard & Jones (1977).

Therefore, a motivational state of 1 or more indicates acceptability thresholds will become lower and a value less than one indicates acceptance thresholds will be higher. The egg-load effects in this model may be thought of as affecting *P. rapae* in the following way. In this model, the current motivational state of the insect will be high in the early part of the day and will decline as eggs are laid. If eggs are not laid then motivational state increases. Evidence for this view comes from the observation that overcast days inhibited oviposition by not allowing flight, but if the next day was sunny then the number of eggs laid increased by 78% (Gossard & Jones, 1977). These findings may be interpreted as indirect evidence that egg-load influences decisions upon which plants to oviposit. More direct evidence of egg-load influencing searching behaviour is available in other species notably, *Battus philenor*.

For example, Odendaal (1989) reports that the proportion of time spent searching by a female is significantly higher when that female has a high egg-load as compared to a female with a lower egg-load. Odendaal & Rausher (1990) reported that *B. philenor* females increase their searching intensity, host selectivity and clutch size in response to egg-load or some internal factor correlated with egg-load.

As data are not currently available for the changes in motivational state due to egg-load, I would suggest that if the model is implemented this variable should be examined to investigate its effect on searching and subsequent acceptance behaviours.

Host plant discrimination

Host discrimination in phytophagous insects has two components pre- and post-alighting discrimination. Pre-alighting discrimination is thought to rely on visual and olfactory cues and post-alighting discrimination occurs after alighting during which the insect gathers mechanosensory information on the plants physical and chemical characteristics.

Pre-alighting discrimination

The way in which sensory input is used by the insect in selecting a plant for alighting is that visual cues, more specifically, the intensity of light reflected at 550 nm which exhibit a linear relationship with the number of landings, (Chapter Three) are used by the insect to determine the plant's acceptability for landing. The size class of the plant is also used to calculate the acceptability of the potential host plant. This value is then compared to the motivational state of the insect and the plant is alighted upon if the probability of alighting is above the current threshold.

Volatile cues have affect pre-alighting discrimination in this model. They influence whether a plant will be alighted upon only in terms of host plant recognition, for example a plant with host plant volatile cues is more likely to be landed upon than a plant without host plant volatile cues.

The size of the plant is also taken into account, once the plant has been assessed on the basis of colour and volatile cues, the resulting alighting probability is adjusted for the size class of the plant (as defined by Jones, 1977; Table 6.3.). The overall probability of alighting is mediated by the insect's current motivational state and a decision to accept or reject the plant for alighting is made.

Learning

The decision regarding on which plant to alight may also be influenced by a female's prior experience (Traynier, 1979) as colour is learned in association with contact stimulants. Therefore, it can be assumed that the effect of learning is most important in terms of influencing the decision to alight on a plant. In this model it can be assumed that if a plant is alighted upon then the same species is more likely to be alighted upon in the next encounter unless the time between encounters is large i.e. if a host plant of species A has been accepted then the individuals will preferentially alight on species A, unless species A has not been encountered in the last ten minutes. This discrimination period comes from the work of Tabashnik (1987) who found that the discrimination period for *P. rapae* in his experiments was between 10-15 minutes.

POST CONTACT DISCRIMINATION

Plant chemistry

The major factor in the post-alighting discrimination of host plants is the plant's chemical acceptability. This is mediated by the balance between stimulatory and deterrent compounds (Huang & Renwick, 1993). The chemical acceptability of a host is dependent upon the preference hierarchy devised by Huang & Renwick (1993) and is shown in Table 6.2. The data in Table 6.2. is used to assign a probability of accepting the plant for oviposition.

Table 6.2. The effect of cruciferous and non-cruciferous host plants on oviposition by *Pieris rapae* (Data from Huang And Renwick, 1993). A= No. of eggs on plant/No. of eggs on cabbage x 100.

SPECIES	WHOLE PLANT (A)
A. saxatile	22
B. juncea	155
C. spinosa	100
E. cheiranthoides	0.89
I. amara	6.4
I. tinctoria	100
L. annua	7.1
T. majus	100

Plant size

For the purpose of this model there are five plant size classes, these are as defined by Jones (1977). The size of a plant has also been shown to influence ovipositional acceptability over and above the effect of alighting preferences (Ives, 1978). Jones (1977) provides data on the probability of plants of different size classes being acceptable (Table 6.3.). The probabilities in Table 6.3. are used in conjunction with the probabilities from host plant chemistry to give an overall probability of oviposition. As in the case of pre-alighting discrimination, this probability of oviposition is compared with the insect's motivational state and a decision to accept or reject the plant is made.

Table 6.3. The effect of host plant size on alighting and oviposition probabilities to *Pieris rapae* females (Data from Jones, 1977).

Plant Size*	Alighting probability	Oviposition probability
1	0.67	0.18
2	0.18	0.13
3	0.28	0.28
4	0.36	0.43
5	0.06	0.63

* Plant size refers to classes of sizes, the age of the plants are as follows:- (1) large old, 9-14 weeks since transplanting; (2) medium old, 9-14 weeks since transplanting; (3) medium medium, 6-10 weeks since transplanting; (4) Medium young, 4-8 weeks since transplanting; (5) small very young, less than 4 weeks since transplanting.

Clearly this model is a simplified version of the events and decisions made by *P. rapae* females engaged in pre-ovipositional behaviours. Simplification has been necessary from a conceptual point of view to allow the implementation of this model and also to maintain the tractability of the process being simulated. In particular, the model is a simplified version of pre-alighting discrimination in so far as no deterrent cues are incorporated into the model and it is assumed that there is no interaction between sensory modalities. (Table 6.4.).

Table 6.4. Summary of the cues utilised in this model by a female *Pieris rapae*.

	Experimental Evidence		
Cues Utilised	**Field Studies**	**Laboratory Studies**	**Effect**
		Alighting	
Volatiles			
Host Derived	NA	Yes	Increases alighting probability
Non-host derived	NA	Yes	Decreases alighting probability
Colour	Yes	Yes	Increases alighting probability
Size	Yes	NA	Affects alighting probability
		Post-Contact	
Plant chemistry	Yes	Yes	Affects probability of acceptance
Size	Yes	NA	Affects probability of acceptance

The characteristics of both butterflies and host plants have been split into those characteristics which can be construed as being species specific, i.e. the same for all individuals, for example the female butterfly's perceptual abilities or the host plant's glucosinolate profile, and others which are deemed to be individual traits; these tend to be variables which are determined or influenced by that individual's prior history, for example pupal weight is a major determinant of egg-load (Gossard & Jones, 1977). The preference hierarchy of hosts in this model is assumed to be a species characteristic which does not vary between individuals. However, many studies have demonstrated that preference hierarchies are, in fact, individual characteristics which although repeatable for a given individual, do show variation between individuals (Ng, 1988; Singer, 1982). Therefore, the conceptualisation of the model is as shown in Figure 6.6.

Figure 6.6. A conceptual summary of the model as currently developed.

Butterfly →		**Host Plant**	
Individual Characateristics Affecting Motivational State		**Individual Characteristics Determining Acceptability**	
Age	Days	Eggs Present	f(butterfly behaviour)
Egg load	f(pupal weight)-Ovipositional history	Colour	f(species * nutrient status)
Last Plant Accepted		Size	f(age * species)
Food Reserves	f(Time Since Last Feeding)	Larvae Present	f(butterfly behaviour * Weather)
Butterfly Species Traits			
Hierarchy Of Preference			
Perceptual Abilities	**Independent Variables**	**Species Traits**	
	Number & Arrangement Of Host Plants	Chemistry	
		Volatile Cues	

DISCUSSION

This chapter has developed and conceptualised a model of the pre-oviposition behaviour of *P. rapae* to the point where implementation is possible. The model can be construed as being individual based and also an Alife simulation as the interactions between the insect and the plants are not specified at any level above that of the individuals themselves. The usefulness of this approach will only become apparent after implementation when the results of the simulations are available. However, the value of the current exercise lies in formalising what may be considered to be the fundamentals of the processes by which a female finds and subsequently accepts a host plant. This model also, I believe, concurs with the suggestions of Hassell and Southwood (1978) in which they argue that models of insect foraging must be based upon a hierarchy of spatial scales.

In addition, I believe that this model, if implemented, as currently proposed offers the opportunity to make specific predictions about the pre-oviposition behaviour of *P. rapae*. These predictions can then be useful in implementing experimental approaches to the problem. Since this model is largely conceptualised in terms of the current orthodoxy of the theoretical aspects of insect plant interactions, the interplay of simulation and experimentation may offer exciting opportunities to refine the understanding of host plant finding behaviours of *P. rapae* and phytophagous insects in general.

The prospect of the validation of the model remains open, however, because of the necessary scale of field experiments and the large number of measurements on each individual plant and butterfly used in the experiments the model may not be validated. Although, this limits the usefulness of any implementation in terms of using the model as a simulation tool. Its usefulness remains as an aid to understanding and in the development of experimental approaches. It was intended that this model would be fully developed and tested during the course of this research, but unfortunately as time was limited this did not prove possible.

CONCLUSIONS

This chapter has proposed and outlined an Alife simulation based on the pre-oviposition behaviour of *P. rapae* in sufficient detail to allow implementation in an object orientated programming environment, for example C++, Delphi, Smalltalk etc.

Chapter Seven
Thesis Discussion and Future Research

In this discussion, I shall attempt to integrate the results from the preceding Chapters into a coherent argument of the effect of sensory cues on the searching behaviour of *P. rapae*. Clearly, it is not possible to expand this subject by a great deal as the appropriate place to study searching behaviour is in the field. Additionally, the types of experiments conducted here have done little but to show how variations in host plant characteristics may affect the searching behaviour of an insect. This discussion will assume that the effects of the laboratory treatments are largely transferable to the field, but that interactions between them will probably occur.

The problems of searching in a diverse environment are serious for an insect herbivore. Jones (1991) has suggested that, to a moving insect, a diverse plant community is a complicated mosaic from which the insect must pick out cues from plants which are potential hosts from among a large number of non-host plants. Most insects are able to perceive and use cues from a distance to direct their encounters with host plants. Observations of this type, inevitably lead to the question of how insects manage to filter incoming sensory information to provide useful cues for host location.

This question has recently been addressed (Bernays, 1996; Bernays & Wcislo, 1994). As noted, an organism's environment contains more sources of sensory information than can be used at any one time. Additionally, an organism's information gathering capacity also exceeds its capacity to process this information and to act upon it. Therefore, the ability to select information which is relevant is of the utmost importance for regulating behaviour (Bernays, 1996; Bernays & Wcislo, 1994). Having behaviour largely influenced by a restricted set of stimuli is hypothesised as affording three major benefits to the individual; increased accuracy, speed and maintenance of specific neural processing (Bernays, 1996). This type of economy and precision in the processing of information may increase the efficiency and precision of the insect's searching behaviour and its decision-making process (Bernays & Wcislo, 1994).

Therefore, in the following discussion, I will attempt to outline how this concept of efficient searching may be used by *P. rapae* and how it affects the use of sensory cues whilst engaging in a searching bout.

The other set of factors which I wish to enlarge upon are that host plant selection is generally considered to be a hierarchical process in which the female searches for a suitable habitat and then a patch and finally for an individual host (Hassell & Southwood, 1978; Judd & Borden , 1991). The categorisations of the levels of the hierarchy have been defined by Hassell & Southwood (1978) as:-

- Habitat is defined as a collection of patches.
- Patch is defined as a spatial sub-unit of the foraging area in which aggregations of food items occur.
- Food items are defined as the individual items which the insect makes use of, for example the leaves on which a caterpillar feeds.

For a butterfly the gross vegetation type constitutes the habitat, and the patch is then the clump of vegetation that provides the resource being searched for, for example oviposition plants, with the food item being the oviposition sites. Therefore, the searching behaviour of a phytophagous insect may be best examined in this hierarchy. Additionally, the process of host plant finding should be studied at two distinct levels, the first requires that the external and internal factors which influence searching behaviour are understood and the second requires an analysis of the movements and behaviour of individual insects under resource-stimulus and non-stimulus conditions (Judd & Borden, 1988).

At present some of this data is available for *P. rapae*, however, the cues utilised in this progression from habitat to patch to individual food items are not known. However, for *D. antiqua* the process is perhaps better understood (Judd & Borden, 1988; 1991). Visual stimuli play little part in the host plant location process at the habitat patch level as orientation to odour occurs at distances over 100 m, it is considered that long range host plant finding is almost entirely by odour. However, once within a patch visual cues are considered to be important in the decision to alight (Judd & Borden, 1991; Judd *et al.*, 1988). Judd & Borden (1991) have argued that the processing of visual stimuli by the neural system occurs as follows: firstly the shape of the object is compared to a neural template. If the shape matches the template then the information is further processed at a higher level, for example spectral discrimination.

For *P. rapae* it is possible to establish a similar hierarchy for the process of host plant finding. At the habitat level, females may use volatile odour cues to carry out long range orientation. The results from Chapter Two and Four would suggest that the counter-turning behaviour of females and their increased number of flights made in response to volatile odour cues would allow them to reach the patch level. Obviously this does not rule out visual cues as being important but the usefulness of visual cues at such ranges is likely to be severely limited. Once the patch has been reached, the female will almost certainly discriminate on the basis of colour alone. This assumption rests on previous work which showed that *P. rapae* flies directly to green objects (Traynier, 1979) and on the basis that the spectral reflectance of *Brassica* plants are generally considered to be distinctive (Hahn & Muir, unpublished; Judd & Borden, 1991; Prokopy *et al.*, 1983). Additionally, Renwick & Radke (1988) found that there was no discrimination on the basis of shape by *P. rapae*. The results from Chapter Three would indicate that a number of wavelengths may be involved in the discrimination of the plant.

At this stage of the host plant finding process once the visual information has been processed and visually assessed the plant is deemed to be acceptable, then volatile cues are used to establish that the plant is a host plant. Therefore, volatile cues may be used as aids to recognising host plants (short range attraction) but do not actively participate in the discovery of plants. For example, Jones (1987) found that female *P. rapae* from the UK were less likely to land on a non-host plant than females derived from an Australian population. The obvious cue to mediate this discrimination is chemical, most probably olfaction. However, the females may have "learned" the colour of the host and/or the Australian females may discriminate less actively; both of these can explain the behavioural differences observed.

The patterns of movement *P. rapae* have received some attention, and as pointed out in Chapter One the movement of females within a patch is considered to be random with respect to host plants (Fahrig & Paloheimo, 1987; Jones, 1977; Kareiva & Shigesada, 1983; Root & Kareiva, 1984). This behaviour would add credence to the notion that volatiles are not particularly important directional cues at the patch level. However, until a satisfactory explanation of *P. rapae's* searching behaviour is forthcoming the functional significance of this random movement in the patch is unknown.

Once landed the female uses contact chemicals to identify and assess the host plant, with the decision to oviposit being due to the relative degree of stimulation and deterrence from the leaf surface chemicals (Huang & Renwick, 1993; Huang *et al.*, 1995).

The impact of prior experience on this process is perhaps best understood by the arguments of Bernays (1996) and Bernays & Wcislo (1994). In their view the process of host identification and the decision to land and oviposit can be simplified to one sensory cue, spectral reflectance, and any object matching this pattern of reflectance is a suitable host. This not only simplifies the process of host plant finding but also may increase the speed and searching efficiency of *P. rapae* females.

To turn now to the arguments of Bernays (1996) and Bernays & Wcislo (1994) in terms of the more general searching behaviour of *P. rapae* and the cues utilised. Brassica foliage is considered to have a distinctive pattern of spectral reflectance in comparison to other plants. This may allow the shape of the plant to be disregarded in terms of discrimination, and would obviously reduce the amount of information processed. Similarly, for *D. radicum* leaf parameters have been shown to be important but are considered to play a subordinate role (Degen & Stadler, 1996). This may be interpreted as a narrowing of perception for these insects and using the sensory modality which supplies the most information. Additionally, the lack of a response to volatiles during pre-alighting discrimination may also be considered in the same light. However, it is necessary to point out that the above discussion contains a great deal of speculation, and much more experimental work is required in order to test these hypotheses, particularly in the field.

Therefore, in summary, the host plant finding process of *P. rapae* can be characterised as a two stage process as follows: volatiles lead females to the host plant patch and the decision to land on a plant is due to the pattern of spectral reflectance of the plants present, with the possibility that volatile cues act as a sign for host recognition. Once alighted host plant chemistry is central to the process of acceptance. This description is not very different from one which could have been written before this research was started and completed, in fact the only real contribution to this description is that host derived volatiles are somehow implicated in this process, whereas prior to this thesis, this would had an assumption. However, even after this contribution is accepted questions remain. These include; are volatiles actually involved in the attraction of females to host plants or more precisely are *P. rapae* females in any way attracted to host plants from a distance ? The behaviourally active constituents of the volatile odours need to be identified, the similar levels of activity for volatile extracts in Chapter Four, would suggest that such a class of compound common to crucifers is responsible. Additionally, are some volatiles from healthy plants repellents in the same way as different leaf surface compounds in a host plant act as ovipositional stimulants and others act as ovipositional deterrents ?

Similarly the results of Chapter Four provoke many unanswered questions. The finding that colour was dominant in controlling the landing behaviour has long been recognised with Hovanitz & Chan (1964) showing that blue green colours were the most preferred for oviposition and Traynier (1979) also showed that *P. rapae* performed a direct and rapid flight towards green objects. Renwick & Radke (1988) and Myers (1985) showed that the reflectivity at 550 nm and the ratio of red to infrared reflectance were important in determining the number of landings and eggs respectively. Therefore, the only real contribution to the study of colour in *P. rapae*'s behaviour was the finding that the narrower band of significant relationships between the RNQ of light reflected at different wavelengths in the presence of host plant phytochemicals as compared to the untreated cards. This would indicate that although responses are identical, the sensory perception of plant characters may be altered in the presence of such chemicals, and the obvious suggestion that the wavelength specific behaviour for drumming and oviposition demonstrated by Kolb & Scherer

(1982) for *P. brassicae* also occurs for *P. rapae*. However, the present study in no way demonstrated that wavelength specific behaviour occurs in *P. rapae*.

Therefore, chemicals may have greater and more subtle effects on the interactions between a host plant and a phytophagous insect than simply eliciting or deterring behaviours. However, the interaction between the sensory perception of light and resultant behaviour needs more clarification. The obvious way of investigating this interaction is through the use of monochromatic light in the presence of host plant phytochemicals and comparing the resultant action spectrums for landing behaviour. Additionally, I think that field studies investigating the possible interactions between behaviours and visual characteristics would be valuable.

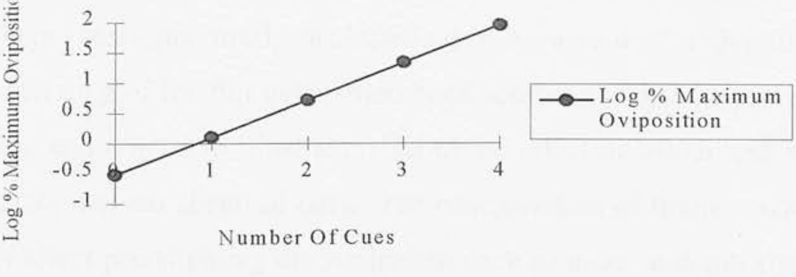
In a more positive light, the results from these experiments have clearly shown that host plant choice in *P. rapae* is more than a simply chemically mediated interaction in which glucosinolates stimulate oviposition and the presence of deterrents in host plants deter oviposition, with the choice of oviposition site resulting from the interaction of these two factors. This result in itself is not surprising as many authors have argued that insects respond to the gestalt of a plant rather than a single factor (Dethier, 1982; Harris & Foster; 1995; Miller & Strickler, 1984).

At present most research on the cues utilised by females in selecting plants for oviposition have been studied independently. This type of research reveals very little about the relative importance of each sensory modality or interactions between them. Selection of oviposition sites generally involves some interplay between sensory modalities, however, for most insects the understanding of this interplay is rudimentary (Judd & Borden, 1991).

So although the study of single sensory inputs allow the elucidation of the stimuli which bring about a particular behaviour, they can by their very nature show nothing about how the behaviour is organised in the central nervous system and what the causes of that behaviour are (its mechanism) rather than what elicits a behaviour. Additionally, the process of sensory integration is inevitable when the large number of sensory inputs (both internal and external) which are known to influence behaviours are considered (Harris & Foster, 1995).

Therefore, Harris & Foster (1995) argue that no behaviour's immediate cause can be determined by whether or not one stimulus elicits a response. Over 30 years ago Kennedy (1965) commented that host selection is a catenary process " a chain of behaviour made up of different responses, usually to different stimuli ... each of which is received as a result of the insect making the previous response in the chain". The weakness of such a scheme in Kennedy's opinion is the idea "that each response has one self-sufficient kind of stimulus, as if each kind of stimulus acted on its own". Whereas, "One response not only brings in the external stimulus for the next following response in the chain, but may also prepare the insect for it by lowering the response threshold" (Kennedy, 1965). There is little experimental evidence to suggest the relative importance of the different sensory modalities. However, Miller & Peters (unpublished) have shown that in *D. antiqua* the addition of cues from different sensory modalities to model plants results in an increase in the number of ovipositions as shown in Figure 7.1.

Figure 7.1. The relationship between the number of sensory modalities and the percentage maximum ovipositions in *Delia antiqua* (Data from Miller & Peters unpublished).



An additional problem in the understanding of the insect's host finding and acceptance phase is the enthusiasm prevalent amongst researchers for identifying active compounds but not showing fully how the compounds identified are mediated by other factors. For example, the responsiveness of females to oviposition sites is known to be affected by egg-load and host deprivation yet very few reports exist which have clarified how this increased responsiveness is mediated in the insect and its processing of information. So although, chemical ecology has been defined in the broadest sense as "the study of chemical interactions between organisms and their environment, which includes other organisms" (Feeny, 1992), in practice, chemical ecologists have primarily concerned themselves with the roles of chemicals which act as mediators of recognition and resistance, ignoring the role of chemicals in nutrition and primary metabolism (Feeny, 1992). An example relating to *P. rapae* will perhaps explain more fully what is meant by this statement. Initially, Renwick and co-workers started by testing single compounds and comparing them with extracts from hosts. This work has been very successful, in my opinion, and demonstrates a progression from a simple concept of what is the effect of this compound on oviposition (for example Renwick & Radke, 1983), to the concepts of stimulants and deterrents mediating oviposition (Huang & Renwick, 1993; Huang *et al.*, 1995), to the present state in which the effects of both glucosinolates and cardenolides on receptors has been correlated to their behavioural activity as stimulants or deterrents respectively (Stadler, Renwick, Radke & Sachdev-Gupta, 1995). Indeed, Renwick & Huang (1994) argued that the *Pieris* crucifer system could be used as a model for unravelling the effects of leaf surface chemicals on host plant acceptance. However, they also noted that they were barely scratching the surface of these complex interactions.

Overall, this research has demonstrated that for *P. rapae* the factors affecting oviposition have been investigated in some detail, both herein and by other scientists. Although, areas previously neglected have been studied, redressing the bias in the understanding of the full oviposition behaviour of *P. rapae*, e.g. the role of volatiles, learning and a more detailed appraisal of the effect of colour and its interaction with host plant derived chemical cues. The concentration of future research on the factors which affect pre-alighting discrimination such as more in-depth studies of the role of plant volatiles, particularly dose-response relationships and the identification of the behaviourally active volatile constituents, in host plant selection, the pre-dominance of research in post-alighting discrimination can be further redressed. However, an insect responds to the "gestalt" of a potential host plant and although showing behavioural responses to volatile extracts can be viewed as worthwhile such experiments add little to the overall understanding of the process of host plant finding and acceptance by *P.* rapae or of other phytophagous insects.

An equally important and challenging research area is to investigate the role of prior experience on the ovipositional choices and preferences of females. Learning has been shown to increase the searching efficiency of phytophagous insects. This may be a major factor in influencing a female's alighting preferences during an ovipositional bout. However, the role of learning in *P. rapae* has not been investigated in 'field situations'. I assume that since learning is important in the ovipositional behaviour of other insect species, it may also be similarly important for *P. rapae*. However, without field studies; a major gap is apparent in the searching behaviour of *P. rapae* for host plants.

I believe that the most fruitful approach, where possible, for future studies is in the field. A study of behaviour in the laboratory may result in findings which may not be generally applicable to the field. Whilst field studies are more complex and less controlled, thereby making it harder to manipulate variables independently with a commensurate loss of clarity, they do reveal more realistically the complexities of host plant exploitation by butterflies.

Therefore, perhaps one of the largest and most profitable areas for future research is a shift from the mechanistic view of the interactions between an insect and a particular host plant species to the wider ecological backdrop of these interactions. At present, virtually all of the research effort is directed towards a greater understanding of the factors influencing ovipositional decisions. Whilst this interaction is important and a greater understanding of these interactions is useful, the wider context cannot be ignored. Where this type of research has been attempted, some interesting patterns have emerged. For example, the interaction between the different trophic levels including other herbivorous competitors may be viewed as crucial in explaining observed ovipositional patterns in the field. In Europe, one can immediately think of the laboratory results from the oviposition deterring pheromone of *P. brassicae* affecting the ovipositional decisions of *P. rapae* and the fact that plants damaged by grazing herbivores are less acceptable for oviposition than undamaged plants.

The effect of parasitoids is probably important since they are known to utilise both plant and herbivore derived cues to direct their searching behaviour to encounter caterpillars. Therefore, in theory at least, an effect on ovipositional decisions may also be exposed. In this context, the searching behaviour of *P. rapae* may not be paradoxical if females do indeed reduce the impact of parasitoids on their own eggs by widely distributing them.

Thesis Conclusions

The experiments carried out show that *P. rapae* females exhibit both pre-and post alighting discrimination of potential host plants. The sensory cues responsible for this discrimination have been evaluated, the results for pre-alighting discrimination would suggest that it is possible that both visual and volatile odours are involved. However, the relative contribution of both of these sensory modalities is unknown.

The effect of colour on landing was further investigated using a regression analysis of the spectral profiles of the coloured cards. This analysis indicated that the frequency of landings on each colour of card could be predicted, in most cases by the relative reflectance at 550 nm or by the ratio of red to infrared reflectance. Furthermore, it may be that the relative reflectance at 550 nm and the ratio of red to infrared relative reflectance are proxies for the suitability of a host for larval development. However, the multiple regression models developed explain a far greater degree of the variability associated with the data from the choice experiments and as such may show that a number of regions of spectral reflectance are important in determining the selection of substrate based on its pattern of spectral reflectance.

Additionally, in the presence of volatile odour cues *P. rapae* females exhibit an increase in the number of flights and the total duration of time spent in flight. When exposed to non-host plants treated with volatiles there was an increase in the number of alightings on such plants as compared to controls. However, it is postulated that visual cues are dominant, with volatile odours acting as host recognition cues; that is, they define a plant as a potential host.

A female's prior experience affects subsequent oviposition behaviours and the quality of the host (in terms of its stimulatory chemicals) would appear to have a large influence on the subsequent acceptability of the host with which the butterflies had prior experience.

Furthermore, the behaviour affected appears to be the choice of landing site, and from what is known of the cues learned in *P. rapae* it appears that prior experience of a host allows the females to search preferentially for that host in subsequent search bouts on the basis of its colour.

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