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Multisensory Foraging by Hymenopterous Parasitoids

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Proefschrift

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Stellingen

1 Het feit dat onderzoek naar visuele orientatie zich over het algemeen beperkt tot de individuele visuele componenten (kleur, vorm, patroon of beweging) leidt tot een onderschatting van de betekenis van visuele stimuli.

Dit proefschrift.

2 Het camoufleren van vraatschade door herbivore insekten kan behalve door de selektieve druk van vogels, verklaard worden als een adaptatie aan visueel fouragerende arthropoden.

Dit proefschrift.
Heinrich, B. (1979). *Oecologia* 42:325-337.

3 Onderzoek naar sensorische preferenties zijn alleen dan interpreteerbaar wanneer rekening gehouden wordt met de ervaring en de fysiologische toestand van het onderzochte organisme.

Dit proefschrift
Bell, W.J. (1990). *Ann. Rev Entomol.* 35:447-467.

4 Gedragstudies zijn ongeschikt om aan te tonen dat een stimulus *niet* wordt waargenomen.

Dit proefschrift
Salt, G. (1934). *Proc. Roy. Soc. London B* 144:450-476.

5 Bij de beoordeling van het belang van verschillende voedselbronnen in de voedselvoorziening van natuurlijke vijanden, dient behalve met de criteria beschikbaarheid en kwaliteit ook rekening gehouden te worden met de waarneembaarheid.

Dit proefschrift
Jervis, M.A. and Kidd, N.A.C. (1986). *Biol. Rev.* 61:395-434.

6 Vooral volgevreten vrouwtjes van *Cotesia rubecula* houden van spruitjes.

Dit proefschrift

7 Het verdient aanbeveling de benaming van visuele stimuli onderteverdelen analoog aan de indeling en naamgeving van chemische signaalstoffen.

Dicke, M. and Sabelis, M.W. (1988). *Funct. Ecol.* 2:131-139.

8 Het objektieve imago van de wetenschap ontkent de subjektiviteit van onderzoekers en geldschieters.

9 Door de algemene tendens om zogeheten 'negatieve' resultaten ongepubliceerd te laten, gaat veel waardevolle informatie verloren.

10 Het in geval van nultolerantie vereiste "ziektevrij" zijn van produkten zegt meer over de onbetrouwbaarheid van de gehanteerde bemonsteringsmethodes, dan over de feitelijke aantasting.

11 Doordat de procedure voor toelating van biologische bestrijdingsmiddelen van de toelatingsaanvrager standaardonderzoek eist, tenzij deze kan beargumenteren dat de gevraagde onderzoekingen irrelevant zijn, wordt het gebrek aan beleid afgewenteld op de schouders en portemonnee van de aanvrager.

12 De term "permanente verblijfsvergunning" is misleidend, aangezien allochtonen (zelfs wanneer zij in Nederland geboren zijn) deze samen met verdere rechten onherroepelijk kwijtraken bij zes maanden buitengrenzelijk verblijf.

13 Door overmatig misbruik dreigt het begrip "duurzaamheid" bij het klein terminologisch afval te belanden.

Stellingen behorend bij het proefschrift:

Multisensory foraging by hymenopterous parasitoids

Wageningen, 26 januari 1994 Felix Wäckers

"Alles Sichtbare ist Ausdruck,
alle Natur ist Bild,
ist Sprache und farbige Hiëroglyphenschrift ..."

(Hermann Hesse)

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Part 2: Host foraging; host location

Part 3: Host foraging; host discrimination

Chapter 1

General introduction

Insects possess various sensory modalities, which they can use for orientation (Schöne, 1983). The research into sensory orientation of hymenopterous parasitoids has long focused on the capacity of parasitoids to detect and learn chemical information. Ever since the early reports of parasitoids being attracted to odors from their hosts (Hase, 1923), literature has abounded with reports of how chemical information governs various aspects of parasitoid behavior. Chemosensory information has been found to be involved during search and selection of food (Jervis, *et al*, 1993), mates (Askari and Alishah, 1979), as well as hosts (Vet and Dicke, 1992). Since our interest in parasitoids stems primarily from their ability to parasitize invertebrates noxious to man, research on parasitoid foraging has traditionally focussed on aspects of host location. Concerning the host foraging sequence, Doutt (1959) distinguished three subsequent phases: host habitat location, host location, and host selection. Within each of these phases, chemoreception has been demonstrated to be involved in the initiation and guiding of various behaviors (van Alphen and Vet, 1985). At the long range, volatile chemical cues are involved in the initiation of flight, guiding of upwind flight and hovering, and initiation of landing. Common responses at the short range (following landing) include orthokinesis, klinokinesis, antennation, ovipositor probing, and stimulation of oviposition (Vinson, 1984). Although different concentrations of one chemical could theoretically elicit this complete foraging sequence, the systems that have been studied in sufficient detail show that various chemicals are involved during the different phases of host location (Turlings *et al.*, 1993).

Parasitoids operate within a multitrophic system. Price *et al.* (1980) classified primary parasitoids (together with predators) as the third trophic level, their hosts as the second, and the host's food as the first trophic level. Both the hosts and the substrates on which these hosts can be found, provide potential information to the foraging parasitoid. A seperate category of foraging information is formed by stimuli deriving from parasitoids themselves (the third trophic level). Parasitoids are known to leave odor marks during or after oviposition and during investigation of foraging sites (Price, 1970). The behaviors that may be invoked by these marking pheromones are usually opposite to the behaviors elicited by host derived stimuli. Marking pheromones may deter antennation, probing, and or oviposition, and may induce parasitoids to leave the marked site.

It is beyond doubt that the available research on the chemical ecology of parasitoids has elucidated a wide range of foraging stimuli and mechanisms which are vital to our understanding of parasitoid behavior. The emphasis on chemical stimuli, however, has sometimes overshadowed the interest in the role of other sensory modalities in parasitoid foraging. In comparison to the in-depth investigation of olfactory and chemotactile cues, the attention given to other sensory modalities has been peripheral at best. Little or nothing is known about orientation by parasitoids to heat, gravity, electric or magnetic fields, while the role of mechanoreception (tactile and acoustic) and visual stimuli in parasitoid foraging has been only touched upon (Lawrence, 1981; Sugimoto *et al.*, 1988; Wäckers and Lewis, 1993).

Reviews of sensory orientation in parasitoids often justify this one-sidedness with claims that chemoreception is the main, or dominant, perceptive modality. Since no comparative studies are available that specifically address the relative role of different sensory modalities in parasitoid foraging, this claimed dominance remains unsubstantiated and may reveal an investigatory bias as much as it reflects ecological facts. Furthermore, the complexity of (multi-) sensory ecology makes it difficult to generalize about the relative significance of different modalities. The role of individual modalities may vary depending on the species, the foraging mode of the individual, the phase of the foraging process and the environmental conditions. In addition to variation within individual modalities, different sensory modalities may also interact during foraging (Prokopy, 1986).

Comparing sensory modalities

Even though foraging parasitoids can draw upon olfaction, chemotaction, vision, mechanoreception, and probably additional sensory modalities, the utility of individual modalities will be contingent on characteristics of both receptor and emitted stimulus. In combination they determine the range of perception, as well as the power of discernment. Because of the interdependence of stimulus and receptor during the process of perception, both should be considered when comparing sensory modalities. Since this work deals with chemical and visual orientation, the comparison will focus on these sensory modes.

- Receptor characteristics

Chemosensory organs combine an extreme selectivity with a high degree of sensitivity (Cardé and Charlton, 1984). The antennae of the male silkmoth perceive female sex-pheromones at concentrations as low as one thousand molecules per cubic centimeter (Kaisling and Priesner, 1970). The olfactory sensitivity of bee antennae is about 6 orders of magnitude lower (Schwarz, 1955).

Besides their antennae, parasitoids are known to possess chemosensory receptors at their ovipositor (Vinson, 1984), tarsi (Salt, 1937) and palpae (gustation). Parasitoid antennae serve as receptors for both olfactory and chemotactile cues. The other chemosensory cues are thought to be used for chemotaction only.

In comparison to vision, which has an inherent directionality, olfaction is more limited in supplying information on the direction of the source, as well as on the position of the receiving organism (Prokopy 1986). The fact that chemosensory organs are believed to be intensity sensitive, rather than direction sensitive, makes them suitable for orientation to odor gradients. Honeybees, for instance, when trained to a certain odor concentration, will seek out that specific concentration within an odor gardient. The fact that both antennae detect odors seperately, moreover, enables insects to perceive spatial odor patterns (Kramer, 1976).

The compound eye. In comparison to the uniform single lens eye of vertebrates, the design of the compound eye shows a wide morphological and physiological diversity. This great variability provides the flexibility for selective adaptation among species exhibiting different life styles and existing in different environments.

Compound eyes are capable of distinguishing shape, pattern and colors. Color vision is based on three, or in some species four, types of visual cells (Bernard, 1979). The spectrum of perceivable wavelengths usually ranges from ±300 nm to ±700 nm. Thereby it is as wide as the human spectrum, but shifted towards the shorter wavelengths (Kühn, 1927; Menzel, 1971). As far as hue discrimination is concerned, the honey bee has been demonstrated to be able to distinguish between wavelengths which differ by only 4.5 nm (Helversen, 1972).

The angular resolution of compound eyes lies within the range of 1°-10°, which is two orders of magnitude lower in comparison to the human eye. However, when we consider that the distance between objects and the receptor is usually substantially less in interactions between insects and their environment, this visual acuity is likely more than adequate. The eyes of many insects contain areas specialized for high resolution. Eyes may possess areas with increased visual acuity, so-called "fovea", while other areas may have a lower visual acuity, but higher movement sensitivity (Wehner and Srinivasan, 1984).

A definite advantage of compound eyes is their wide field of view. Since compound eyes are shaped to create a convex retina, they can provide an almost omni-directional perception (Wehner and Shrinivasan, 1984).

Visual perception is suited for both directional orientation as well as positional orientation. Only visual perception can provide reliable information on the distance of the source. Distance estimation at close range is possible by binocular triangulation (Rossel, 1983), while motion parallax enables appraisal of distance at longer ranges (Collett, 1978).

- Stimulus characteristics

The utility of various stimuli as foraging cues depends both on their detectability to the forager and on their reliability in indicating resource presence, accessibility and suitability (Vet *et al.*, 1991; Wäckers and Lewis, 1993). Aspects of both stimulus detectability and reliability should therefore be taken into account when considering the potentialities and limitations of a stimulus as a foraging cue.

Chemical stimuli. The extreme selectivity of chemical perception makes chemical cues especially suited in situations in which reliable identification is crucial. This explains why behaviors that are directly linked to reproductive success of parasitoids, such as mate recognition and the identification of suitable hosts seem to be primarily governed by chemical stimuli.

The detectability of olfactory stimuli is a function of the rate of molecule emission, the release area, the distance between insect and odor source, wind speed, turbulence and probably also contrast against background odors.

In the absence of wind, odor dispersal is determined by diffusion, which creates a spherical odor field with gradients of decreasing odor concentration at increasing distance to the odor source. Under natural conditions, however, air is generally moving at speeds exceeding diffusion (Levi, 1978). As a consequence, olfactory information is usually transmitted in meandering odor plumes which are only detectable downwind from the odor source (Elkinton and Cardé, 1984). Insects, therefore, will experience olfactory information in bursts, which makes detection of olfactory stimuli inconsistent over time and distance to the source. Upwind orientation within an odor plume, however, enables insects to trace the source of an odor plume over a distance of meters.

Unlike sounds and most visual signals, chemical signals placed on other organisms or objects in the environment continue to be transmitted in the absence of the marking individual. This high durability makes them suitable for marking objects and organisms for subsequent retrieval or avoidance. Non-chemical marking signals are rare in insects. Hoverflies are known to mark their territory through their physical presence (Collett and Land, 1975).

Visual stimuli. The visibility of an item is a function of the item's dimensions, pattern, and contrast against the background, and is furthermore dependent on the distance between the insect and the item, as well as on the intensity and type of illumination (Prokopy and Owens, 1983). Visual detection is independent from air movement, and visual stimuli are consistent with small changes in distance to the source (Miller and Strickler, 1984). Unlike olfactory stimuli, which are usually transmitted in moving air, visual cues can be perceived omni-directionally. Visual stimuli are furthermore easy to locate. Animals with anything but the most primitive visual receptors locate the source of a visual stimulus in the very process of perceiving it (Marler and Hamilton, 1966). In comparison to olfactory cues, whose production may fluctuate with environmental conditions, visual cues are often more stable (Loper, 1972; Pham-Delegue *et al*, 1989; Turlings, unpublished data). This stability increases the reliability of visual stimuli. The often noticeable visual change induced by pollination or aging of flowers (Gori, 1983) is a variable that only adds to the reliability of visual stimuli, since it allows pollinators to discriminate between rewarding and unrewarding flowers.

Color, shape and pattern can be distinguished as individual categories of visual stimuli. Of these, only color is independent of the viewing angle, while pattern and especially shape often change with varying angle of view. While the limited angular resolution of the compound eye limits detailed recognition of shape and patterns at longer distances, color detection is less dependent on a fine resolution. Shape and pattern perception, on the other hand, are relatively independent of illumination, whereas illumination is a crucial determinant of color characteristics (Levi, 1968). Vision, in general, has limited value in dim light or at night. Fireflies have overcome these restrictions, by providing their own light source (Case, 1984).

The limitations of insect visual acuity, in combination with the limited size of insects, may have restricted the role of visual signalling between insects. Most visual adaptations found in insects (both aposematic coloration, as well as mimicry), are thought to be directed to vertebrates, birds in particular (Heinrich, 1993). Still, intra-specific visual signalling have been described in various insects, with the function of either mate and/or species recognition (Obara and Hidaka, 1968; Thörig, *et al.*, 1981; Collett and Land, 1975; Case, 1984).

The lack of investigatory interest in the role of visual stimuli in parasitoid foraging is especially remarkable, considering that most of our knowledge of insect visual ecology is based on work done with other Hymenoptera. Besides the prominent work on vision in honeybees (for an overview see Gould and Towne, 1988), aspects of visual orientation have been studied extensively in digger wasps (Tinbergen and Kruyt, 1938; van Iersel, 1975; Rosenheim 1987) and ants (Hölldobler and Wilson, 1990 and references within).

Two factors may explain the large focus on visual stimuli in these groups of Hymenoptera, in comparison to parasitoids (Turlings et al., 1993). The interest in visual orientation of flower pollinators was early on aroused by the striking visual display of insect-pollinated flowers towards their pollinators (Darwin, 1876). Von Frisch (1915) showed that bees can indeed use the displayed visual information to locate nectar sources, while learning of visual differences enables them to specialize on the most rewarding source. His comprehensive work set the stage for an extensive line of ecological and physiological research on visual orientation and perception in honeybees (Gould and Towne, 1988; Menzel *et al.*, 1993).

In comparison, the interaction between herbivores and their natural enemies is less conspicuous. Parasitoids and predators are actually likely to put a selection pressure on their hosts to minimize its chances of being detected. Therefore, in contrast to the visual display evolved in the mutualistic interaction between plants and their insect pollinators, parasitoids and their hosts are involved in an evolutionary game of hide and seek.

The second factor that drew the attention to visual orientation in bees, ants and digger wasps, was the fact that they commute between a home base and foraging sites. This central place foraging confronted investigators with the inevitable question of how these insects are able to find their way back to their nests or to profitable foraging locations. Subsequent research has revealed such intriguing visual orientation mechanisms as landmark learning, orientation to the sun, moon and polarized light (Gould and Towne, 1988; Hölldobler and Wilson, 1990).

Host foraging parasitoids, on the other hand, are expected to abandon host sites when prolonged search no longer contributes to fitness optimization (Mac Arthur and Pianka, 1966). Consequently, parasitoids are not expected to utilize spatial orientation to retrieve previously visited host sites.

These differences between parasitoids and honey bees do not, however, preclude the use of visual information by foraging parasitoids. Instead of using spatial orientation to retrieve profitable sites, parasitoids might use spatial memory to avoid repeated parasitization of the same host (visual host discrimination), or to avoid duplication of searched area. It seems also conceivable that parasitoids, in analogy to honey bees, use the olfactory as well as the visual display of flowers to locate floral nectar.

Outline of the research

This research addresses the role of olfactory as well as visual stimuli during various phases of parasitoid foraging.The experiments were done with two solitary parasitoids of lepidopteran larvae: *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoïd of the cotton bollworm en related species (*Helicoverpa* spp.), and *Cotesia rubecula* (Hymenoptera: Braconidae), a solitairy parasitoïd of the small cabbage white (*Pieris rapae*). Some of this work corresponds with studies on honey bee foraging, which allows for comparisons, and reveals contrasts or analogies. In addition, this work attempts to address unique aspects of parasitoid sensory ecology that relate to facets specific to parasitoid foraging, such as host discrimination or the switch between food and host foraging.

This report on sensory orientation in parasitoids is divided in three parts, each dealing with an individual aspect of parasitoid foraging, namely, food foraging, host location, and host discrimination.

Food foraging. Even though the ecological research on parasitoids has concentrated on the question of how parasitoids locate their hosts, food foraging can be just as essential to the fitness of the parasitoid. Most parasitoids need external energy sources both to survive and for the production and maturation of eggs (Bartlett, 1964; Lum, 1977). In the field parasitoids are known to feed on various nectar sources (Jervis *et al.*, 1993). Nevertheless, there are very few data with regard to the stimuli by which parasitoids actually detect their food sources. It was investigated whether parasitoids possess innate preferences for flower fragrances and for flower colors.

Behaviour can be externally triggered (signals) or internally triggered (physiological state). To determine the effect of hunger on sensory preferences, innate visual and olfactory preferences were determined both for both hungry and satiated individuals.

Host foraging; host location. Parasitoids can use different strategies to circumvent the low detectability of their hosts (Vet *et al.*, 1991). One of these strategies is the parasitoid's ability to learn to link highly detectable substrate cues to reliable host derived cues.This associative learning could also enable parasitoids to employ detectable visual information from the host's environment as foraging cues. It was studied whether parasitoids are capable to learn visual parameters (color, shape and pattern) in association with host presence.

It was investigated whether parasitoids can distinguish between profitable and unprofitable structures on the same plant on the basis of olfactory and/or visual learning. Multisensory learning was compared to learning of individual stimuli, and the hierarchy of individual stimuli during multisensory conditioning was determined.

Host foraging; host discrimination. The final part of this study focused on the last phase of host foraging: the detection and acceptance of the individual host. It was investigated how parasitoids employ both olfactory and visual information either to recognize hosts which have been previously parasitized, or to avoid sites which have been previously searched.

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Chapter 2

Finding floral nectar and honeydew in *Cotesia rubecula*: Random or directed?

SUMMARY

Several aspects of food foraging were investigated for *Cotesia rubecula*. Provision of sugar water was demonstrated to prolong the average life span of male and female parasitoids by a factor 9 and 14 respectively. The response of parasitoids to flowers (floral nectar) and aphid infested leaves (honeydew) was tested in a y-tube olfactometer. Irrespective of their state of hunger, parasitoids were attracted to flower odors. Parasitoids did not respond to aphid infested leaf material.

INTRODUCTION

The importance of finding food for survival has been described for many parasitoid species (Zoebelein, 1955; Leius, 1967; Jervis and Kidd, 1986). However, the question of how parasitoids find their food sources under natural conditions has only been investigated to a limited extent.

Since *Cotesia rubecula* feeds on nectar, while parasitizing herbivorous *Pieris* spp., the process of food foraging is dissociated from host foraging and should be considered as an individual foraging process. In the field, nectar-feeding parasitoids have various sugar sources available. Besides floral nectar, they can use honeydew, and extra-floral nectar for feeding (Leius, 1960). In numerous field studies parasitoids have been recorded on nectar secreting structures, and laboratory feeding studies have confirmed that nectar from various sources can prolong the life span of parasitoids (Jervis et al., 1993). These studies, however, fail to identify and quantify the factors that determine food encounter and food acceptance. Only by clarifying these factors it will be possible to elucidate the respective role of various food sources in the diet of parasitoids.

Kidd and Jervis (1989) stressed the importance of the availability (abundance; distribution) and the quality (nutritional value) of food sources. These two factors are sufficient to describe the food encounter rate for dystropic insects, whose random search gives them an equal chance to encounter any exposed food source. In eutropic insects, however, food detectability is an additional crucial factor. Food sources that can be easily perceived from a distance are more likely to be visited than food sources that are only detected upon contact. The latter is especially relevant for insects like *C. rubecula* that do the majority of their foraging in flight, relying on long-range cues, while covering only a limited area after landing on the plant.

In this study, we first determined the effect of feeding on parasitoid survival. Subsequently we investigated the olfactory detectability of flowers (floral nectar) and aphid infested leaves (honeydew) to *C. rubecula*. This was done by testing both starved and satiated parasitoids for their response to the odors of these food sources.

MATERIALS AND METHODS

Cotesia rubecula had been collected in Brussels sprouts and red cabbage fields from *Pieris rapae* larvae and subsequently reared for ca. seven generations on *P. rapae* larvae feeding on brussels sprouts. The parasitoid pupae were collected three times per week and were allowed to emerge in plexiglass cages (30 x 40 x 37 cm) at 25°C, 50-70% RH and a 16L:8D photocycle. Daily transfer of the pupae assured cages with parasitoids of uniform age. Satiated parasitoids were provided with sugar water (70%) and water. Starved parasitoids were given water only.

Survival experiment

To determine the survival of *C. rubecula* in the presence and absence of sugar water, one hundred individuals of either sex were divided upon emergence over ten plastic containers (12 x 12 x 8 cm) and kept at 25°C and a 16L:8D photocycle. Daily, one half of the containers was provided with both water and sugar water (70%), presented on separate cotton-wool plugs (satiated). The other half was given water only (starved). The number of surviving individuals per treatment was counted daily.

Olfactometer experiment

One-two day old females were used in the olfactometer experiments.

Y-tube olfactometer. The olfactometer used was comparable to the one described by Steinberg *et al.*, (1992). Here, Erlenmeyer flasks were used as odor containers. Both flasks were placed in a black plastic dish (height 4 cm) to rule out visual perception of the plant material. Air pressure generated an airflow through both arms at a rate of 3 l/min. At the base of the olfactometer the air was extracted by the vacuum system of the building at a rate of 6 l/min. Flow meters were used to control and attune both air import and air extraction in order to ensure constant air pressure and a laminar airflow within the olfactometer. Olfactometer experiments were done at 25 ± 2 °C, and 40-60% RH. The light intensity was 600 lux, provided by two 16 W TLD fluorescent tubes, located behind and in front of the set-up. Various odor sources were tested in the y-tube olfactometer for their attractiveness to starved and/or satiated parasitoids.

Flowers and leaf material used in the Y-tube olfactometer were collected from the field. Flowers and leaf material were selected from plants free of any herbivore damage.

Ground-elder (*Aegopodium podagraria* L.; Umbelliferae) was chosen since flowers from the family of the Umbelliferae are known to be frequently visited by various parasitoid species (Kevan, 1973). Their exposed nectaries provide accessible nectar to nectar feeders with short mouth parts (Leius, 1960). A single umbel was used as an odor source in the choice experiments.

Rape seed (*Brassica napus* L.; Cruciferae) was chosen since it is known to be a host plant for *Pieris* spp. To match the biomass of one ground-elder umbel, ten rape seed flower heads were used as an odor source.

Myzus persicae **on lettuce (*Lactuca sativa* L.) and rape seed leaves** were obtained from the greenhouse culture as described by Reinink *et al.*, (1988). Infested leaves were covered with honeydew and contained 100-200 aphids of different instars, and their exuviae. Uninfested leaves were taken from aphid-free plants.

Test procedure

Parasitoid females were introduced into the central tube of the olfactometer, 1 cm from a start line. The observation started as soon as the parasitoid passed this start line. Walking upwind, the parasitoid could choose at the bifurcation between both olfactometer arms. The observation was counted as a choice when the individual passed the finish line in one of the arms for a period of 15 seconds. The small fraction (no more than 10% in any of the tests) of individuals that had not made a choice within 2 minutes was discarded. The connections between the odor source containers and the olfactometer arms were exchanged after every five parasitoids tested. Odor sources were renewed after every ten parasitoids tested. At the end of each day odor containers were cleansed with 70% ethanol.

RESULTS

Survival experiment. The availability of sugar water increased the life span of *C. rubecula* significantly for both sexes (Wilcoxon, $p<0.005$) (fig 1). Starved parasitoids lived an average of only 1.6 days (females) and 2.2 days (males), while the average life span for fed parasitoids was 23.2 days (females) and 19.5 days (males). When sugar water was available, females lived longer than males (Wilcoxon, $p<0.05$).

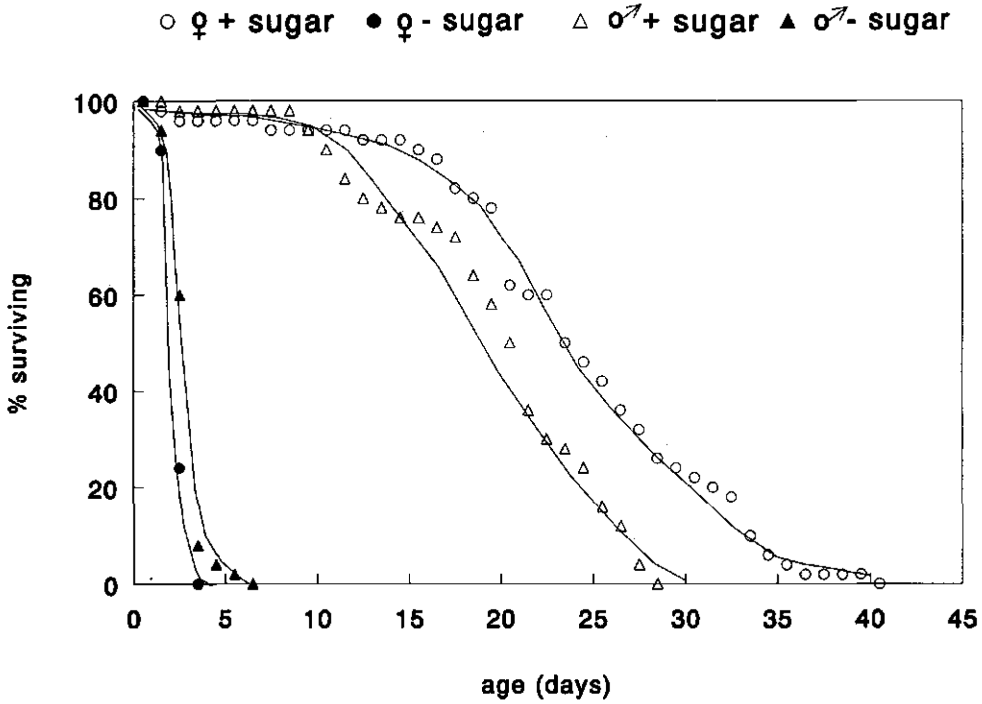

Fig. 1: Survival (in %) of *C. rubecula* in presence and absence of sugarwater.

Olfactometer experiments. Starved as well as satiated parasitoids were attracted to flower odors. Both flowers tested (rape seed and ground elder) were chosen significantly more often than the corresponding undamaged leaf material (fig 2).

Starved parasitoids were not attracted to aphid infested rape seed- or lettuce leaves when tested against clean leaf material (fig 2).

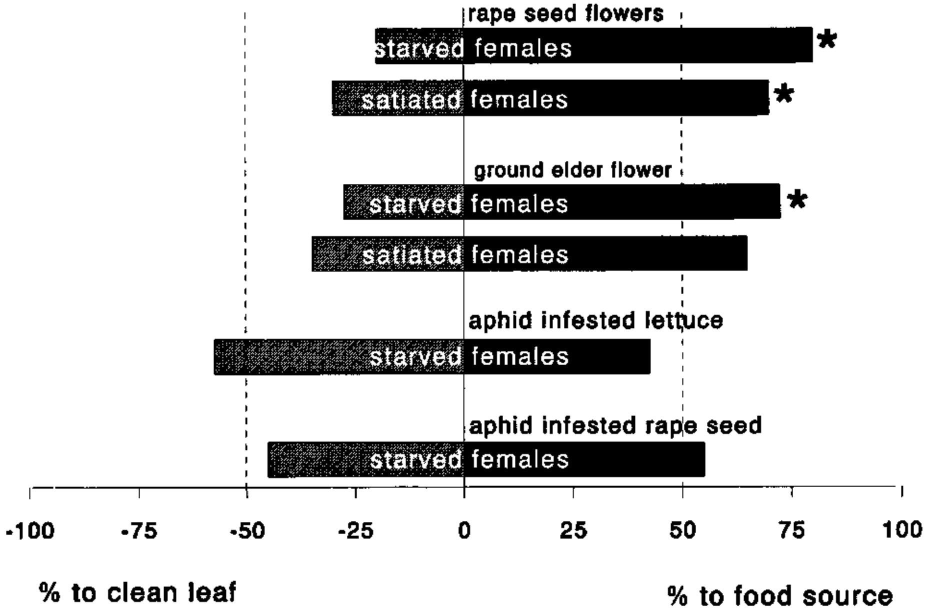

Fig. 2: Odor preferences of *C. rubecula* when given a choice in a y-tube olfactometer between various food sources and corresponding leaf material. N=40 for each comparison. Stars indicate a significant preference (test for binomial distribution; p=0.05).

DISCUSSION

<u>Survival experiment</u>: Our data show that feeding increases the average life span of male and female *C. rubecula* by a factor nine and fourteen respectively. This finding confirms the importance of food foraging for the survival of parasitoids and underlines that the availability of food sources can be a crucial element in biological control. It should be considered that laboratory studies may actually overestimate the life span of starved parasitoids. Since flight is the behavior which requires by far the most energy (Elton, 1966), free ranging parasitoids are likely to use more energy than individuals confined to small cages in the laboratory.

__Olfactometer experiments__: **Flower odors.** Both starved parasitoids and parasitoids satiated on sugar water were attracted by flower odors. This innate response will enable inexperienced parasitoids to locate floral nectar. The fact that *C. rubecula* responded both to flowers of a cruciferous and an umbelliferous plant, indicates that their flower odor preference is not restricted to the plant family on which these parasitoids find their hosts. Such "flower generalism" is adaptive when host infestation and nectar availability are not synchronized within a given plant species.

Aphid infested leaves. Besides floral nectar, parasitoids can feed on a variety of sugar sources (Kevan, 1973). Honeydew can be an important source of food and moisture, especially when flowering plants are scarce (Leius, 1960). Several papers report field observations of a wide variety of parasitoid species feeding on honeydew (Györfi, 1951; Zoebelein, 1955). Zoebelein (1955) showed that honeydew was indeed a suitable food source, increasing parasitoid longevity in all parasitoid species tested. Although honeydew will often be the most available and accessible sugar source in the field, we did not find attraction of starved *C. rubecula* to odors from honeydew, or aphid infested leaves. This lack of response, rather than reflecting a lack of interest, probably indicates that the parasitoids cannot perceive the presence of the food source. The latter is supported by the observation that starved *C. rubecula* readily assume feeding once honeydew has been contacted.

Unlike flowers, that advertize their nectar with notable scents and visuals in order to attract pollinators, there is usually little benefit to honeydew producers in attracting attention to their sugar excretion. To the contrary, since volatiles in honeydew can serve predators and parasitoids as kairomones leading to its producers, the latter are subject to a strong selection pressure to minimize honeydew detectability. This could explain the fact that even parasitoids of honeydew producing insects, to which honeydew could be a reliable indicator of host patches, do not seem to perceive honeydew volatiles (Sheehan and Shelton, 1989; Noldus and van Lenteren, 1990; Budenberg, 1990; Hågvar and Hofsvang, 1991).

Since *C. rubecula* neither responds to honeydew nor to volatiles of aphid infested leaves, finding honeydew is reduced to a random process. The chances of walking into honeydew are further reduced by the fact that *C. rubecula* mainly forages in flight and covers only a limited area after landing on the plant. This means that honeydew will be of only limited value as a sugar source compared to the highly detectable floral nectar that the parasitoid can actively seek out.

For parasitoids like *C. rubecula*, that feed on food sources dissociated from the host sites, feeding represents a disruption of the host foraging process. When food is available in the direct vicinity of the host, this disruption will be minor. However, in the situation in which the host habitat does not provide food sources, food foraging can interfere considerably with parasitization efficiency. It is the latter situation that often applies to the agro-ecosystems in which natural enemies are released for biological control. Lack of suitable food sources could be an important cause of failure in biological control programs (Clausen, 1956). To overcome this obstacle, various approaches could be used. The availability of suitable food sources could be increased through: diversification of the agro-ecosystems (introducing, or preserving flowering weeds); selecting crop cultivars with a higher (or more extended) production of food sources; timing the release of natural enemies into the season in which the agro-ecosystem provides sufficient food sources; introduction of artificial food sources.

All these strategies, however, require knowledge of the relative suitability of potential food sources under field conditions. Since this relative suitability is not only determined by the availability and quality of different food sources, but also by their detectability, it is crucial to identify the stimuli and mechanisms involved in food detection by natural enemies.

The detectability of an item is determined by characteristics of the emitted stimuli, the transmitting medium, and the receptor. In combination, these parameters describe a field of perception, which represents the space over which a specific sensory receptor perceives a given stimulus. This field of perception is therefore reciprocal to the concept of "active space" (the volume which the searcher must enter for detection to occur (Dusenbery, 1992)), which takes the stimulus rather than the detecting organism as the point of origin. Since this study deals with olfactory cues, the olfactory field of perception will be considered.

Olfactory information is transmitted in cone shaped odor plumes (Elkinton and Cardé, 1984). From the view of the receiving organism, this translates to a (reversed) cone shaped field of perception. The field of olfactory perception can therefore be described by the formula for cone volume $[1/3\ \pi * (\tan \alpha)^2 * D^3]$, in which α is the angle of perception and D represents the distance of detection. This means that both the field of olfactory perception (detectability) increases by the third power with increasing range of detection.

A comparison between potential nectar sources can demonstrate the extent to which detectability determines the role of food sources in parasitoid food foraging: honeydew, for one, can be abundant but is difficult to detect (Hagvår and Hofsvang, 1991; present study). The outstanding floral fragrances and visual stimuli, in contrast, result in a high detectability of floral nectar. Under the (conservative) assumption that the distance of detection for a honeydew site and a flower are 1 cm and 5 cm respectively, this means that a single flower is 125 (5^3) times as likely to be encountered than a honeydew patch.

This example shows the importance of identifying and quantifying the factors that determine food encounter and food acceptance in order to come to a complete understanding of the respective role of food sources under natural conditions. Knowledge of the key stimuli in parasitoid food foraging are not only crucial in selecting the most suitable natural or artificial food sources, but may also be applied for evaluatory trapping.

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Chapter 3

The effect of hunger on the innate visual and olfactory preferences in *Cotesia rubecula*

SUMMARY

The response of *Cotesia rubecula* (Hymenoptera: Braconidae) to olfactory and visual flower stimuli was tested. It was demonstrated that parasitoids can use both flower-odors and -colors during food foraging. The response of parasitoids to food-indicating stimuli depended on the hunger state of the individual. Given a choice in a y-tube olfactometer between flower odors and odors from host-infested leaves, starved individuals chose flower odors, while satiated individuals preferred host associated odors. In flight chamber experiments, starved parasitoids landed more often and spent more time searching on yellow targets, while satiated individuals displayed a higher overall foraging activity, without reacting differentially to yellow.

INTRODUCTION

It is generally appreciated that parasitoid feeding is not restricted to their larval stages. Most parasitoid adults require food as an energy source, especially for flight (Elton, 1966), while many synovigenic parasitoids require food for the production and maturation of eggs (Bartlett, 1964; Lum, 1977). Feeding has been demonstrated to increase longevity and fecundity in numerous parasitoid species (Zoebelein, 1955; Jervis and Kidd, 1986). In the case of *C. rubecula*, sugar-water feeding extends longevity of both sexes by a factor of 15-20 (Wäckers and Swaans, 1993).

On the basis of food foraging, we can distinguish two groups of parasitoids. First, there are parasitoids that either feed directly on their hosts, on host products (honeydew), or on host associated substrates. For these parasitoids, host- and food foraging are a single process. The second group is comprised of those parasitoids, like *C. rubecula*, that feed on food sources that are not associated with host sites. These parasitoids face a far more intricate foraging situation, since they have to commute between host patches and food sites. Compared to the in-depth investigations of host location by parasitoids (van Alphen and Vet, 1985; Turlings *et al.*, 1993), there are very few data regarding the stimuli and mechanisms involved in food foraging. The vast majority of food foraging studies in parasitoids consists of collections and observations in the field (Jervis *et al.*, 1993). Although field studies can demonstrate the occurrence of parasitoids on different food sources, they usually do not elucidate how these sites were located. In general, field studies merely record the presence of parasitoids at or near food sources. Parasitoid presence, however, does not prove attraction to long-range stimuli, since parasitoids might have encountered the food source randomly, while differential distribution of parasitoids over food and non-food resources can be due to arrestment by contact stimuli. Moreover, field recordings give no insight in the motivational state and the feeding history of the observed parasitoids. Parasitoids recorded at food sites might be searching for items other than food, such as shelter, heat, mates, or hosts. These confounding factors make field data unsuitable for drawing conclusions on the stimuli and mechanisms involved in food foraging.

Laboratory studies addressing food source location by parasitoids, on the other hand, have been scarce (Leius, 1960; Shahjahan, 1974; Syme, 1975; Elzen *et al.*, 1983). These reports agree in their finding that parasitoids are attracted to their food sources. Regrettably, however, none of these studies controls for the feeding experience of the parasitoids. Parasitoids are either field collected, reared on honey (dilutions), or tested repeatedly with unknown feeding experiences. Consequently, it remains unresolved whether the reported attraction reflects innate preferences, or learning behavior (due to either associative learning, pseudoconditioning, or sensitization).

Since starved parasitoids tested in this study lacked any feeding experience, it was possible to address the question of how feeding-inexperienced parasitoids locate nectar sources. Innate preferences to both olfactory and visual flower cues were studied in choice experiments. Y-tube olfactometer experiments were used to address olfactory preferences, while the innate response to visual stimuli was examined by studying free-ranging parasitoids in a flight chamber.

By testing and comparing the responses of both unfed parasitoids and parasitoids fed on sugar water, it was examined whether olfactory and visual preferences change relative to the parasitoid's state of hunger.

MATERIALS AND METHODS

Cotesia rubecula had been collected in Brussels sprouts and red cabbage fields from *Pieris rapae* larvae and subsequently reared for ca. ten generations on ***P.*** *rapae* larvae feeding on brussels sprouts (for details see Wiskerke and Vet, 1991). The parasitoid pupae were allowed to emerge in plexiglass cages (30 x 40 x 37 cm) at 25°C, 50-70% RH and a 16L:8D photocycle. Daily transfer of pupae to a new cage assured parasitoid groups of uniform age.

Females were inexperienced with regard to flower and host odors. Satiated parasitoids were provided with water and a 70% saccharose solution as a food source. Starved parasitoids were given water only. Since starved females of *C. rubecula* will only live an average of 1.6 days (Wäckers and Swaans, 1993), one to two day old mated females were used in the experiments. To assure sufficient fitness of the starved parasitoids, only individuals that showed normal flight behavior were used in our experiments.

Olfactometer experiments

Y-tube olfactometer. For a detailed description of the olfactometer, see Sabelis and van de Baan (1983) and Takabayashi and Dicke (1992). Here, Erlenmeyer flasks were used as odor containers. Both flasks were placed in a black plastic dish (height 4 cm) to rule out visual perception of the plant material. Air pressure generated an airflow through both arms at a rate of 3 l/min. At the base of the olfactometer the air was extracted by the vacuum system of the building at a rate of 6 l/min. Flow meters were used to control and attune both air import and air extraction in order to ensure constant air pressure and a laminar airflow within the olfactometer. Olfactometer experiments were done at 25 ± 2 °C, and 40-60% RH. The light intensity was 600 lux, provided by two 16 W TLD fluorescent tubes, located behind and in front of the set-up.

Odor sources

Flowers and leaf material, used as odor sources in the Y-tube olfactometer, were collected from the field. It was assured that flowers and leaf material were selected from plants free of any herbivore damage.

Rape seed (*Brassica napus* L.; Cruciferae) was chosen since it is known to be a food plant for *Pieris* spp. To approximately match biomass ten flower heads and one leaf from a single rape seed plant were used as odor sources.

Ground-elder (*Aegopodium podagraria* L.; Umbelliferae) was chosen as an alternative flower odor since flowers from the family of the Umbelliferae are known to be frequently visited by various parasitoid species (Kevan, 1973). Their exposed nectaries provide accessible nectar to nectar feeders with short mouth parts (Leius, 1960). To match the biomass of the rape seed flowers, a single umbel was used as an odor source in the choice experiments.

P. rapae feeding damage was obtained by placing 10 first instar *P. rapae* on a young leaf of a rape seed plant and allowing them to feed overnight. A freshly cut damaged leaf, including the feeding larvae, was subsequently used in the Y-tube experiments.

Test procedure. Using a small glass vial, individual parasitoids were introduced into the central olfactometer tube, with the opening of the vial positioned 1 cm from the start line. The observation started as soon as the wasp passed this start line. Walking upwind, the parasitoid could choose at the bifurcation between both olfactometer arms. The observation was counted as a choice when the individual passed the finish line in one of the arms for a period of 15 seconds. Individuals that had not made a choice within 2 minutes were counted as "no choice". When comparing starved and satiated parasitoids, individuals of both treatments were tested alternatively. The connections between the odor source containers and the olfactometer arms were exchanged after every five parasitoids tested. Odor sources were renewed after every ten parasitoids tested. At the end of each day odor containers were cleansed with 70% ethanol.

All choice experiments consisted of 20 replicates per treatment, except the choice test between rape seed flowers and *P. rapae* damaged leaves, which consisted of 44 replicates per treatment. Data were analyzed by Binomial test (α=0.05).

Flight chamber experiment

The flight chamber design was identical to that described by Takken (1994). The test area was 2.05 meters long with a 60 x 60 cm cross section. The floor was white. Overhead lighting was provided by eight 32 W TLD/48HF fluorescent lights and four 200 W Philips softtone light bulbs. Lights were placed in a hemispherical top (205 x 60 cm) located 40 cm above the flight arena. Fluorescent lights were shielded and the spotlights aimed upward to assure indirect lighting of the test arena. The inside of the top was coated with aluminium sheets (Stuccodessin R) for an even light reflection. The light-intensity inside the flight chamber was controlled at 2000 lux. Within the flight chamber a plant patch was created by placing four individual Brussels sprouts plants in the test arena. The distance between plants was 25 cm, allowing sufficient space for the flying parasitoid to move freely among plants. The experiments were conducted in still air since this is likely to be conducive to omnidirectional visual orientation by the parasitoids. Climatic conditions were controlled at 25 ± 1 °C and 40-60% RH.

Brussels sprouts were grown individually in plastic pots (10 cm diameter) under glasshouse conditions (20-30°C, 50-80% RH, and a 16L:8D photocycle). Plant sets of uniform age and growth stage (14th leaf stage; 25-30 cm in height) were used in the experiments.

Targets consisted of "Pantone" paper (2.5 x 2.5 cm) in the basic colors "Pantone Yellow U" and "Pantone Cool Grey 2". One target of each color was attached to the four cabbage plants.

The Pantone colors were selected on the basis of their spectrophotometric characteristics measured in a Licor Li 1800 Portable Spectroradiometer with an integrating sphere. "Pantone Yellow U" has a color spectrum similar to that of rapeseed-flowers (Fig 1). Furthermore, the spectral maximum at 550 nm corresponds with one of the sensitivity maxima described for Hymenoptera (Peitsch *et al.*, 1992). "Pantone Cool Grey 2" on the other hand has a uniform spectrum (Fig 1). The shade of the "Pantone Cool Grey" was chosen to match the overall reflection of "Pantone Yellow U" (calculated over the insect's visual spectrum). To the parasitoid, both types of colored paper should consequently be of similar brightness. Any distinction made by the parasitoid is therefore likely based on wavelength characteristics, either hue (dominant wavelength) or saturation (% dominant wavelength). Both the grey and the yellow targets stood out against the background of the cabbage leaves due to their higher intensity (overall brightness) (Fig 1).

Test procedure. Individual parasitoids were taken out of the rearing cage in a small glass vial and subsequently released by placing the vial in between the plants on the flight chamber floor. After take-off, the parasitoid's behavior was observed continuously for a period of 30 minutes. Using software for behavioral research (Noldus, 1991) the following test parameters were recorded: landing choices (yellow targets, grey targets, plant tissue, flight chamber walls); parasitoid behavior on the various substrates (search, non-search); retention time before renewed flight; and overall foraging activity (flight, search, non-search). "Search" was defined as walking, usually accompanied with drumming of the antennae. "Non-search" was defined as any other behavior after landing, such as cleaning or being stationary.

Plants and targets were renewed after every four parasitoids tested. Starved and satiated parasitoids were tested alternatively. Each treatment consisted of ten replicates.

The percentage landings as well as the percentage time spent searching was calculated for each individual parasitoid and subsequently averaged over the parasitoids tested in each experiment. Data were analyzed by Wilcoxon signed rank test ($\alpha=0.05$).

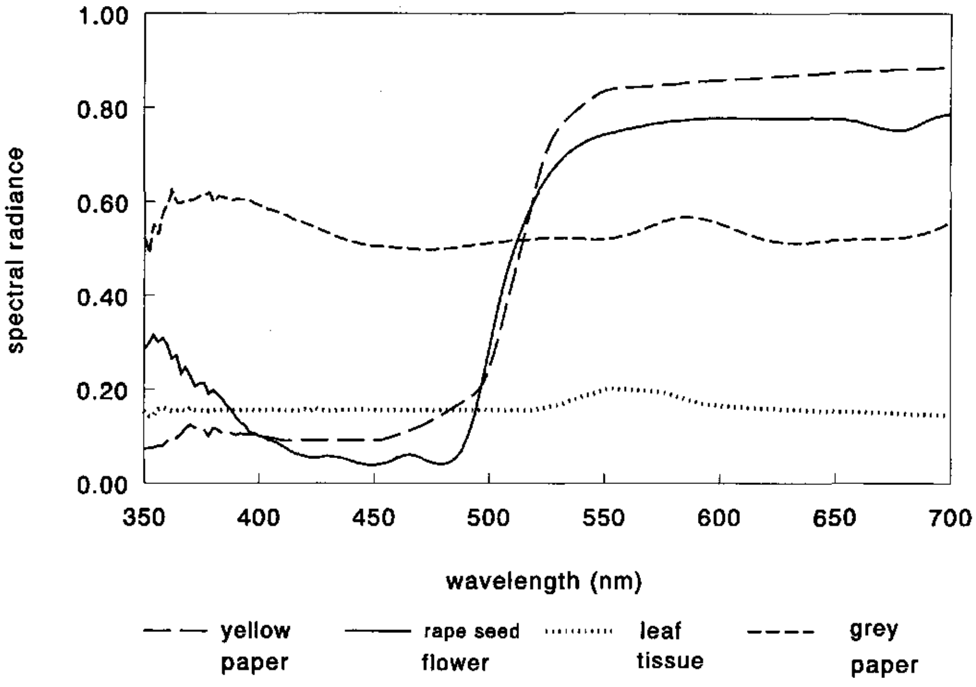

Fig 1. Spectral reflectance curves of rape seed flowers, brussels sprouts leaves and both yellow and grey paper targets. Measurements conducted using a Licor Li 1800 Portable Spectroradiometer with an integrating sphere.

RESULTS

Olfactometer experiments

Starved as well as satiated parasitoids were attracted to flower odors. When given a choice between undamaged leaf material and rape seed flowers, both parasitoid categories chose the latter significantly more often (Fig 2). The innate flower odor response was not restricted to the plants on which *C. rubecula* find their hosts. In a direct comparison between rape seed and ground elder, starved parasitoids did not show a preference for one of the two flower odors (Fig 2).

Starved and satiated parasitoids showed different preferences, however, when given a choice between rape seed flowers and hosts feeding on rape seed leaves (Fig 3). Starved parasitoids predominantly chose the flower odors, while satiated parasitoids preferred the odor of larval feeding damage.

Flight chamber experiments

Landing choices. Starved parasitoids on average made 2.1 landings on the yellow targets, while only two of the ten starved individuals landed on a grey target once. On average 24.7% of the total number of landings were made on the yellow targets (Fig 4). The majority of alightments (74.3%) were made on other parts of the cabbage plants. However, since the area of the yellow target comprised only approximately 0.5% of the total plant surface area, the deviation from random landing demonstrates that starved parasitoids seek out the yellow color (sign test, $p=0.001$).

Satiated parasitoids showed no interest in either of the paper targets. None of the landings were made on the grey, while only a single landing was observed on the yellow targets. In the far majority of cases (98.4%) satiated parasitoids landed on the cabbage plants (Fig 4).

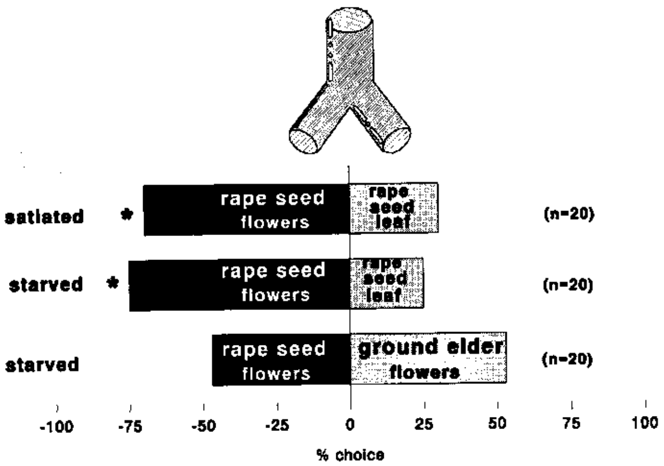

Fig 2. Innate odor preferences (% choice in a two-arm olfactometer) of satiated and starved parasitoids. Odor alternatives were either rape seed flowers and an undamaged rape seed leaf (tested both for starved and satiated parasitoids), or rape seed flowers and flowers of ground elder (tested for starved parasitoids only). Significant preferences at p=0.05, (binomial test, n=20 per treatment) are indicated by a "*".

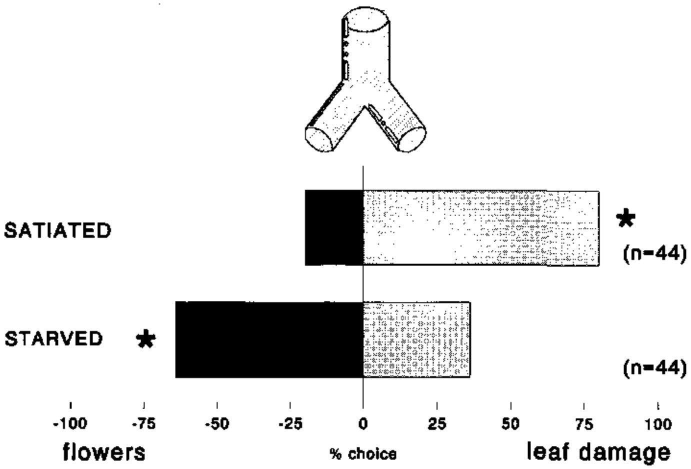

Fig 3. Innate odor preferences (% choice in a two-arm olfactometer) of satiated and starved parasitoids when given a choice between rape seed flowers and a *Pieris rapae* damaged rape seed leaf. Significant preferences at p=0.05, (binomial test, n=44 per treatment) are indicated by a "*".

Search behavior following landing. After landing on a yellow target, starved parasitoids generally searched the yellow paper intensively. Searching parasitoids typically scraped their mouth parts over the target surface. On average, 77.9% of the total time on the yellow targets was spent searching (Fig 5). The time budget was markedly different when parasitoids landed on plant tissue. In those cases parasitoids spent the majority of their time (76.7%) resting or cleaning (non-search), while only 23.3% of their time was used searching (Fig 5). Parasitoids searching the leaves only occasionally scraped the leaf surface with their mouth parts.

Since satiated parasitoids did not land on grey targets, and only a single landing on yellow targets was observed, their searching behavior on colored targets relative to their behavior on cabbage leaves could not be tested. When satiated parasitoids landed on plant tissue they spent 48.3% of their time searching. In comparison with the starved individuals this represents a significantly higher searching activity on plant tissue (Wilcoxon, Z=0.02).

Overall foraging activity. Calculated over the whole observation period as well, starved parasitoids were less active than satiated parasitoids. Satiated parasitoids spent 66.7% of their total time searching, while this figure was only 22.6% for starved individuals (Fig 6). This lower activity was principally due to a reduction in the number and duration of flights. In contrast to satiated parasitoids, who hovered at close distance around the plants in extensive flight bouts, starved parasitoids spent most of their time resting (Fig 6). This reduced activity is likely due to energy shortage in starved individuals.

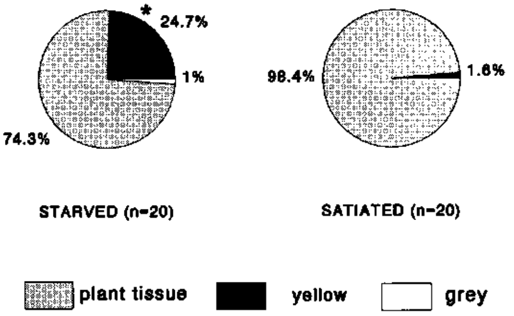

Fig 4. Innate landing choices of starved and satiated parasitoids on yellow and grey targets (each 6.25 cm^2) or the remaining plant surface (± 1250 cm^2). Yellow targets received a significantly higher percentage of landings than to be expected from their percentage of the total surface area (sign test, $p=0.001$). Starved parasitoids landed significantly more often on yellow compared to satiated parasitoids (Wilcoxon, $p=5.9*10^{-3}$, $n=10$ and $n=10$ respectively).

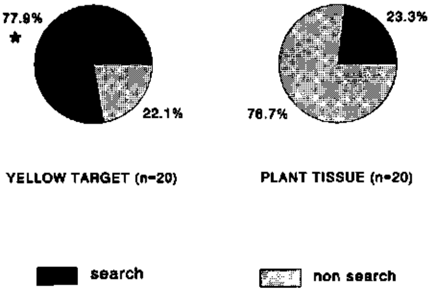

Fig 5. Average search activity of starved parasitoids on both yellow targets and plant tissue. The percentage searching on the yellow targets was significantly higher as compared to the plant tissue (Wilcoxon, $p=9.1*10^{-3}$ $n=10$ and $n=10$ respectively).

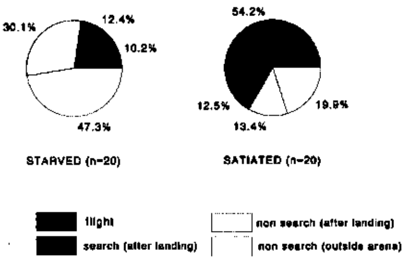

Fig 6. Overall time budget of starved and satiated parasitoids foraging in a plant patch. Satiated parasitoids spent a significant higher percentage of their time flying (Wilcoxon, $p=5.9*10^{-3}$ $n=10$ and $n=10$ respectively).

DISCUSSION

The distinction between innate behavior and learning is often difficult to make. The inconsistent terminology in reference to the issue has added to the confusion (Bateson, 1983; Papaj, 1993). Not only have various terms been used to describe predetermined behavior, but individual terms have acquired multiple and often conflicting meanings (Bateson, 1983).

Although not free from controversy, here the term innate will be used in its most common connotation of "unlearned", referring to any behavior that is manifested in response to a stimulus to which the organism has not previously been exposed. The difficulty inherent in this definition is the exclusion of previous exposure. Since organisms are inevitably exposed to stimuli during their development it is usually impossible to disentangle truly innate preferences from (pre-) imaginal conditioning. In bees, for instance, their standard exposure to pollen and nectar during pre-imaginal development strongly determines their initial foraging choices (Menzel, 1985; Dobson, 1987). This has been an important factor precluding the study of innate odor preferences in this otherwise thoroughly studied organism.

The study of innate odor and visual preferences in insect parasitoids, in contrast, makes it possible to circumvent the factor of pre-imaginal conditioning. Since parasitoids in our experiments were reared from folivorous hosts, and kept without food after eclosion, any exposure to flower odors and yellow colors could be precluded. The demonstrated olfactory preference of starved parasitoids for rape seed flowers over rape seed leaves and the visual preference for yellow are therefore likely to be innate.

What should become genetically incorporated?

It is usually assumed that a stimulus has to be consistently linked to foraging success for a behavioral response to this stimulus to become genetically incorporated, (Lewis *et al.* 1990). It is true that many innate responses are triggered by stimuli directly from the host or food, which obviously are reliable foraging indicators. Nevertheless, parasitoids foraging for hosts also demonstrate innate responses to such unreliable indirect stimuli as plant image (McAuslane *et al.*, 1990) or green color (Ma *et al.*, 1992). In themselves, these indirect stimuli are evidently poor predictors of host presence and thus foraging success. Innate responses to such indirect stimuli can be understood, however, when we consider that the highly reliable direct host stimuli are often undetectable at longer ranges (Vet *et al.*, 1991). The lack of direct host stimuli at the long range creates the need for responses to less reliable (but more detectable) indirect stimuli to become genetically incorporated.

Unlike herbivores, that (other than for mating) have little benefit in attracting attention to their presence, flowers advertize their nectar with notable scents and visual stimuli. This means that the problem of low detectability does not apply to flower foraging. Since the direct flower cues combine reliability with high detectability, it is unlikely that additional (indirect) flower stimuli will be genetically incorporated.

Another factor determining what will become genetically incorporated is the degree of foraging specialization. Generalist foragers should incorporate responses to those stimuli which are held in common by all of its potential resources (common denominators), while missing in most non-resources. Specialist foragers, on the other hand, should incorporate responses to the most discriminating, species-specific stimuli. Because of the usually intimate interactions between developing parasitoids and their hosts, most parasitoids are restricted to a relatively limited host range. This host foraging specialization creates the need for responses to very specific host stimuli to become congenitally fixed.

In contrast to host foraging, parasitoids foraging for nectar can choose from a broad range of resources. Nectar can be obtained from a wide range of flower species, as well as from honeydew and extra floral nectaries (Leius, 1960). This means that innate food preferences have to be sufficiently broad to allow the use of various nectar sources they may encounter. The innate preferences displayed by starved *C. rubecula* seem to fit the pattern of generalist food foraging. Its innate odor preference covered responses to flowers of such disparate species as rape seed and ground elder, while the innate response to the common floral color yellow covers 30-40% of the flowering species (Weevers, 1952; Kevan, 1972). These broad innate responses will allow inexperienced parasitoids to maximize their chances of finding floral nectar.

Sources of individual flexibility

What is most reliable over generations is not necessarily the optimal indicator of profitable resources during the foraging life of an individual parasitoid. Innate preferences, however, may not be as fixed as some of its common synonyms ("congenitally fixed", "genetically programmed") suggest. Learning processes can modify innate responses to host related stimuli (Vet *et al.*, 1990; Wäckers and Lewis, 1993) as well as food stimuli (Lewis and Takasu, 1990) allowing the individual to incorporate responses to stimuli that are only temporarily or locally reliable. In this way parasitoids have the flexibility to adjust their initial preferences according to the variability of their foraging environment (Lewis et al., 1990).

In addition to this extrinsic variability, a parasitoid is faced with the intrinsic variability of its constantly changing physiological state. The present study is the first to show that parasitoids possess different sets of innate preferences, which take priority relative to the physiological needs of the individual. This second type of flexibility provides parasitoids with the plasticity to adjust innate preferences according to internal conditions as well.

The full extent of plasticity of innate responses is realized when both extrinsic- and intrinsic-flexibility work in concert. Lewis and Takasu (1990) demonstrated that the parasitoid *Microplitis croceipes* can learn different novel odors in association with separate host and feeding rewards and use them subsequently in accordance with the parasitoids relative host and food needs.

Physiological state

The physiological state of an individual organism is a collective term referring to intrinsic variables, reflecting the organism's condition relative to its various needs (Bell, 1990). Besides the state of hunger, physiological factors in parasitoids include age (Hérard *et al.*, 1988), mating status (Stouthamer and Luck, 1991), and egg load (Minkenberg *et al.*, 1992). The organism's temporary state relative to these needs interacts with the genotypic and phenotypic constitution of the individual organism in shaping its foraging decisions. Despite the fact that the physiological state has often been recognized as a determinant in parasitoid foraging behavior, it has rarely been studied in detail. The available studies demonstrate how various physiological parameters affect (innate) preferences within a single foraging mode. The present data show that the physiological state can also act as a two-way switch between foraging modes. This internally controlled shift between foraging modes requires both the possession of separate sets of preferences, as well as mechanisms to activate preferences relative to the physiological state of the individual.

Olfactory preferences

Volatiles released by plants in response to herbivore damage have been proven to be highly atractive to hymenopterous parasitoids (for a recent overview see Turlings *et al.*, 1993). Here it was shown that in starved parasitoids the attractiveness of host odors is overruled by innate flower odor preferences. This constitutes the first conclusive record of innate responses to food odor in parasitic Hymenoptera. The fact that *C. rubecula* responded equally to flowers of a cruciferous and an umbelliferous plant, indicates that the innate flower odor preference in this parasitoid is not restricted to the plant family on which they find their hosts. In order to elucidate the specific chemicals that trigger innate responses in parasitoids, further research is required, linking floral chemistry analysis to behavioral studies.

Entomophilous flowers advertise their nectar with notable scents in order to attract insect pollinators. The odoriferous phase requires high amounts of chemical energy converted from starch reserves (Meeuse and Buggeln, 1969). This high energetic investment in odor signals indicates that flower volatiles play a significant role in attracting pollinators. Odor is likely the most discriminating characteristic of flowers (Gould and Towne, 1988). Among the wide range of nectar producing flowers, individual species, genotypes, or even sexual stages differentiate themselves through a specific odor profile (Williams, 1983; Dobson, 1988; Pham-Delegue *et al.*, 1989; Patt *et al.*, unpubl. manuscript). In addition to these chemical variables that are stable over time and space, floral chemistry can fluctuate depending upon plant phenology and the environmental conditions (Loper, 1972; Pham-Delegue *et al*, 1989). Despite these sources of variability, common components in floral chemistry can be identified (Williams, 1983; Dobson, 1988; Pham-Delegue *et al.*, 1989). It is to be expected that these common components will be the main triggers of innate flower responses in generalist flower foragers, such as insect parasitoids. Both the flower specific chemicals and their specific ratio could enable learned discrimination between concomitantly flowering species (Gould, 1993).

Visual preferences

The studies that have addressed visual orientation in parasitoids (for an overview see Wäckers and Lewis, 1993) have been restricted to host foraging. Several studies have reported innate visual preferences for host related stimuli. Parasitoids were demonstrated to be attracted to visual characteristics of either the host environment (Goff and Nault, 1984; Mc Auslane *et al.*, 1990; Leyva *et al.*, 1991; Ma *et al.*, 1992), feeding damage (Sugimoto, 1988; Faeth, 1990; Wäckers, 1992) or the host itself (Pak and de Jong, 1987; Schmidt *et al.*, 1993).

The present study is the first to show that, in addition to these host-related preferences, parasitoids use innate visual preferences during food foraging. The fact that starved parasitoids seek out yellow targets and display intensified searching behavior on this color, while satiated individuals concentrate their foraging on green leaf tissue may indicate an adaptation to nectar foraging. Since yellow is the most common flower color (Weevers, 1952) as well as the primary signal in the advertisement of pollen (Osche, 1983), innate attraction to yellow seems to be an adaptive strategy for flower visitors.

Attraction to yellow over green colors is not only found in unspecialized nectar feeders (Kugler, 1951), but has also been frequently demonstrated for herbivorous insects (reviewed by Prokopy and Owens, 1983). Since yellow has its peak reflectance in the same bandwidth as green foliage, but at a greater intensity, Prokopy and Owens (1983) explain these yellow preference by suggesting that yellow is a "super normal foliage type stimulus" (sensu Tinbergen, 1948). This explanation implies that herbivores perceive yellow as a more intensely reflecting (and therefore more attractive) hue of green foliage.

In the present study, however, parasitoids are demonstrated to be well capable of distinguishing between foliage hues and yellow, preferring either stimulus according to their physiological needs. This finding shows that the theory of yellow as a super normal foliage type stimulus does not necessarily apply. The concept of hunger-dependent visual preferences could give an alternative explanation for some of the reports of yellow preferences in insect herbivores. Knowing that many adult herbivores visit flowers for food (Proctor and Yeo, 1973; Kevan and Baker, 1984), attraction to yellow might simply be an indication of flower foraging.

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Chapter 4

Olfactory and visual learning and their combined influence on host site location by *Microplitis croceipes.*

SUMMARY

The host foraging behavior of the larval parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), was studied in response to various assemblages of hosts and associated cues distributed over a patch of cotton plants located in a flight chamber.

Females of the parasitoid developed a preference for stimuli experienced during host encounters. To study odor learning, frass from *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), feeding on either of two different parts of the cotton plant were offered as volatile stimuli. During training sessions only one of the frass odors was associated with the host. Subsequent choice evaluations revealed that parasitoids preferred whichever frass odor had been associated with the host during training sessions. Thus, it was shown that females can learn to distinguish between frass odors from hosts feeding on different parts of the plant. In the same manner it was shown that parasitoids can be conditioned to visual stimuli. This study demonstrates that parasitoids use olfactory as well as visual learning to concentrate their search on plant structures that are most profitable in terms of host encounters.

Visual and olfactory learning proved to be additive: parasitoids conditioned to a combination of visual and olfactory stimuli displayed a stronger preference than individuals conditioned to either sensory component alone. When conditioned to a combination of stimuli, olfactory learning was demonstrated to be dominant over visual learning. To our knowledge this study is the first account of multisensory conditioning and summational learning effects in insect parasitoids. The significance of multisensory learning in parasitoid foraging is discussed.

INTRODUCTION

Microplitis croceipes (Hymenoptera: Braconidae), a solitary parasitoid specialized on *Helicoverpa* (Lepidoptera: Noctuidae) species, can locate its host by orientation to host-associated volatiles being released from frass and larval feeding damage (Drost *et al.*, 1988). The polyphagous nature of its host, occurring on more than 200 plant species (Fitt, 1989), confronts the parasitoid with a wide array of potential host habitats. The parasitoid's ability to learn olfactory cues experienced in association with hosts or host products (Lewis & Tumlinson, 1988) can serve as an effective strategy to cope with this variability. Eller *et al.* (1992) showed that associative learning of frass odors enables the parasitoid to differentiate between frass from *H. zea* feeding on different plant species. Moreover, within a plant species *Helicoverpa* spp. can feed on various structures. First and second instars can be found feeding on young shoots, but overall flowers and fruiting structures are the preferred feeding sites (Farrar and Bradley, 1985).

In this study we investigated whether parasitoids could use associative learning of both olfactory and visual cues to differentiate host sites at the level of plant structures. Such a differentiation would be adaptive since plant parts represent disparate profitabilities to the foraging parasitoid. Not only do plant structures differ in their frequency of infestation, if infested, host accessibility can vary greatly between plant structures. While *Helicoverpa* larvae are exposed when feeding on leaves and open flowers, they are often concealed when excavating buds and fruiting structures.

Using flight chamber experiments we studied whether free ranging parasitoids could be conditioned to distinguish between profitable and non-profitable sites within one plant species. Since plant structures not only differ in their chemical composition (e.g. Turlings *et al.*, 1993), but also in their visual properties (Gates, 1980), we investigated wether parasitoids learn to distinguish between plant structures on the basis of olfactory as well as visual stimuli.

Under natural conditions specific olfactory stimuli are usually experienced by the parasitoid in association with specific visual stimuli. A cotton flower, for instance, combines a characteristic odor with a characteristic visual appearance. Studying learning only at the level of singular sensory modalities is therefore a simplification of the complex reality in which the parasitoid operates. To get a more accurate impression of the impact of learning on parasitoid foraging, we studied multisensory conditioning. In two additional experiments we conditioned parasitoids to a combination of an olfactory and a visual stimulus (multisensory conditioning). We first examined whether multisensory conditioning would further increase the preference level above the level of preference achieved after conditioning the parasitoid to the individual sensory components. In a subsequent experiment we determined the hierarchy in which visual and olfactory learning are learned.

MATERIALS AND METHODS

Microplitis croceipes were reared from *H. zea* larvae as described by Lewis and Burton (1970). The parasitoids were kept in acrylic cages (30 x 30 x 17cm), according to the day of their emergence and had access to honey and water. Rearing conditions were set at 28°C, 50-70% RH and a 16L:8D photoperiod. Three day-old mated females without oviposition experience were used in the experiments.

***Helicoverpa zea* larvae** were reared on a pinto-bean based diet according to Burton (1969). Late third instars were used in the experiments as an oviposition reward. Frass was collected from larvae reared on plant material.

Cotton flower frass (CFF) and cotton leaf frass (CLF), were used as kairomone sources. Frass was collected immediately before the experiment from 5th instar *H. zea* feeding individually in small petri-dishes (5cm) on cotton flowers or cotton leaves.

The flight chamber was designed similar to the flight chamber described by Drost *et al* (1986). The test area was two meters long with a 75x75 cm cross section. The floor was covered with plain green cardboard. Overhead lighting was provided by four 80 Watt fluorescent bulbs. The experiments were conducted at 27°C and a wind speed of 31 (± 2) cm/sec.

Cotton plants were grown individually in plastic pots (10 cm diameter), using a mixture of 1/2 potting soil, 1/3 sand and 1/6 peat moss. Growth conditions were controlled at 25-35°C and a 14L:10D photocycle. Plant sets of uniform age and size (about 30 cm in height; fifth leaf stage) were used to create a plant patch in the flight chamber.

The plant patch consisted of 4 cotton plants placed pairwise in the flight chamber. The distance between pots was 30 cm, allowing sufficient space for the flying parasitoid to move freely among the plants. Host sites were simulated by means of removable targets consisting of a rectangular piece of paper (4 x 2.5 cm) with three pellets of frass (± 25mg) placed at the base of the paper as a volatile attractant. The paper targets differed visually (plain orange, versus black and white stripes), olfactorily (CFF versus CLF), or both visually and olfactorily (for instance plain orange with CFF versus black and white stripes with CLF). In each of our experiments we used two types of targets, only one of which was being reinforced. Reinforced targets had a *H. zea* larvae pinned to the paper as a reward, allowing the foraging parasitoid to be conditioned to the visual and olfactory stimulus presented on that target. Larvae were affixed to the top of the reinforced target with a # 000 insect pin pushed through the last abdominal segment. Using an identical pin in the unreinforced targets, both types of targets were attached to the upper leaves of the cotton plants. One reinforced and one unreinforced target were placed on each of the four plants in the plant patch. The targets were positioned downwind on two upper leafs to assure maximum accessibility and visibility to the flying parasitoid.

General procedure: To increase the initial foraging motivation, parasitoids were allowed to antennate *H. zea* frass for a period of 30 seconds (Eller *et al.*, 1992) before their introduction into the plant patch. To rule out possible sensitization effects, the frass used in this pre-flight treatment was always of the same type as offered on the unreinforced target in an experiment. Five minutes after the pre-flight experience the parasitoid was released into the flight chamber from a 2-dram shell vial placed 40 cm downwind from the first pair of plants. Parasitoids were allowed to forage freely within the plant patch during two training sessions before they were tested in a subsequent experimental trial. In the visual and the olfactory learning experiments (experiment 1 and 2) the average number of landings on both types of targets during the initial training session was recorded to establish the parasitoid's initial preference for each of the visual and the olfactory stimuli. Training sessions were ended as soon as three out of the four available hosts had been parasitized. After each training session, the targets were renewed before the parasitoid was reintroduced. During the experimental trial both types of targets were offered without larvae to determine whether parasitoids had developed preference for the reinforced target. The number of landings made on both previously reinforced and unreinforced targets was recorded for each individual. Experimental trials were ended when a parasitoid left the plant patch (phototactic flight to the top of the flight chamber, or by its landing and resting on a flight chamber wall) for a period of two minutes. Experiments 1 and 2 consisted of 10 replications, while 12 parasitoids were trained and tested in experiments 3 and 4. The fraction of landings on the previously reinforced target was calculated per individual and subsequently averaged over each experiment. To assure a balanced experimental design, equal numbers of parasitoids were conditioned to each of the target types. Preference learning was concluded when the overall fraction of landings on the reinforced target was significantly higher than the number of landings on the non-reinforced targets.

Statistical analysis: The overall data from experiments 1, 2 and 4 were analyzed in a Wilcoxon signed-rank test. To test for summational learning effects in multisensory conditioning (experiment 3), the level of preference exhibited by individuals conditioned to a combination of visual and olfactory stimuli was compared to preference levels in both experiments on single-component conditioning (experiment 1 and 2), using a Wilcoxon signed-rank test with the Bonferroni adaptation.

EXPERIMENTS AND RESULTS

Experiment 1: Olfactory learning. To investigate whether *M. croceipes* can use olfactory learning to distinguish between hosts feeding on different plant parts, we conditioned parasitoids to either cotton flower frass (CFF) or cotton leaf frass (CLF) presented on visually identical targets (both plain white). Host larvae were added to only one of the two targets. Ten parasitoids were trained, five to each target, and subsequently tested for olfactory preference. In the test, the frass was placed on the back of the target to exclude the possibility of visual distinction between frass pellets.

In the initial training session, parasitoids made 59% of their first five landings on CFF, while the remaining 41% were made on CLF. During the test, all individuals, irrespective of the frass they had been conditioned to made the majority of their landings on the reinforced frass type. Parasitoids conditioned to CFF made 73% of their landings on the reinforced frass type, while this figure was 70% in the case of CLF (Fig. 1). Calculated over all individuals, parasitoids displayed a conditioned preference (71%) (Wilcoxon, Z=0.006) for the previously reinforced frass odor.

Experiment 2: Visual learning. To determine whether *M. croceipes* can learn visual stimuli, we used two types of targets, which differed in color as well as in pattern (plain orange versus black and white stripes), while both contained identical olfactory stimuli (CLF). Ten parasitoids were trained, five to each target, and subsequently tested for visual preference.

In the initial training session, parasitoids made 54% of their first five landings on black and white striped targets, and the remaining 46% on the orange targets. In the test trial, nine out of the ten parasitoids made the majority of their landings on the previously reinforced target. On average, parasitoids conditioned to orange targets made 65% of their landings on this target (Fig. 2). This figure was 87% for parasitoids trained to the black and white targets. Calculated over all individuals, parasitoids displayed a significant conditioned preference (76%) (Wilcoxon, Z=0.01) for the image of the previously reinforced target.

To exclude the possibility that parasitoids were actually learning odor differences between the two types of paper, we trained six parasitoids (three to orange; three to black and white) using the same training routine as described earlier. We then attached the targets to the underside of the leaves, to prevent visual discrimination by the upwind-oriented parasitoid. In this control experiment, only 52% of the landings were made on the reinforced targets. The significantly higher (Kruskal Wallis H-test, 95%) overall preference for the visible reinforced target consequently allows the conclusion that *M. croceipes* indeed learns visual stimuli associated with profitable host sites.

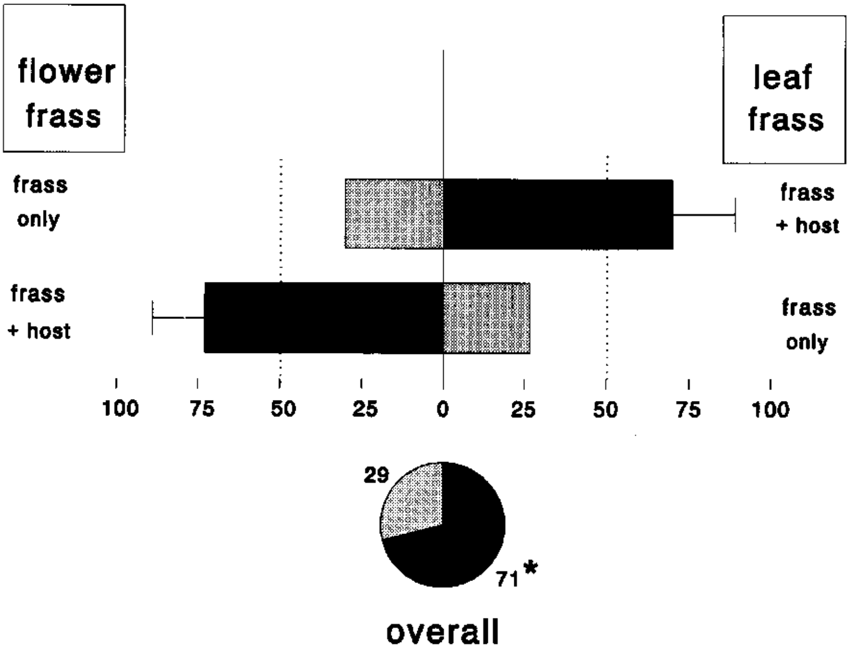

Fig. 1: Olfactory learning by *M. croceipes* foraging in a flight chamber plant patch. Treatments (according to training): 1. leaf frass reinforced; 2. flower frass reinforced; 3. overall. Bars indicate the percentage of landings on previously reinforced and unreinforced targets following training. Percentages were calculated per individual and subsequently averaged over the individuals tested in each treatment. The dotted lines indicate the distribution of landings in a no-preference situation. The overall preference for the reinforced odor target (Wilcoxon signed-rank test, Z=0.006) is evidence of associative odor learning.

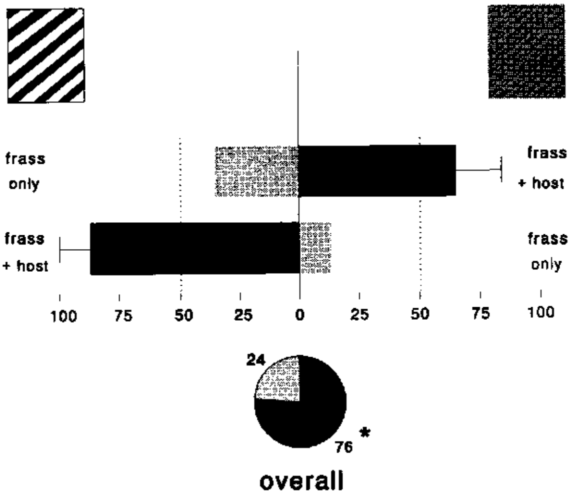

Fig. 2: Visual learning by *M. croceipes* foraging in a flight chamber plant patch. Treatments (according to training): 1. orange reinforced; 2. black stripes reinforced; 3. overall. Bars indicate the percentage of landings on previously reinforced and unreinforced targets following training. Percentages were calculated per individual and subsequently averaged over the ten individuals tested in each treatment. The dotted lines indicate the distribution of landings in a no-preference situation. Parasitoids demonstrated an overall preference for the visual target that had been reinforced during training (Wilcoxon signed-rank test, Z=0.01).

Experiment 3: Multisensory conditioning. In this experiment, we determined whether the learning of olfactory and visual stimuli was integrated, i.e., whether the conditioned preference of the parasitoid could be raised even further if the reinforced and unreinforced targets differed both visually and olfactorily. Twelve parasitoids were tested, divided in groups of three individuals. One group was trained to each of the four possible combinations of the two visual and two olfactory stimuli.

All tested individuals, irrespective of the combination they had been conditioned to, landed more often on the previously reinforced combination. Overall, 88% of the landings were made on the target the parasitoids had been conditioned to (Fig. 3). This preference level was significantly higher than the preference exhibited by the parasitoids that were trained to either visual or olfactory stimuli alone (experiment 1 and 2).

Experiment 4: Hierarchy of olfactory and visual stimuli in multisensory conditioning. This experiment was designed to determine if one sensory component dominates during multisensory conditioning. We conditioned 12 parasitoids, three individuals to each of the four possible combinations of the two visual and olfactory stimuli. Visual and olfactory components of the reinforced and the unreinforced target were interchanged in the test (Fig. 4). Parasitoids consequently faced the choice between the reinforced visual stimulus in combination with the unreinforced odor, and the reinforced olfactory stimulus combined with the unreinforced visual stimulus.

Ten out of twelve individuals made the majority of their landings on the previously reinforced olfactory stimulus, while two individuals landed more often on the reinforced visual stimulus. On average, parasitoids trained to a combination of olfactory and visual stimuli landed significantly more often (Wilcoxon, Z=0.04) on the reinforced olfactory component (69%), as compared to the visual component (31%). This shows that, at least under these experimental conditions, learning of the olfactory stimulus is dominant.

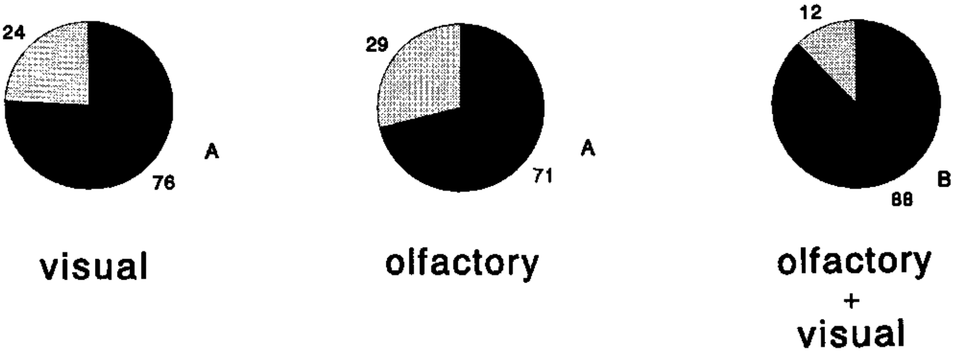

Fig. 3: Multisensory learning by *M. croceipes*. The black sections indicate the level of conditioned preference following training. Percentages were calculated per individual and subsequently averaged over the twelve individuals tested. Different letters indicate significant differences among treatments (Inequality of Bonferroni, α=0.05).

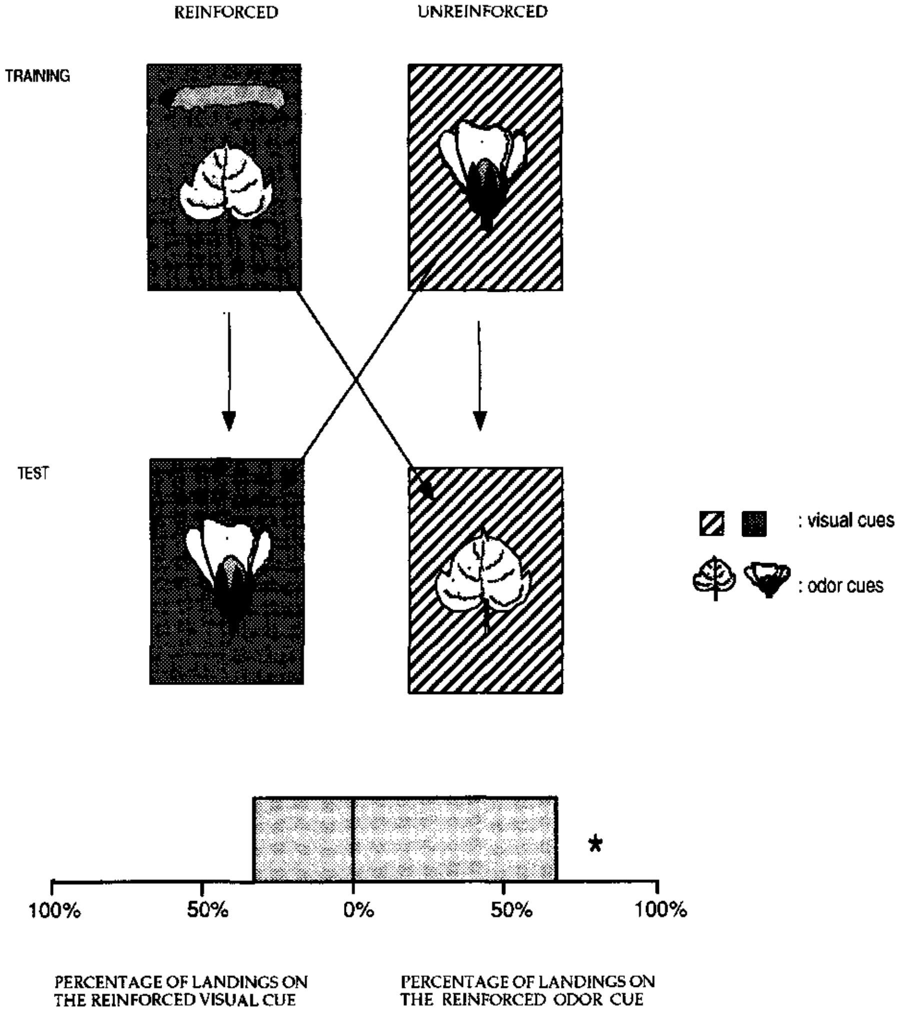

Fig. 4: Hierarchy of stimuli in multisensory conditioning in *Microplitis croceipes*.
Twelve parasitoids were trained in a flight chamber plant patch containing a reinforced and an unreinforced combination of olfactory and visual stimuli. To deteremine the hierarchy of the combined stimuli, the visual and olfactory components of the reinforced and the unreinforced target were interchanged during testing.
The horizontal bar indicates the percentage of landings on the previously reinforced olfactory and visual stimulus respectively. Dominance of the olfactory component in multisensory conditioning was concluded from the significant higher number of landings made on the previously reinforced odor component (Wilcoxon signed-rank test, Z=0.04).

DISCUSSION

Research on sensory ecology of parasitoids has long focused on olfactory stimuli, providing insight in the prominent role that behavioral chemicals play in parasitoid orientation (for an overview see Vet & Dicke 1992). The effectiveness of olfactory orientation is determined by the specificity of the olfactory information in indicating profitable host sites. Olfactory stimuli released by plants as a result of herbivore feeding can provide a high level of specificity. Plant released synomones can enable parasitoids to distinguish between one herbivore feeding on different plant species and at least in some cases between different herbivore species feeding on the same plant (Dicke and Takabayashi, 1991; Turlings *et al.*, 1990). Our study shows that *M. croceipes* can learn frass odors to differentiate between one host species feeding on different structures of the cotton plant. This finding both underscores the specificity of olfactory information originating from interactions between the first and second trophic level, as it shows the specificity of olfactory perception in parasitoids.

The emphasis on olfactory stimuli in parasitoid foraging has overshadowed research into the potential role of other sensory modalities. Only a limited number of studies has investigated the use of visual cues by parasitoids during host location (Askew, 1961; Takahashi and Pimentel, 1967; Weseloh, 1972, 1986; Cardé, 1984; Sugimoto *et al.*, 1988; McAuslane *et al.*, 1990, 1991), while the role of visual stimuli in nectar foraging by parasitoids has only been studied indirectly (Hassan, 1966; Kevan, 1973). Learning of visual information by hymenopteran parasitoids was first demonstrated by Arthur (1966, 1967), who showed that the ichneumonid *Itoplectis conquisitor* (Say) could learn to discriminate between microhabitats on the basis of visual characteristics. Only recently has visual learning in parasitoids been examined in further detail. Studies investigating visual learning in the parasitoid *Exeristes roborator* (F.) have unambiguously demonstrated that this parasitoid could be conditioned to color as well as shape (Wardle, 1990; Wardle and Borden, 1990). Both shape and pattern learning were shown in *M. croceipes* (Wäckers unpublished data). Van Giessen *et al.* (1993) demonstrated location learning in *M. croceipes* and suggested this as a mechanism through which parasitoids could avoid already exploited sites (visual host discrimination) (see also Wäckers and Lewis 1993; Sheehan *et al*, 1993).

The limited attention given to visual learning in hymenopteran parasitoids is particularly striking considering the extensive investigation of visual learning in other Hymenoptera, especially honey bees (for an overview see Gould and Towne, 1988) and digger wasps (van Iersel, 1975). Our experiments show that free foraging parasitoids can differentiate between potential host sites both by learning their olfactory and visual characteristics. Although the benefits of visual and olfactory learning may overlap, multiple sensory learning is not necessarily redundant. Multisensory learning may enhance the learning efficiency along several lines:

- **Summation in multisensory conditioning:** In the field, insects usually don't experience stimuli as discrete singular entities. Resources represent a combination of sensory information to the foraging insect. This sensory multiplicity is also reflected in insect learning. Insects are known to learn various sensory stimuli which may interact in multisensory conditioning (Prokopy, 1986). As a rule, the response to a conditioned compound exceeds the response to either component (summation) (Weiss, 1972). Although there is ample discussion about the exact type of interaction between sensory components to describe summation best (Kehoe and Gormenzano, 1980), the generality of the phenomenon shows that multisensory conditioning will usually enhance the learned preference compared to single-sensory conditioning.
- **Increased differentiation:** The more information is incorporated into the search profile [search profile being defined as the array of stimuli evoking searching behavior in a foraging parasitoid], the better the parasitoid will be able to distinguish between profitable and unprofitable sites. When information is restricted to a single sensory modality (for instance olfaction), differentiation is limited to one sensory dimension (odor). With each additional sensory category learned, however, differentiation increases by one dimension. For instance, by learning color and shape in addition to odor, the search profile, and consequently the differentiating power, increases by two dimensions (odor x color x shape).

- **Combining best of both sensory worlds:** Each sensory modality has its own characteristics which brings on specific advantages and limitations when being used by a foraging insect (Miller and Strickler, 1984). As our study shows, olfactory stimuli produced as a result of interactions between the first and the second trophic level can convey extremely specific information to parasitoids. Under natural conditions, however, olfactory stimuli occur in meandering plumes carried in the moving air (Elkinton and Cardé, 1984). Consequently they are only detectable downwind from the odor source. Turbulence can interrupt the odor plume, which may cause a searching parasitoid to lose track. Since visual perception is independent from windspeed and wind direction, visual stimuli can be perceived omnidirectionally and their detectability is not affected by turbulence. Consequently, when parasitoids scan their host-habitat by flying over the canopy, visual stimuli are expected to be a main source of information (Miller and Strickler, 1984). Visual orientation, however, has its limitations as well. Visual stimuli (especially spectral reflectance) are dependent on light intensity (Prokopy, 1986), while the visual parameters shape and to pattern, change when varying the angle of view. Moreover, visual information is inaccessible when objects are obstructed. When vision is blocked, such as in dense canopy, olfactory information can be more effective to track down an object.

By learning more than one sensory characteristic, a parasitoid can combine the advantages of different sensory modalities. A parasitoid can be solely guided by the odor plume in situations where the object is barred from vision, while visual orientation will enable the parasitoid to stay on course when it loses the odor trail. Moreover, in situations where the accessibility of one type of sensory information is impeded (visual obstruction; turbulence disrupting odor plumes), or one of the sensory receptors becomes impaired, other modalities can take over.

In conditioning experiments with choice alternatives (Skinner, 1938; Staddon *et al.*, 1981), animals are trained to choose among equally detectable stimuli in accordance with their probability of reinforcement (i.e. the reliability of the conditioned stimulus as an indicator of the reward). Here we like to propose that the value of a stimulus to the forager is not only determined by its **reliability** in indicating presence, accessibility and suitability of resources, but also by its **detectability** (see also Vet *et al.*, 1991). In the example of parasitoid foraging, stimuli derived directly from the host are obviously most reliable as indicators of host presence. Their reliability over generations has allowed for genetical incorporation of responses to these stimuli (Lewis *et al.*, 1990). What is most reliable over generations, however, is not necessarily the optimal indicator of profitable host sites for the individual parasitoid. For example, the reliability of host-derived stimuli can be decreased by evasive behavior of the host, such as concealed feeding inside plant structures. Moreover, larvae of many species are known to frequently desert feeding sites (Heinrich 1979; Heinrich and Collins 1983). The second factor constraining the use of direct host stimuli by foraging parasitoids is their limited detectability. The latter as a direct consequence of the usually relatively limited host size, but likely also as a result of ongoing selection of the host for inconspicuousness to escape detection.

Associative learning enables a parasitoid to incorporate additional stimuli, proven to be temporarily or locally reliable indicators of host presence, into their search profile. Thus, associative learning may improve a parasitoid's foraging efficiency along two lines:

1. Increasing the detectability of profitable host sites by linking highly detectable stimuli to less detectable host-derived stimuli. Associative learning of well perceivable volatiles, such as the herbivore-induced synomones (Turlings *et al.*, 1990) will increase the range over which hosts can be detected downwind from a feeding site.

Highly detectable visual stimuli, such as the outstanding image of the cotton flower, are by themself usually unreliable indicators of host presence. However, when these visual stimuli are associated with the less detectable, but highly reliable host-derived odors, the association combines high reliability and high detectability. Since visual stimuli can be perceived in all directions, learning of prominent visual stimuli will increase both the range and radius of host detectability.

2. Increasing the reliability of stimuli indicating potential host sites. Associative learning of stimuli experienced at rewarding sites enables the parasitoid to differentiate between potential host sites on the basis of their profitability. Learned stimuli can serve as modifiers for genetically fixed responses to host-derived stimuli. This was the case in the visual learning experiment in which host-derived frass was learned only to be profitable in the presence of a specific visual stimulus (the modifier). In the field, learning of modifiers would, for instance, enable a parasitoid to pursue flower frass located in the flower, while ignoring flower frass dropped onto leaves below.

Various stimuli can be used by foraging parasitoids during separate stages of the resource location process (Prokopy, 1986). In *M. croceipes*, target-oriented flight is usually initiated by olfactory cues (Drost *et al.*, 1986). This study shows that during subsequent target orientation, parasitoids not only orient to olfactory stimuli, but also navigate by focusing on visual target characteristics (see also McAuslane, 1990).

Both visual and olfactory cues seem to be involved during alightment: host associated olfactory stimuli usually initiate alightment, while outstanding visual targets improve the accuracy of the landing (Wäckers, unpublished data).

After landing, we observed that *M. croceipes* uses both olfactory stimuli (odor trailing), visual cues (the parasitoid assumes an attack posture in response to moving objects), as well as mechanosensory cues (attack posture induced by surface vibrations) in host location. Host recognition and host acceptance seem to be mainly governed by chemical cues perceived through receptors on the ovipositor (Tilden and Ferkovich, 1988).

The dominance of olfactory stimuli in multisensory conditioning (experiment 4), corresponds with the learning hierarchy found in honeybees (Gould, 1985). Learning hierarchies, however, are not necessarily fixed. Therefore, when comparing learning rates of olfactory and visual cues, the following should be taken into consideration:

- Different stimuli might be dominant during different stages of the host location process. Since diurnal insects are primarily attracted by visual cues at a longer distance, while odor is more important at close range and as an initiator of landing (Faegri and van der Pijl, 1979), measuring landing choices as a preference parameter will bias towards odor learning.
- An organism's learning capability is likely to be affected by the quality and the quantity of the tested stimuli (Miller and Strickler, 1984), as well as by environmental conditions. The relatively laminar windstream in the flight-chamber, for instance, will facilitate the use of olfactory cues by parasitoids. Under more natural conditions, however, turbulence is likely to make olfactory orientation less reliable and foraging parasitoids might increasingly rely on visual stimuli to locate potential host sites.

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Chapter 5

Learning of color, shape and pattern during host foraging by *Microplitis croceipes.*

SUMMARY

Visual learning of the larval parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) was studied in a flight chamber set-up. Free ranging parasitoids were offered two visual alternatives, only one of which was associated with a host larva. In subsequent choice evaluations we tested whether parasitoids had developed a preference for the visual stimuli experienced during host encounters. By using alternatives that differed in either color, shape or pattern, it was shown that parasitoids can learn to distinguish host sites on the basis of each of these visual elements. This is the first report demonstrating that visual learning of parasitoids is not necessarily restricted to color, but can also includes structural characteristics. When parasitoids were conditioned to a combination of shape and color, the latter was learned dominantly.

It is discussed how learning of the three visual parameters can enable parasitoids to concentrate their search on the plant structures that are most profitable in terms of host encounters.

INTRODUCTION

Microplitis croceipes (Hymenoptera: Braconidae) is an obligatory parasitoid of *Helicoverpa* (Lepidoptera: Noctuidae) species. The polyphagous nature of its host confronts the parasitoid with a wide array of potential host habitats (Fitt, 1989). Not only do *Helicoverpa* spp. occur on a broad range of plant species, but within a single plant species they can feed on various plant parts. Feeding sites include young shoots, stems, flowers and fruiting structures (Farrar and Bradley, 1985). Various plants and plant parts represent disparate profitabilities to the foraging parasitoid. Not only do plants and plant structures differ in their frequency of infestation, they also vary in host accessibility. While *Helicoverpa* larvae are exposed when feeding on leaves and open flowers, they are often concealed when excavating stems, buds, and fruiting structures.

The ability of parasitoids to learn stimuli experienced during oviposition success (Lewis and Tumlinson, 1988) enables them to differentiate between the characteristic sensory properties of profitable and unprofitable sites. Previously it has been demonstrated that parasitoids can learn odor differences to distinguish between herbivore infested plants (Eller *et al.*, 1992), or hosts feeding on different plant structures (Wäckers and Lewis, 1993). Individual plants and plant structures, however, also present the foraging parasitoid with distinct visual characteristics such as color, shape, or patterns of feeding damage.

Out of the three visual parameters, color, shape, and pattern, only the former has been studied in any detail in parasitoids. A number of studies have shown differential responses in hymenopteran parasitoids to differently colored surfaces (Hollingsworth *et al.*, 1970; Weseloh, 1972, 1986; Moreno *et al*, 1984; Ma *et al.*, 1992), without addressing whether these visual response are innate or learned. The ability of hymenopteran parasitoids to learn color was first examined by Arthur (1966). Studying *Itoplectis conquisitor* (Say), he demonstrated that parasitoids could learn visual cues to discriminate between differently colored habitats. However, since the colors in his experiments differed not only in color, but in brightness as well, learning of the latter could not be excluded. The same applies to the study by Schmidt et al. (1993), who demonstrated that the pupal parasitoid *Pimpla instigator* could be trained to associate colored cylinders with the presence of hosts. The only unambiguous proof of color learning in parasitoids has been reported by Wardle (1990), who conditioned *Exeristes roborator* (F.) to different colors of equal intensity.

Although visual learning of shapes and patterns has long been demonstrated and studied extensively in honey bees (von Frisch 1915, Wehner, 1967; Schnetter, 1972;

Gould, 1986), it has yet to be demonstrated in parasitoids. Drost and Cardé (1992) established that the pupal parasitoid *Brachymeria intermedia* can learn to distinguish between habitat positions. When presented with Petri dishes on the floor and on a vertical structure, with hosts present in only one of these two positions, parasitoids would eventually concentrate their search on the reinforced position. Although shape parameters of the petridish structure might have been one of the stimuli learned by these parasitoids, the experiments did not address the question which conditioned stimuli underlie the reported site-discrimination.

Arthur (1967) reported that the parasitoid *Itoplectis conquisitor* could be conditioned to differentiate cylinders with and without hosts by their size and overall configuration. However, since this study was based on indirect probing counts and lacked direct behavioral observations, it was not elucidated which information about the cylinders was learned. Form learning was also addressed by Wardle and Borden (1990), who showed that the parasitoid *Exeristes roborator* could be conditioned to differentiate between a styrofoam cylinder and sphere. Parasitoids, however, did not distinguish between the different forms during orientation or landing (shape learning sensu von Frisch, 1915). Learned differentiation was displayed after landing only, through a longer searching time on the previously reinforced form, and a (correlated) higher number of probes. Although this experiment proves that parasitoids learned to distinguish between forms upon contact, again it does not clarify which sensory cues are being learned. The reported form recognition could be visual (positional orientation to light, orientation to surroundings, recognition of surface shape), tactile (size, surface curvature) or gravital.

In previous experiments with *Microplitis croceipes*, we demonstrated that this parasitoid could be conditioned to distinguish between host sites on the basis of both olfactory and visual cues (Wäckers and Lewis, 1993). These visual learning experiments, however, did not address visual elements individually. Here we used flight chamber experiments to study whether free ranging parasitoids could be conditioned to the individual visual parameters of color, shape, and pattern. In a subsequent experiment we determined the hierarchy of color- and shape learning.

MATERIALS AND METHODS

Microplitis croceipes were reared from *H. zea* larvae as described by Lewis and Burton (1970). The parasitoids were kept in acrylic cages (30 x 30 x 17cm), according to the day of their emergence, with access to water and honey. Rearing conditions were set at 28°C, 50-70% RH and a 16L:8D photoperiod. Three day old mated females without oviposition experience were used in the experiments.

***Helicoverpa zea* larvae** were reared on a pinto-bean based diet according to Burton (1969). Late third instars were used in the experiments as an oviposition reward. A separate group of larvae was reared on cotton leaves for frass collection.

Host Frass (fecal pellets) was used as a kairomone source. Frass pellets were collected immediately before the experiment from 5th instar *H. zea* feeding individually in small Petri dishes (5cm) on cotton leaves.

The flight chamber was designed similar to the chamber described by Drost *et al.* (1986). The test area was two meters long with a 75x75 cm cross section. The floor was covered with green paper. Overhead lighting was provided by four 80 Watt fluorescent bulbs. Experiments were conducted at 27°C and a wind speed of 31 (±2) cm/sec.

Cotton plants were grown individually in plastic pots (10 cm diameter), using a mixture of 1/2 potting soil, 1/3 sand and 1/6 peat moss. Growth conditions were controlled at 25-35°C and a 14L:10D photocycle. Plant sets of uniform age and size (about 30 cm in height; fifth leaf stage) were used to create a plant patch in the flight chamber. The plant patch consisted of 4 cotton plants placed pairwise in the flight chamber. The distance between pots was 30 cm, allowing sufficient space for the flying parasitoid to move freely among the plants.

Targets. Host sites were simulated by means of removable targets. Targets consisted of a piece of paper, containing three pellets of frass (± 25mg) placed at the base of the paper as a volatile attractant. The paper targets differed either in color, shape or pattern. In each of our experiments we used two types of targets, only one of which was being reinforced. Reinforced targets had a *H. zea* larvae pinned to the paper as a reward, allowing the foraging parasitoid to be conditioned to the visual stimulus presented on that target. Larvae were affixed to the top of the reinforced target with a # 000 insect pin pushed through the last abdominal segment. Using an identical pin in the unreinforced targets, both types of targets could be attached to the cotton plants. One target of both target types was placed on each of the four plants. The targets were positioned downwind on two upper leaves to assure maximum accessibility and visibility to the flying parasitoid.

Colors. Colored targets were made out of "Pantone" paper (2.5 x 2.5 cm) in the basic colors "Pantone Yellow U" and "Pantone Cool Grey 2". The Pantone colors were selected on the basis of their spectrophotometric characteristics, measured in a Licor Li 1800 Portable Spectroradiometer with an integrating sphere. "Pantone Yellow U" has a spectral maximum at 550 nm, which corresponds with one of the sensitivity maxima described for Hymenoptera (Peitsch *et al.*, 1992). "Pantone Cool Grey 2" on the other hand has a uniform spectrum. The shade of the "Pantone Cool Grey 2" was chosen to match the overall reflection of "Pantone Yellow U" (calculated over the insect's visual spectrum). To the parasitoid, both types of colored paper should consequently be of similar brightness. Any distinction made by the parasitoid is therefore likely based on wavelength characteristics, either hue (dominant wavelength) or saturation (% dominant wavelength). Both the grey and the yellow targets stood out against the foliage due to their higher intensity (overall brightness).

Shapes. Square paper targets (2.5x2.5cm) and triangular targets (5x2.5cm; base and perpendicular) were used in the shape learning experiment (Fig 2). Both target types were made out of plain white paper. The surface area of both targets was equal (6.25 cm^2), while they differed only slightly with respect to contour length (spatial frequency) and contour density (ratio of contour length to enclosed area).

Patterns. For the pattern learning experiment we used a checkered and a striped target as pattern alternatives (Fig 3). Patterned targets were of equal size (both 2.5x2.5 cm) and color (black on white). The checkered pattern was chosen since it has been shown to be very attractive to honey bees (Hertz, 1929; 1931). The striped pattern was chosen to match the brightness (50% black, 50% white) while closely approximating the contour length and the contour density of the checkered pattern.

General procedure: To increase the initial foraging motivation, parasitoids were allowed to antennate *H. zea* frass for a period of 30 seconds (Eller *et al.*, 1992) before their introduction into the plant patch. One minute after the pre-flight experience the parasitoid was released into the flight chamber from a 2-dram shell vial placed 40 cm downwind from the first pair of plants. Parasitoids were allowed to forage freely within the plant patch during two training sessions before they were tested in a subsequent experimental trial. Training sessions were ended as soon as three out of the four available hosts had been parasitized. After each training session, the targets were renewed before the parasitoid was reintroduced. During the experimental trial both types of targets (again one of each per plant) were offered without larvae. To determine whether parasitoids had developed preference for the reinforced visual stimulus, we recorded the number of landings made on both previously reinforced and unreinforced targets. Parasitoids were allowed to make up to 15 consecutive landing choices during the experimental trial. Experimental trials were ended prematurely when a parasitoid left the plant patch (phototactic flight to the top of the flight chamber, or by its landing and resting on a flight chamber wall) for a period of two minutes.

Statistical analysis: All experiments consisted of 20 replications (i.e. 20 different females). To assure a balanced experimental design, equal numbers of parasitoids were conditioned to each of the target types. In the test, we recorded both the number of landings on the previously reinforced target and on the unreinforced target. The percentage of the target landings made on the reinforced target was calculated per individual and subsequently averaged over each experiment. Preference learning was concluded when the percentage of landings on the reinforced target was significantly higher than the percentage of landings on the non-reinforced targets (Wilcoxon signed-rank test (α=0.05)).

EXPERIMENTS AND RESULTS

Experiment 1: color learning. To investigate whether *M. croceipes* can learn to distinguish host sites by their color, we conditioned parasitoids to either yellow or grey targets. Twenty parasitoids were trained, ten to each target, and subsequently tested for visual preference.

During the test, 19 out of the 20 individuals made the majority of their landings on the reinforced color. Parasitoids conditioned to yellow made 78% of their landings on yellow, while in the case of parasitoids with oviposition experience on grey, 69% chose for grey (Fig. 1). Calculated over all individuals, parasitoids displayed a significant conditioned preference (73%) (Wilcoxon, n=20, Z=2.0*10^{-4}) for the previously reinforced color.

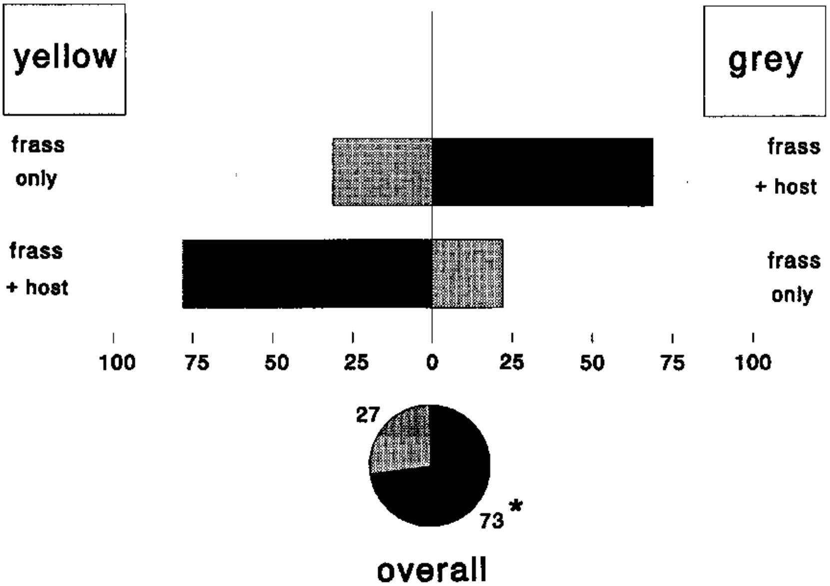

Fig 1. Color learning by *M. croceipes* foraging in a flight chamber plant patch. The percentage of the target landings made on the reinforced color (black bars) and unreinforced color (grey bars) was calculated per individual and subsequently averaged over each experiment. The pie chart shows the overall distribution of landings. Parasitoids landed significantly more often on the reinforced color (Wilcoxon, n=20, Z=2.0*10^{-4}).

Experiment 2: shape learning. Out of the 20 parasitoids conditioned to distinguish between triangles and squares 19 made the majority of their landings on the previously reinforced target. On average, parasitoids conditioned to square targets made 76% of their landings on this target (Fig. 2). The ten parasitoids trained to the triangular targets made an average of 83% of their landings on triangles. Calculated over all individuals, parasitoids displayed a significant conditioned preference (79%) (Wilcoxon, n=20, $Z=1.1*10^{-4}$) for the shape of the previously reinforced target.

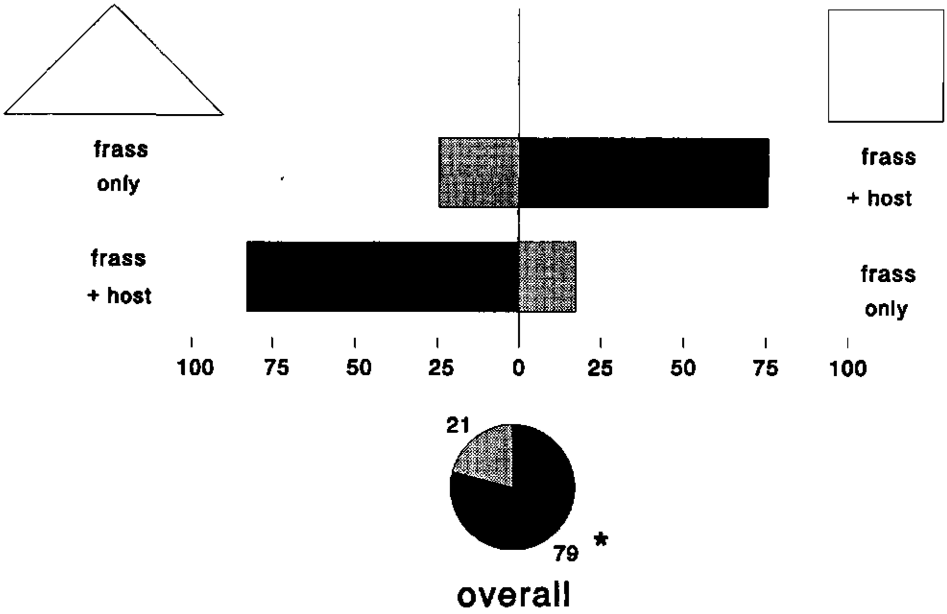

Fig 2. Shape learning by *M. croceipes* foraging in a flight chamber plant patch. The percentage of the target landings made on the reinforced shape (black bars) and unreinforced shape (grey bars) was calculated per individual and subsequently averaged over each experiment. The pie chart shows the overall distribution of landings. Parasitoids landed significantly more often on the reinforced shape (Wilcoxon, n=20, $Z=1.1*10^{-4}$).

Experiment 3: pattern learning. Of the 20 parasitoids, 15 landed more often on the previously reinforced pattern. Parasitoids conditioned to the checkered target made on average 58% of their landings on this target. When the striped targets had been reinforced 62% of the landings were on the stripes (Fig. 3). Calculated over all individuals, the majority (60%) of the landings were made on the target to which parasitoids had been conditioned (Wilcoxon, n=20, Z=0.002).

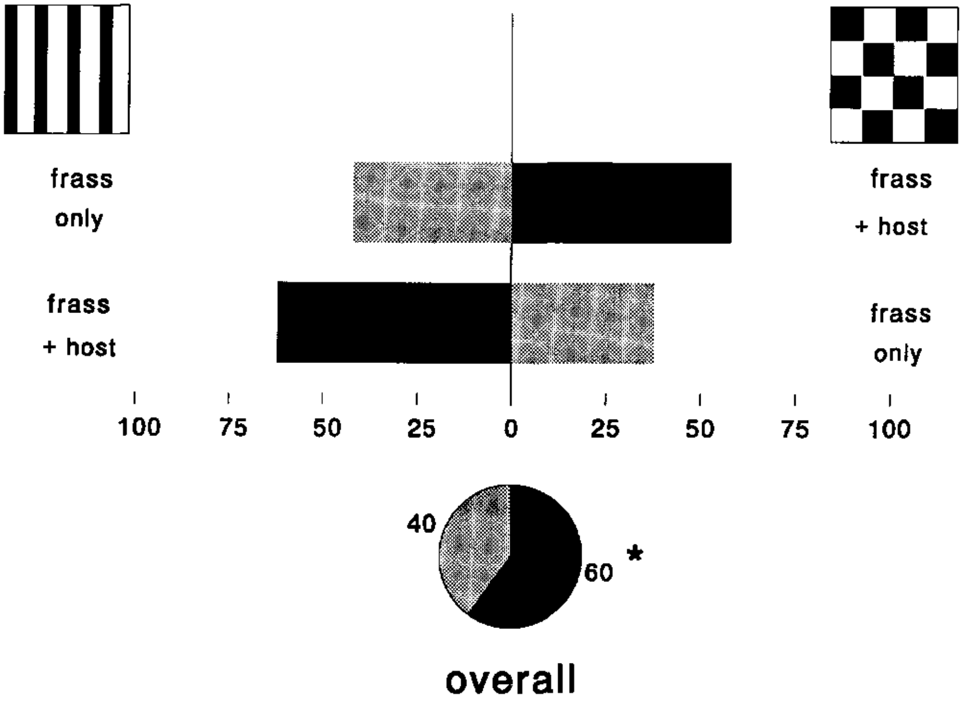

Fig 3. Pattern learning by *M. croceipes* foraging in a flight chamber plant patch. The percentage of the target landings made on the reinforced pattern (black bars) and unreinforced pattern (grey bars) was calculated per individual and subsequently averaged over each experiment. The pie chart shows the overall distribution of landings. Parasitoids landed significantly more often on the reinforced pattern (Wilcoxon, n=20, Z=0.002).

Experiment 4: hierarchy of color and shape in visual learning. This experiment was designed to determine if one sensory component dominates during multisensory conditioning. We conditioned 20 parasitoids, five individuals to each of the four possible combinations of the two color and shape stimuli. Color and shape components of the reinforced and the unreinforced target were interchanged in the test (Fig. 4). Parasitoids consequently faced the choice between the reinforced color stimulus in combination with the unreinforced shape, and the reinforced shape combined with the unreinforced color.

Out of 20 individuals 15 made the majority of their landings on the previously reinforced color stimulus, one was tied, while the remaining four individuals landed more often on the reinforced shape. On average, parasitoids trained to a combination of color and shape landed significantly more often (Wilcoxon, n=20, Z=0.01) on the reinforced color component (61%), as compared to the shape component (39%).

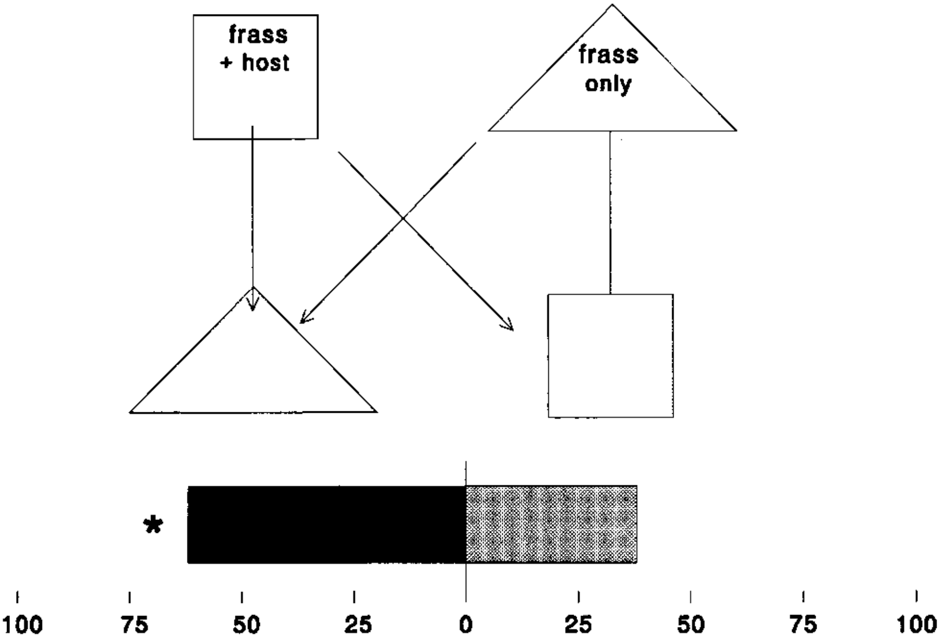

Fig 4. Hierarchy of color and shape in visual learning by *Microplitis croceipes*.
Twenty parasitoids were trained in a flight chamber plant patch containing a reinforced and an unreinforced combination of color and shape stimuli. To deteremine the hierarchy of both visual parameters, the color and shape components of the reinforced and the unreinforced target were interchanged during the test.
The horizontal bar indicates the percentage of landings on the previously reinforced color and shape stimulus respectively. Dominance of the color stimulus was concluded from the significant higher number of landings made on the previously reinforced color (Wilcoxon, n=20, Z=0.01).

DISCUSSION

The detectability of an object by a visual system depends on (i) the object's dimensions and its contrast with the background, (ii) the medium and the distance between emitter and receiver, (iii) the illumination and (iiii) the sensitivity of the visual receptor (Prokopy and Owens, 1983). The limited resolution of the insect's compound eye (1°-2°) precludes detailed visual recognition of pattern and shape at longer distances (Wehner and Srinivasan, 1984). Color detection is less dependent on a fine visual resolution. For the visually orienting parasitoids this means that the general outline of the plant (Mc Auslane *et al.*, 1990) or color stimuli can be used at longer distances, while information about the specific shapes of plant structures and patterns of feeding damage can be used at close range.

In our experiments long- and short-range orientation by the flying parasitoid could be clearly distinguished. At the longer range (5-40 cm), experienced parasitoids display a linear target-directed flight, which is followed by hovering at close range (1-2 cm from the plant). The fact that conditioned parasitoids display this linear target-directed flight, even in absence of the frass odor, shows that *M. croceipes* uses visual target information (likely the contrasting target color) during long-range target orientation. It is likely that recognition of shape and pattern occurs during the short-range hovering flights. The typical hovering movements in the parallel plane to the targets could facilitate shape and pattern recognition. The fact that experienced parasitoids were repeatedly observed to land directly on the host could indicate close-range visual orientation to the host image.

Individual visual parameters

Color. The vast majority of the visible plant biomass contains chlorophyll, which strongly absorbs blue and red light. As a consequence, the reflectance of foliage is remarkably consistent over a wide range of plant species (Gates, 1980). Insects perceive this foliage as mostly grey, due to the equally intense reflection of leafgreen in all regions of the insect's visual color spectrum (Daumer, 1958). Against this achromatic background, any speck of color achieves an optimal contrast. The bright coloration displayed by insect- and bird-pollinated flowers, as well as by fruits aiming for seed dispersal by birds, are obvious examples in which plants use this color contrast to advertise their rewards and attract their mutualistic associates.

The color signals displayed by flowers and fruits, however, are equally available to others besides the intended receivers. Flower- and fruit coloration can also serve as cues to herbivores foraging for mating-, oviposition-, or feeding sites (Prokopy and Owens, 1983), while natural enemies, for their part, could use displayed colors to locate nectar (Wäckers, 1993), or to find their herbivorous hosts. Since only circumstantial evidence has been reported with respect to the latter (Varley, 1941; Askew, 1961), use of flower- or fruit color as indirect host foraging cues has yet to be demonstrated for parasitoids. The only investigation to our knowledge (Leyva *et al*, 1991) did not find an effect of fruit color on fruit selection by *Diachasmimorpha longicaudata*, a larval parasitoid of tephritid fruit flies.

Whenever the coloration of plant structures is consistently and specifically altered due to herbivory (e.g. galls, or leafminers), the color of infested plant structures could become an especially reliable host foraging stimulus.

The demonstrated ability of *M. croceipes* to learn colors could facilitate differentiation by the parasitoid between profitable and unprofitable host sites. Color information could be used to differentiate between open flowers (exposed host) and flower buds (concealed hosts), between differently colored flower species, or between flowers and green plant structures.

Color is composed of the components hue (dominant wavelength), saturation (% dominant wavelength) and intensity (reflected energy) (Levi, 1968). Honey bees distinguish colors mainly on the basis of their hue (Menzel 1967), while color preferences in the case of bumblebees are based on color saturation, rather than on hue (Lunau, 1990). Unlike hue and saturation, the intensity of light reflected from a plant surface can vary considerably with angle and degree of illumination (Gates, 1980). This makes intensity an unreliable factor for orientation. Nevertheless, in systems in which host hues lie beyond the insect's visual spectrum, intensity can become the most important color component in host recognition. *Rhagoletis* *pomonella*, for instance, ovipositing on fruits whose red hue it cannot perceive, locates these fruits primarily on the basis of fruit intensity (Owens and Prokopy, 1986).

Since the parasitoids in both our experiments, and in the experiments reported by Wardle (1990), could be conditioned to distinguish between differently colored targets of comparable intensity, it is likely that parasitoids learned to distinguish spectral qualities. Wardle (1990) corroborated this by demonstrating that parasitoids in her experiments failed to distinguish between shades of grey. Whether this spectral learning is based on hue discrimination, or on saturation-based learning can not be concluded.

Shape. In contrast to flower foragers, host foraging parasitoids are usually faced with a mainly monochrome world in which the spectral quality of foliage is unlikely to constitute a plant-specific character (Gates, 1980). Together with the fact that the majority of herbivores generally do not affect the coloration of the plant structures they feed on, this will obviously limit the use of color as a host foraging cue. Shape, on the other hand, can be a highly discriminating cue in almost any habitat. Not only do plant *species* differ in their morphology, individual plant *structures*, as well, can be distinguished by their characteristic shape. These specific structural qualities could be effective cues for parasitoids to locate host habitats. Furthermore, when plant structures are specifically transformed by the herbivore, the characteristic shape of infested plant structures can also become an effective stimulus during the phase of host location.

Many insects respond to structural features from their feeding- or oviposition sites. Kügler (1955) reported that flower visiting Diptera prefer fringed shapes which resemble the specific morphology of sapromyophilous flowers. The stick insect *Carausius morosus* approaches models of the silhouette of a food plant (Jander and Volks-Heinrichs, 1970). Size and shape of fruits have been shown to be important foraging parameters for the fruit fly *Ragholetis pomonella* (Prokopy, 1968; 1977), while its parasitoid *Diachasma alloeum* seems also attracted to the visual image of hawthorn fruits (Glas and Vet, 1983).

Learning of shape stimuli has been extensively studied in bees (see Barth, 1991 and Gould, 1993 for an overview). Aside from this work, little is known about the extent to which shape preferences in other arthropods are affected by experience. Fruit size is learned by the apple magot fly, *Rhagoletis pomonella* (Papaj and Prokopy, 1986) and the Mediterranean fruit fly, *Ceratitis capitata* (Papaj *et al.*, 1988) in association with oviposition experience. Leaf shape preferences found in the pipevine swallowtail (*Battus philenor*) were also demonstrated to be primarily due to visual learning (Rausher, 1978; Papaj and Rausher, 1987). To our knowledge this is the first report proving shape (silhouette) learning in parasitoids. The demonstrated ability of *M. croceipes* to associate shapes with an oviposition reward could enable the parasitoid to concentrate its foraging on those structures that are most profitable in term of host encounters. Although shape in itself is usually not a reliable indicator of host presence, in combination with other stimuli, such as synomones released by plants in response to herbivore damage, it can help parasitoids to select suitable host sites among the wide range of plants and plant structures. Amongst infested plant structures, shape learning could enable parasitoids to avoid plant structures on which hosts are concealed and to concentrate foraging on more accessible exophytically feeding larvae.

Patterns. Plant morphology may lead to a variety of structural patterns. Leaf venature, defensive leaf structures and variegated coloration can cause distinguishable patterns on the leaf surface. Patterns resulting from herbivory are detectable and the most reliable structural indicators of herbivore presence, making them likely candidates to be used by natural enemies. Birds, who forage entirely by vision, have the capacity to use the image of leave damage as a cue to locate catterpillars (Heinrich and Collins, 1983; Real *et al.* 1984).

Unlike birds, invertebrate predators and parasitoids can use both olfactory and visual cues to locate herbivorous insects (Raveret Richter, 1990; McPheron, pers. comm.; Wäckers and Lewis, 1993). Even though herbivory induced synomones may generally be the primary leaf damage cues used by parasitoids and arthropod predators (Dicke and Sabelis, 1988; Turlings *et al.*, 1990), the visual profile of damage patterns could serve as supplementary information to the herbivore-induced synomones. Not only will this multisensory foraging improve the efficiency by which parasitoids can locate herbivore sites, it could also improve differentiation between herbivore sites in the following ways:

- The fact that landing responses by host foraging parasitoids are usually contingent upon synomone presence will prevent parasitoids from visiting sites that visually resemble feeding damage. Also will it enable parasitoids to distinguish occupied from deserted feeding sites, when synomone production at the latter drops sharply within a short period after feeding ceases (Turlings and Tumlinson, 1992).
- Learning of damage patterns could enable parasitoids to distinguish between plant-herbivore complexes when these are indistinguishable by their volatile profiles only, e.g. different herbivore species, or -instars feeding on the same plant (McCall *et al.*, 1993).
- Herbivore accessibility often coincides with the visibility of primary damage (endophytic larvae usually leave only an entrance hole, while feeding damage of exophytic larvae can be quite prominent). The combination of host-induced synomones and visible damage patterns, therefore, not only ensures the presence of herbivores but can also their accessibility.

Learning rate and hierarchy

In comparison with the learning rates reported for honey bees, *M. croceipes* seems to need more conditioning trials to achieve the same accuracy of choice. To realize a level of odor preference comparable to the level of accuracy achieved by honey bees after single trial conditioning (95%), *M. croceipes* requires four positive reinforcements (Menzel, 1985; Eller *et al.*, 1992). After six ovipositions on yellow in our color conditioning experiment the parasitoids made 78% of their landings on this color. This accuracy level corresponds with the level honey bees reach after two exposures (Menzel, 1967).

Although these comparisons of learning ability between different species can be of obvious ecological interest, such correlations are intricate. The learning experiments on which these comparisons are based usually differ in more variables than the species under comparison. Additional variables may lie within the organisms (their genetic learning disposition, experience, and physiological state), the unconditioned stimulus, the quality and quantity of conditioned stimuli, the experimental procedure and the environmental conditions. Even though our procedures are highly comparable to the methodology used in the corresponding bee-conditioning experiments (sensu von Frisch, 1915 and subsequent studies), they do, for instance, use host rewards instead of the nectar rewards in bee studies. This and other differences make direct comparisons of learning rates difficult to interpret. Indirect comparisons, on the other hand, of relative (intra-species) learning rates, offer a more appropriate way to contrast learning abilities between species.

Considering the relative learning rates in honey bees, shape learning seems to be an interesting exception to the generally higher learning rate. Von Frisch (1915) reported that bees could not be conditioned to distinguish between a class of solid figures (square, triangle or circle), nor between a class of "open" figures, while bees would readily learn to distinguish between the two classes of figures. Comparable limitations in shape discrimination of honey bees were described by Hertz (1929, 1930, 1931). Schnetter (1972) demonstrated that the learning rate in the case of shapes depends on the spatial frequency of the object. Four-pointed stars (low spatial frequency) required 18 trials to be selected at a 80% accuracy level, while this same accuracy was realized after 8 trials with a 23-pointed figure. Although shapes with a high frequency are learned faster, acquisition rate is still considerably slower than in color learning. This difference in learning rate has been interpreted to reflect the higher reliability of color as a cue for flower foragers (Gould, 1992).

In comparison with the marked difference between the rate of color learning and shape learning in bees honey bees, the relative rate of shape learning in *M. croceipes* is noticably high. Even though the shapes in our experiments had a low spatial frequency, parasitoids learned shape at a rate comparable to the rate of color learning. This relatively high rate of shape learning in host foraging *M. croceipes* could be an adaptation to the monochrome but multishaped environment in which parasitoids have to locate their herbivorous hosts.

The dominance of color over the shape in host foraging (experiment 4), corresponds with the learning hierarchy found in honeybees (Gould, 1985). It should be considered, though, that the low spatial frequency of the tested shapes is likely to result in an underestimation of shape learning. Furthermore it should be considered that the learning propensity and learning biases of *M. croceipes* might not be representative for parasitoids of strict folivores, since *Helicoverpa* spp. do also occur on colored plant parts such as flowers and fruiting structures. Further studies on visual learning in other parasitoid species as well as in invertebrate predators will be required to substantiate possible differences in learning predispositions between flower foragers, such as bees, and insects searching for folivores.

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Chapter 6

Leaf damage from a parasitoid's point of view

SUMMARY

In the present paper experimental data are presented on parasitoids using visual leaf damage stimuli during host foraging and the role of the visual image of leaf damage is extensively discussed in a tritrophic context.

The innate response of *Cotesia rubecula* to the visual component of leaf damage was examined in flight chamber experiments. Parasitoids were observed while foraging among plants containing different types of artificial leaf damage (small punch holes; big punch holes; peripheral damage) and undamaged control leaves. Parasitoids displayed an innate preference for leaves containing small punch holes, compared to leaves with big punch holes, while leaves with visible damage were preferred over leaves without visible damage. This preference for the visual image of herbivore feeding was manifested in a significantly higher number of landings. The subsequent search time, however, did not differ. The innate visual preference was no longer found when the odor component of natural feeding damage was added to the four leaf categories. This indicates that under the given experimental conditions, visual orientation was overruled by herbivore induced volatiles. When free-ranging parasitoids were given repeated oviposition experience on leaves with small punch holes, they displayed a preference for this leaf-category, which was expressed in a higher number of landings, notwithstanding the herbivore induced volatiles.

INTRODUCTION

Herbivore feeding damage provides important foraging cues both for predators and parasitoids (Heinrich and Collins, 1983; Dicke and Sabelis, 1988; Turlings *et al.*, 1990). The research on sensory orientation in insect parasitoids has long focused on the chemical component of herbivore damage. This research has elucidated intriguing tritrophic interactions and led to a general appreciation of the prominent role played by plant released semiochemicals in parasitoid foraging (reviewed by Vet and Dicke, 1992).

Herbivore feeding, however, is not revealed solely through chemical cues. Whenever herbivory leads to deformation of plant structures, feeding damage can also be a distinct and specific visual indicator of herbivore presence. Birds, hunting entirely by vision, can use this visual information as a cue to locate caterpillars (Heinrich and Collins, 1983; Real *et al.*, 1984). Invertebrate predators and parasitoids, on the other hand, may use both visual and olfactory cues to locate herbivores (Montllor and Bernays, 1993; Wäckers and Lewis, 1993). Even though parasitoids are usually considered to respond primarily to the odor component of feeding damage (Vet and Dicke, 1992), there have been indications that they are attracted to the visual image of damaged leaves as well. Sugimoto *et al.* (1988) provided evidence that the leafminer parasitoid *Dapsilarthra rufiventris* uses visual information from host mines during host foraging. Faeth (1990) showed that leafminers (*Cameraria* spp.) suffered a higher mortality due to attack by (unspecified) invertebrates in the presence of odor-sealed leaf damage compared to leafmines in the presence of sealed undamaged leaves. Since predation and parasitism were treated as a single category in the statistical analysis of his data, the impact of the visual damage stimuli on parasitization per se was not demonstrated.

In contrast to the damage by leaf-mining larvae, the feeding damage of leaf-chewing folivores usually consists of leaf perforations. The visual role of this structural damage in the interactions between folivores and parasitoids has not been elucidated. Wäckers and Lewis (1994) suggested that the reported ability of *M. croceipes* to learn color, shape and patterns may enable this parasitoid to employ perforation patterns as host foraging cues. This study deals with the question of whether host-foraging parasitoids actually do respond to the visual component of structural leaf damage. This question was addressed using the tritrophic system of *Cotesia rubecula*, *Pieris rapae*, and *Brassica oleracea* cv. "Titurel" (Brussels sprouts).

C. rubecula is a specialist parasitoid which preferably parasitizes early instars of *Pieris rapae*, but by exception may also accept and develop in *P. brassicae* (Shenefelt, 1972; Brodeur and Geervliet, 1992; Geervliet and Brodeur, 1992). When searching for food this parasitoid has been shown to use both olfactory and visual cues, while satiated parasitoids are attracted to odors from *P. rapae* feeding on Brussels sprouts (Wäckers, 1994).

P. rapae feeds solitarily on Brassicaceae and occasionally on Capparidaceae. The larvae are cryptically colored, but produce detectable feeding damage. Feeding by the caterpillar induces cabbage plants to release plant specific semiochemical blends which play a prominent role in host foraging by *C. rubecula* (Blaakmeer *et al.*, 1994).

To investigate whether the parasitoid uses the image of feeding damage as an additional foraging cue, the innate responses of *C. rubecula* to different categories of artificially damaged leaves were determined in a flight chamber. In a subsequent experiment the odor component of natural feeding damage was added to the visual component of the artificial leaf damage. Lastly, it was investigated whether oviposition experience on one type of artificial damage affects the subsequent preference for that type of artificial leaf damage.

MATERIALS AND METHODS

Cotesia rubecula had been collected in Brussels sprouts and red cabbage fields from *Pieris rapae* larvae and subsequently reared for ca. five generations on *P. rapae* larvae feeding on Brussels sprouts (for details see Wiskerke and Vet, 1991). The parasitoid pupae emerged and were subsequently kept in plexiglass cages (30 x 40 x 37 cm) at 25°C, 50-70% RH and a 16L:8D photocycle. Daily transfer of parasitoid pupae to a new cage assured parasitoid groups of uniform age. Parasitoids were provided with water and honey. Three day old mated females were used in the experiments.

The flight chamber design was identical to that described by Takken (1994). The test area was 2.05 meters long with a 60 x 60 cm cross section. Overhead lighting was provided by eight 32 W TLD/48HF fluorescent lights and four 200 W Philips soft tone light bulbs. Lights were placed in a hemispherical fixture (205 x 60 cm) located 40 cm above the flight arena. Fluorescent lights were shielded and the spotlights aimed upward to assure indirect lighting of the test arena. The inside of the light fixture was coated with aluminium sheets (Stuccodessin R) for an even light reflection. The light intensity inside the flight chamber was controlled at 2000 lux. Within the flight chamber, a plant patch was created by placing four individual Brussels sprouts plants in the test arena. The distance between plants was 25 cm, allowing sufficient space for the flying parasitoid to move freely among plants. Experiment 1 was conducted in still air to induce visual orientation by the parasitoids. The remaining experiments were conducted at a windspeed of 15 cm/sec. Climatic conditions were controlled at 25 ± 1 °C and 40-60% RH.

Brussels sprouts plants *Brassica oleracea* cv. "Titurel", a natural host for *Pieris spp.*, were used in the experiments. Plants were grown individually in plastic pots (10 cm diameter) under glasshouse conditions (20-30°C, 50-80% RH, and a 16L:8D photocycle). Four plants of uniform age and size (40 cm; 8-10 leaves) were used in the experiments.

Mechanical damage. Part of the leaves were mechanically damaged either by punching out leaf material (visible damage) or by cutting a strip along the leaf edge (indistinguishable). Mechanical damage of leaf material inevitably result in the release of green leafy volatiles from the site of damage (Whitman, 1988; Steinberg *et al*, 1993). Since parasitoids are known to be attracted to these plant volatiles (Whitman and Eller, 1990; Steinberg *et al*, 1993), these volatiles constitute an interfering factor in the study of the visual component of leaf damage stimuli. To minimize this interference, damage was administered one week prior to the experiment. Since volatile release by artificially damaged corn plants drops sharply within 24 hours (Turlings and Tumlinson, 1992) and artificially damaged cabbage plants lose their attractiveness to *C. glomerata* almost instantly (Steinberg *et al.*, 1993), this interval was considered to be ample to at least curb the release of plant volatiles. To control for possible residual odor effects, four leaf treatments were used in the experiments (Fig 1), randomly assigned to the eight youngest leaves of each of the four plants:

- Two leaves containing 30 small punch holes (0.3 cm in diameter).
- Two leaves containing 6 big punch holes (1.5 cm in diameter).
- Two leaves damaged by cutting a thin strip along the edge, as a control for possible damage-induced odor cues.
- Two undamaged leaves.

To match the length of the damage contour and thereby the concentration of possible leaf-odors, the number of punched holes was chosen to realize equal circumference of leaf damage in the first two treatments. Holes were punched in between the main leaf veins, so that no wilting would occur in the remaining leaf tissue. Leaves of the latter two treatments were marked with a piece of white masking tape (± 4mm^2) at the base of the petiole.

Natural feeding damage was used in experiment 2 to study the effect of an additional odor source on visual preferences. Feeding damage was obtained from second instar *P. rapae* that had been feeding on separate Brussels sprouts plants for at least 24 hours. Leaf discs (1.5 cm in diameter) containing feeding damage were punched out immediately before the start of the experiment. Eight discs were placed on each plant in the flight chamber (one on each of the eight experimental leaves). To keep leaf discs from drying out, they were applied on wetted filter paper of equal diameter. Leaf discs were renewed after a replicate had been completed.

Hosts were used in experiment 3 to investigate whether parasitoids would display visual preference for damage patterns following oviposition experience on leaves containing small punch holes. For this purpose, a leaf damage disc together with two larvae (second instar *P. rapae*) were added to leaves containing small punch holes, while the remaining leaves received only a leaf-damage disc. Larvae were affixed to a clear plastic disc (2 cm diameter) with a # 000 insect pin pushed through the last abdominal segment. The plastic disc prevented larvae from feeding on the plant tissue and made it possible to position the larvae onto the punched leaves by placing the pin through one of the punch holes.A single parasitoid was allowed to forage in this set-up during two periods of one hour. In between these two foraging bouts the larvae and the leaf damage discs were renewed. To test whether parasitoids had developed a visual preference for the artificial damage pattern, the conditioned parasitoids were tested using a new set of plants. During the test no larvae were present, while each of the four leaf categories contained leaf discs.

General procedure. Individual parasitoids were taken out of the rearing cage in a small glass vial and subsequently released by placing the vial in between the plants on the flight chamber floor. After take-off, the parasitoid's behavior was observed continuously for a period of 30 minutes. Landing choices and retention time before renewed flight were recorded for each of the four leaf categories.

Plants were renewed after every four parasitoids tested. Twenty individuals were tested in each of the experiments, each individual being one replication.

Statistical analysis: The fraction of the target landings made on the reinforced target, as well as the percentage time spent searching, was calculated per individual and subsequently averaged over each experiment. Preference was tested both for the number of landings as for the retention time using the Wilcoxon signed-rank test (α=0.05).

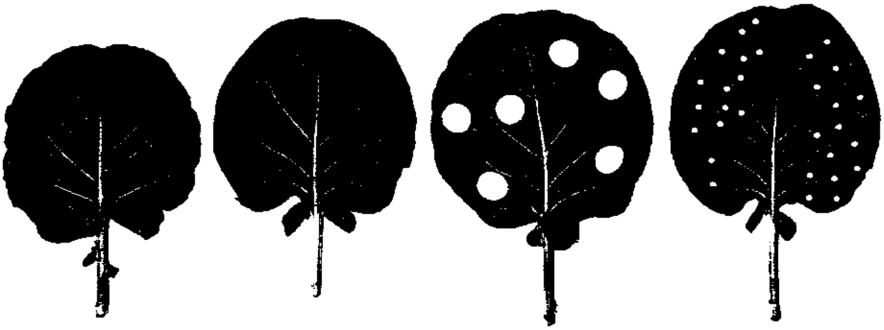

Fig 1. Leaf types of *Brassica oleracea* used in the experiments. From the left: undamaged, peripherally damaged, big punch holes, and small punch holes.

Fig 2. Natural feeding damage by *Pieris* spp. on *Brassica oleracea* leaves: early second instar *P.brassicae* (left); early second instar *P. rapae* (right).

EXPERIMENTS AND RESULTS

Experiment 1: the visual component of leaf damage

The initial response of *C. rubecula* to the visual component of feeding damage was examined by observing the foraging behavior of inexperienced females within the plant patch in which eight leaves of each leaf category were present. Females were released into the flight chamber, and allowed to forage for a maximum of 60 minutes. Both the number of landings on the different leaf categories as well as the subsequent searching time were recorded.

Parasitoids exhibited an initial landing preference for leaves containing small punch holes over leaves containing big punch holes. Parasitoids also landed more frequently on the two visible damage categories combined when compared to the two categories lacking visible damage (Table 1). Searching time, however, did not differ between the contrasted leaf categories.

Table 1. Response of free flying *Cotesia rubecula* to the visual component of leaf damage. Four leaf categories were offered per cabbage plant: leaves containing small punch holes, big punch holes, peripherally damaged leaves (considered invisible), and undamaged control leaves. The response of inexperienced females to each of these leaf categories was determined on the basis of both their landing choices, and the time parasitoids spent searching the leaves before renewed flight. Comparisons by Wilcoxon, n=20.

	LEAF CATEGORY				P		
response (n=20)	1. small holes	2. big holes	3. peripher. damage	4. undamaged	1vs2	3vs4	1+2vs3+4
average number of landings (%)	36.8±9.9	27.2±11.7	19.9±9.1	16.1±10.1	0.02	NS	5.3×10^{-4}
average retention time (s)	43.4±19.7	58.2±28.4	37.6±19.3	51.8±25.0	NS	NS	NS

Experiment 2: the visual and olfactory component recombined

When leaf discs containing natural leaf damage were added to the different leaf categories, parasitoids no longer displayed visual landing preference as demonstrated in experiment 1. Both landing choices and retention times were approximately equal among the four leaf categories (Table 2). This indicates that under the given experimental conditions visual orientation was overruled by herbivore induced volatiles. In comparison with experiment 1 the average retention time showed a fivefold increase. This higher retention was due to the contact kairomones present on the naturally damaged leaf discs, which induces area restricted search in the parasitoid.

Table 2. Response of free flying *Cotesia rubecula* to the visual component of leaf damage in the presence of damage odors. Four leaf categories were offered per cabbage plant: leaves containing small punch holes, big punch holes, peripheral damage (invisible), and undamaged control leaves. As a source of semiochemicals, a naturally damaged leaf disc was added to each of the leaf categories. Both landing choices, and retention time were measured as preference parameters. Comparisons by Wilcoxon, n=20.

	LEAF CATEGORY				P		
response (n=20)	1. small holes	2. big holes	3. peripher. damage	4. undamaged	1vs2	3vs4	1+2vs3+4
average number of landings (%)	26.3±10.1	24.1±13.1	27.1±14.8	20.8±12.3	NS	NS	NS
average retention time (s)	254±149	232±127	221±106	266±115	NS	NS	NS

Experiment 3: experience

It was investigated whether parasitoids would display visual preference for damage patterns after oviposition experience. Parasitoids were given experience in a plant patch in which all four leaf categories contained leaf damage discs, while larvae were present only on leaves containing small punch holes.

Following two foraging rounds, parasitoids in the test demonstrated a preference for the small punch holes, even though herbivore induced volatiles were present on all leaf categories. This preference expressed itself in a higher number of landings, without affecting the search time (Table 3).

Table 3. Response of free flying *Cotesia rubecula* to the visual component of leaf damage after repeated oviposition experience on leaves containing small punch holes. Four leaf categories were offered per cabbage plant: leaves containing small punch holes, big punch holes, peripheral damage (invisible), and undamaged control leaves. The response of experienced females to each of these leaf categories was determined both on the basis of their landing choices, and by the time parasitoids spent searching the leaves before renewed flight.

	LEAF CATEGORY				P
response (n=20)	1. small holes	2. big holes	3. peripher. damage	4. undamaged	1vs2
average number of landings (%)	31.7±9.6	20.8±11.1	24.3±8.9	23.2±10.8	5.8x10^{-3}
average retention time (s)	44.9±31.7	40.0±36.5	29.6±20.7	31.5±21.0	NS

DISCUSSION

Parasitoids have to achieve an astonishing degree of specificity in their foraging decisions. Not only do they have to distinguish hosts from non-hosts, they also usually parasitize no more than a narrow range of host developmental stages. The research into the sensory basis of this differentiation has revealed that olfactory stimuli are specific enough to account for many of the parasitoid's foraging decisions. Nevertheless, the specificity of olfactory cues seems to fall short from providing the whole answer.

The specificity of olfactory cues from herbivore damaged plants is not only due to the fact that their release of semiochemicals is specifically induced by herbivore feeding (Dicke and Sabelis, 1988; Turlings *et al.*, 1990), but also to the fact that these herbivore-induced semiochemicals can be specific in terms of plant species and plant structure (Turlings *et al.*, 1993b). Despite their high degree of plant specificity, however, herbivore-induced synomones are not necessarily herbivore-specific. Examples are known in which volatile profiles from different herbivores feeding on the same plant are specific enough for natural enemies to distinguish between the herbivores (Dicke and Takabayashi, 1991; Turlings *et al.*, 1993b), while other tritrophic systems do not show such specificity: Turlings *et al.* (1993a) reported that both the regurgitate from various caterpillar species as well as from the grasshopper *Schistocerca americana* would induce corn seedlings to release a similar volatile profile. This lack of herbivore specificity was corroborated by the finding that neither *C. marginiventris* nor *M. croceipes* could distinguish between plants exposed to regurgitate from their host *H. zea* and *S. americana*. *M. croceipes* showed an equal lack of discrimination ability when given a choice between cowpea containing *H. zea* and either of the two non-hosts *Trichoplusia ni* or *Spodoptera exigua*. Even after repeated exposure to feeding damage of either caterpillar, including oviposition experience in the case of exposure to host damage, parasitoids landed equally between the two cowpea-herbivore complexes (McCall *et al.*, 1993). Since *M. croceipes* is capable of learning such subtle chemical differences as to distinguish between frass from *H. zea* feeding on different parts of the cotton plant (Wäckers and Lewis, 1993), this randomness indicates a lack of (perceivable) herbivore specificity in herbivore-induced synomones. Investigations on the volatile profiles released by cotton seedlings when damaged by different caterpillar species confirm that volatile blends induced by *H. zea*, and *S. exigua* are indistinguishable by chromatographic analysis (McCall, pers. comm.).

This lack of herbivore specificity is supported by studies on the herbivore-induced synomones in Brassicaceae. In bioassays, *C. rubecula* distinguishes between *P. rapae* feeding on different plants, but exhibits no landing preferences when given a choice between their host *Pieris rapae*, the non-host *Mamestra brassicae*, and *P. brassicae*, all feeding on Brussels sprouts (Geervliet, unpubl. data).

Visual characteristics of feeding damage may supply natural enemies with specific information that could enable them to distinguish between different plant herbivore complexes that are indistinguishable by their volatile profiles only. Feeding damage can differ considerably depending on the herbivore species as well as on the larval instar (Heinrich and Collins, 1983; Feichtinger and Reavy, 1989). The differences between damage of phloem-sucking, leafmining, and leafchewing insects are obvious, but vast differences in feeding damage may also exist between species within each of these herbivore categories (Heinrich and Collins, 1983). Herbivores, moreover, often change their feeding pattern as their development progresses (Feichtinger and Reavy, 1989), thus providing visual information as to which instar is feeding.

The feeding pattern of *P. rapae* is quite characteristic both in terms of the herbivore species and of the instar. Feeding by early instars results in small perforations, while later instars produce increasingly large feeding holes. The fact that *P. rapae* frequently moves away from feeding damage in between feeding bouts (Mauricio and Bowers, 1990), results in a typical configuration of feeding holes. This feeding pattern is distinctly different from the feeding damage by some of the other common folivores on Brassicaceae such as *P. brassicae* (Fig 2). The reported preference of *C. rubecula* for the small leaf perforations over the bigger leaf holes might be an adaptation to the specific feeding patterns of the early instars of its preferred host. The fact that parasitoids show this preference in the absence of host-induced semiochemicals indicates that *C. rubecula* may use leaf damage patterns during host foraging. Experiment 1 furthermore allows the conclusions that the visual information is perceived by the flying parasitoid (landing preference) and that this visual preference is innate (parasitoids were inexperienced). This inate preference, nevertheless, was overruled when larval feeding damage was added (Table 2). This shows that under the given experimental conditions olfactory orientation was dominant. It should be considered, though, that the relatively laminar windstream in the flight-chamber is a misrepresentation of the natural foraging conditions. In the field, turbulence is likely to hamper olfactory orientation and foraging parasitoids might also rely on the visual image of feeding damage to locate host sites.

The visual preference for small leaf perforations seems more pronounced when parasitoids had been given oviposition experience on the small leaf damage (Table 3). It is not evident from our data whether this preference by experienced parasitoids reflects visual learning, or whether it is a repeated manifestation of the innate preference manifested in experiment 1. Regardless of the underlying mechanism, it is noticable that this visual preference was not overruled by the odors from natural feeding damage, present in this experiment.

This study shows that the visual component of leaf damage should not be overlooked when studying tritrophic interactions. The work implies that cryptic feeding strategies found in various caterpillar species (Heinrich and Collins, 1983) may have developed not only to reduce predation by visually hunting birds, but may also be adaptive in reducing parasitization.

The image of damage in a tritrophic context

Feeding patterns reflect more than direct interactions between herbivore and plant. When visually hunting enemies use damage patterns as foraging cues, this constitutes a selective force which may indirectly shape the feeding strategies of herbivores. The fact that three trophic levels have their (interactive) impact on feeding patterns, requests a tri trophic approach (Price, 1980). To discuss the ecological significance of visual feeding patterns, the implications of damage images will be considered in terms of plants, herbivores and natural enemies.

- ***The plant.*** Since feeding by folivorous caterpillars inevitably leads to deformation of leaves, feeding damage often constitutes a noticeable visual indication of herbivore presence. Plants could benefit from this visibility, either when other herbivores visually recognize and avoid herbivore infested plants, or when natural enemies are attracted by the damage patterns. The latter has been demonstrated for a number of natural enemies (as reviewed below). An example of herbivore deterrence is the reduced oviposition by *Pieris brassicae* in response to the image of conspecific egg masses (Rothschild and Schoonhoven, 1977). Available accounts of oviposition deterrence by feeding damage (Rothschild and Schoonhoven, 1977; Faeth, 1988) regrettably do not distinguish between visual and olfactory stimuli.

The fact that plants may benefit from the visibility of herbivore feeding makes it feasible that plants might actively produce visual cues to reduce herbivory, e.g. by mimicing herbivore presence. Gilbert (1975) interpreted the specific extensions on the stipule tips of two *Passiflora* spp. as egg mimics of *Heliconius* butterflies, since they resemble the eggs both in shape and in color. Williams and Gilbert (1981) substantiated this hypothesis by showing that mimetic eggs reduce egg laying by the herbivore. Simultaneously, Shapiro (1981) demonstrated oviposition deterrency by egg mimics of *Streptanthus* towards *Pieris sisymbrii*. Smith (1986) mentioned mimicry of herbivore feeding damage as one of the possible explanations for the color polymorphism found in several herbaceous plants. Variegated color morphs of the tropical subcanopy liane *Byttneria aculeata* suffered less attack by lepidopteran leaf miners than plain morphs. However, since color morphs are likely to differ in their leaf chemistry as well, chemical discrimination by the herbivores can not be excluded. Niemelä and Tuomi (1987) postulated that the irregular leaf shape of several Moraceae species are mimetic of leaves which have been partially eaten by herbivores. They suggested that such feeding-damage mimicry could be an anti-herbivore adaptation or a means to attract predators and parasitoids. The latter proposed function, however, will depend on the ratio of imitation and honest (Maynard Smith, 1982). Since mimetic leaf damage lacks any association with actual herbivore presence, a high ratio of imitation will make the image of feeding damage unreliable as a foraging cue and therefore prone to be ignored by natural enemies. Irregular leaf shapes may in fact benefit herbivores, since they may camouflage any actual herbivore damage, and thereby enable herbivores to elude detection.

- ***Natural enemies***. Herbivores are small components in a complex environment. Their limited size restricts the use of herbivore-derived stimuli by predators and parasitoids (Vet et al., 1991). Visual detection is further impeded by the fact that numerous caterpillars mimic their substrate and restrain their movements (Heinrich, 1979). In cases in which caterpillar feeding results in visible deformations of the plant structures, however, visually hunting predators and parasitoids may be able to use the often prominent image of plant deformations as cues to locate the hidden herbivore. These deformations can be either (i) primary feeding damage, i.e. a direct consequence of chewing or suction, (ii) herbivore-induced reactions by the plant (e.g. galls), or (iii) transformation of the plant structures by the herbivore for the purpose of protection (e.g. leaf rollers, leaf tiers).

A number of studies demonstrate that predators and parasitoids indeed employ this detectable image of feeding damage when foraging for caterpillars. The insectivorous bird *Dacnis cayana* has been observed to focus its foraging onto discolored (herbivore damaged) leaves (Greenberg and Gradwohl, 1980). Heinrich and Collins (1983) showed that Black-capped Chickadees, when foraging for cryptic prey, learn to recognize both tree species and leaf damage simultaneously. Blue Jays can differentiate photographic images of "messy" feeding damage from undamaged leaves (Real *et al.*, 1984).

Unlike birds, invertebrate predators and parasitoids can use both olfactory and visual cues to locate herbivorous insects (Montllor and Bernays, 1993; Wäckers and Lewis, 1993; present study). Even though the use of semiochemicals emitted from feeding damage sites has been more widely reported (Turlings *et al.*, 1993b), a number of studies show that invertebrate predators and parasitoids may also employ the visual profile of feeding damage during herbivore finding (Sugimoto *et al.*, 1988; Faeth 1990).

- ***Herbivores.*** Herbivores, as mentioned previously, display a wide variety of feeding patterns. Besides being determined by physical and physiological attributes of the herbivore, this heterogeneity in feeding strategies has likely been shaped by various (and often conflicting) external forces. The following factors may underlie some of the diversity in patterns of herbivore damage:

- *Nutritional quality.* In order to achieve intake of adequate nutrient quantities, herbivores can adjust their consumption depending on the substrate. Greater consumption can be due to more rapid food intake (increased bite frequency), longer meals and/or more frequent meals (Slansky, 1993), each of which may have its bearing on feeding patterns.
- *Physical plant defenses.* Defensive features at the leaf surface (such as trichomes, glandular hairs; scleromorphy) can be important obstructions to herbivore feeding (Southwood, 1986). Physical defenses will affect feeding patterns by restricting feeding to undefended spaces, while the relative toughness of veins can determine feeding patterns when feeding herbivores leave veins and connected leaf fragments as remnants (Heinrich, 1979).
- *Plant defensive allelochemicals (internal defenses).* Plant chemicals can have a strong impact on herbivory. Toxic allelochemicals may affect insect feeding

directly (Slansky, 1993), while attraction of invertebrate natural enemies to plant-released synomones can constitute an important indirect effect on herbivores (Dicke and Sabelis, 1988; Turlings *et al.*, 1990). Avoidance of both the direct and the indirect effects of allelochemicals may be reflected in herbivore feeding strategies and consequently in leaf damage patterns.

- *Insect pathogens.* Foraging caterpillars commonly encounter insect pathogens on leaf surfaces (Tanada, 1976). Not only may these pathogens have selected for a nutritionally suboptimal rate of feeding (Meisner *et al.*, 1990; Schultz and Keating, 1991), but a possible spatial heterogeneity of these pathogens over the plant could also have a selective impact on the pattern of herbivore feeding.
- *Higher-order natural enemies.* Since natural enemies of herbivores either forage entirely by vision (birds), or may use vision in addition to olfactory cues (invertebrate predators and parasitoids), visually inconspicuous feeding could be of a considerable selective advantage. This latter selective force seems to be reflected in the foraging behavior of *Papilio glaucus*. Relative to the plant species on which it feeds, this caterpillar changes its feeding patterns in such a way that the visual evidence of feeding damage is minimized (Lederhouse, 1990).

In most tritrophic systems, however, it is likely that foraging patterns are the outcome of the trade-off between several of the listed factors, rather than being shaped by any single one of these forces. This entanglement of selective forces makes it difficult to demonstrate the isolated effect of natural enemies on feeding patterns.

By comparing feeding patterns of palatable and unpalatable caterpillar species, Heinrich (1979; 1993) made a case for the importance of visually hunting enemies in the shaping of caterpillar feeding patterns. He reported that palatable caterpillars, unlike unpalatable species, may exhibit one or several of the following behaviors to avoid, or dissociate themselves from visible feeding damage: (i) they restrict feeding to night-time, (ii) eat the whole leaf, paring it down along the side, keeping the leaf-contour smooth, (iii) clip off the leaf remnant at the petiole, (iiii) move away from the feeding damage after a short feeding bout.

Although it is likely that visually foraging predators and parasitoids have had their impact on these caterpillar feeding strategies, other, alternative explanations for the described feeding behavior should not be omitted. Nocturnal feeding, for instance, could also be a strategy to avoid the higher levels of herbivore induced allelochemicals during daylight (Turlings, pers. comm.). When combined with diurnal hiding, nocturnal foraging may be a strategy to escape the destructive effect of photo-active plant toxins (Fields *et al.*, 1990). Both discarding of the leaf damage and abandoning feeding sites might also serve to avoid and dissociate from (toxic) plant allelochemicals. Since most of these allelochemicals are not released earlier than five hours after the onset of the feeding (Turlings and Tumlinson, 1992) both strategies allow herbivores to escape toxification as well as to elude those natural enemies that forage by odor cues. The different responses to plant defensive chemicals in palatable and unpalatable caterpillars could be accounted for if unpalatable caterpillars are less sensitive to induced chemical defenses (for instance when they sequester these defensive chemicals).

The study by Mauricio and Bowers (1990) shows that the assumed function of the behavioral patterns described by Heinrich do not hold in all cases. In their comparison between an aposematic unpalatable caterpillar (*Euphydryas phaeton*) and the cryptic palatable *P. rapae*, they reported no differences in feeding strategies. Both species moved frequently in between feeding bouts and consumed only small fractions of the leaf area, leaving visible feeding damage. This detectable feeding pattern of *P. rapae* and its frequent moving render the undefended caterpillars vulnerable to visually hunting enemies (Schultz, 1983). This could either indicate that the selection pressure by natural enemies is outweighed by one or more of the previously listed factors, or that natural enemies do not constitute a significant selective pressure for visual inconspicuousness. The latter explanation seems contradicted by the fact that *P. rapae* larvae are cryptically colored and exhibit specific behaviors (countershading) to enhance their inconspicuousness which are difficult to explain other than as strategies to avoid visual detection. Alternatively the conspicuous feeding may be explained as a strategy to elude natural enemies. By feeding briefly and moving away in between feeding bouts, *P. rapae* might be able to hide in between self-created decoy sites.

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Chapter 7

Host discrimination in flight and following alightment by the parasitoid *Microplitis croceipes*: a study of sensory mechanisms.

ABSTRACT

Microplitis croceipes (Cresson), while foraging individually in a simulated plant patch, distinguished between unparasitized and parasitized *Helicoverpa zea*. This host discrimination was manifested in a reduced number of alightments on parasitized host targets (in-flight discrimination), as well as in increased host rejection on the target. The role of both visual learning and chemical marking in host discrimination was examined. On the target chemical marking was shown to be the main mechanism underlying host discrimination, while visual avoidance on the target could not be demonstrated. It was shown that chemical marking is also involved in discrimination by the flying female. Furthermore, *M. croceipes* was found to distinguish between self-parasitized hosts and hosts parasitized by conspecifics, indicating that the chemical marker is individualized. The adaptive value of this type of host discrimination is discussed.

INTRODUCTION

Host discrimination, defined as the ability to distinguish between unparasitized and parasitized hosts, is a widespread phenomenon among parasitic Hymenoptera. Van Lenteren (1981) estimated that the ability to discriminate has been established in 150 - 200 parasitoid species, representing all Hymenopteran families and numerous reports of host discrimination have been published since (for a recent overview of the subject see van Alphen & Visser 1990). It is all the more striking, therefore, to find a seeming exception to this rule described for the larval parasitoid *Microplitis croceipes*. Several studies investigating host discrimination in this parasitoid (Lewis & Snow 1971; Vinson & Guillot 1972; Eller *et al.* 1989; Tillman & Powell 1992) agree in their conclusion that *M. croceipes* lacks the ability to differentiate between unparasitized hosts and hosts that had recently been parasitized once. Lewis & Snow (1971), using Petri dish experiments, were the first to study host discrimination in this parasitoid. Results from both direct observations and an indirect experiment, in which clusters of 50 unparasitized hosts were exposed to a single parasitoid and subsequently dissected, led to the conclusion that "females demonstrated no ability to discriminate larvae which had been parasitized previously". Vinson & Guillot (1972) also concluded that *M. croceipes* accepted single-parasitized hosts as well as unparasitized hosts. Nevertheless, they reported the existence of a marking pheromone, produced in the parasitoid's Dufour's gland. This marking pheromone, however, only reduced the acceptance of superparasitized hosts, i.e. hosts that had been parasitized more than once. Two recent studies confirmed that *M. croceipes* did not respond to a chemical mark on single-parasitized hosts (Eller *et al.* 1989; Tillman & Powell 1992). This largely indiscriminate host acceptance by *M. croceipes* seemed to be corroborated by the field collections of Lewis & Gross (1989), showing a randomly (Poisson) distributed number of parasitoid eggs in collected *H. zea* larvae. However, as pointed out by van Lenteren et al. (1978), random distributions can also be obtained trough the combination of small scale non-random distributions and therefore do not disprove a parasitoid's discriminative ability.

Still, the available reports seemed to leave little justification for yet another study of host discrimination in *M. croceipes*. During general observations of free-flying individual parasitoids, though, we noticed obvious avoidance of superparasitism that could not be reconciled with the previously reported findings. In an attempt to explain the arisen discrepancies, we addressed host discrimination in *M. croceipes* once more. However, in contrast to the previous work, in which parasitoids were studied in Petri dishes, we chose to study host discrimination by observing free-foraging parasitoids in a flight-chamber plant patch. These flight chamber experiments not only allowed for investigation of host discrimination after landing, but also enabled us to study host discrimination by flying parasitoids.

Van Giessen *et al.* (1992) reported that *M. croceipes*, following an oviposition experience on a kairomone source, showed a reduced flight response to this particular kairomone site. This reduction in flight response did not occur when the appearance of the kairomone site was changed after oviposition. Since this finding indicates location-avoidance learning as a possible host discrimination mechanism, we designed our experiments to elucidate the respective role of both chemical marking and visual learning in host discrimination. In this paper we address both these discrimination mechanisms with respect to parasitoids searching on the plant. As far as in-flight discrimination is concerned, we will only consider the role of chemical marking here. Visual discrimination by the flying parasitoid was addressed in a subsequent study (Sheehan *et al.* 1993)

MATERIALS AND METHODS

***M. croceipes*.** Parasitoids were reared from *H. zea* larvae as described by Lewis & Burton (1970). The parasitoid pupae were allowed to emerge in acrylic cages (30 x 30 x 17 cm) at 28°C, 50-70% RH and a 16L:8D photocycle. Daily transfer of pupae to a new cage assured parasitoid groups of uniform age. Three day old, naive females were used in the experiments.

***H. zea*.** Host larvae were reared on a pinto bean based diet according to Burton (1969). Young third instars were separated in small Petri dishes (5 cm) containing cowpea leaves as food source. The cowpea-fed late third instars were used in the experiments.

Cowpea frass. Frass pellets were collected right before the experiment from isolated 5th instar *H. zea* fed on cowpea leaves. In each experimental run we used frass pellets from one individual larvae.

In all experiments oviposition was concluded from observed ovipositor insertion. This assumption was based on data by Tillman & Powell (1992), showing that 95-100% of twice-attacked hosts contained two parasitoid eggs. In combination with the fact that *M. croceipes* never deposits more than one egg during oviposition (Lewis pers. comm.), this means that, both in unparasitized and in once-parasitized hosts, ovipositor insertion is a reliable indicator of oviposition.

Flight chamber experiments (experiment 1,2,3 & 5)

Several aspects of host discrimination by *M. croceipes* were examined in flight chamber experiments through direct observation of free-ranging parasitoids foraging behaviour.

Flight chamber. The design of the flightchamber was similar to the chamber described by Drost *et al.* (1986). The test area was two meters long with a 75 x 75 cm cross section and was lighted by four 80 W fluorescent lights. The floor of the flight chamber was covered with green cardboard. The experiments were conducted at 27 2 °C, and a windspeed of 31 2 cm/sec. In order to be able to study free ranging parasitoids under semi-natural conditions, we created a plant patch within the flight chamber.

Plant patch. The plant patch consisted of eight cowpea plants, arranged pairwise in the test arena. The distance between plants was 25 cm, allowing sufficient space for the flying parasitoid to move freely among plants.

Cowpea plants. Plants were grown individually in plastic pots (10 cm diameter) on a mixture of 1/2 potting soil, 1/3 sand, and 1/6 peat moss. Growth conditions were controlled at 25-35°C and a 14L:10D photocycle. Plant sets of uniform age and growth stage (4th leaf stage; 20-30 cm in height) were used in the experiments.

Targets. On each plant we attached one target, consisting of a rectangular piece of white paper (4 x 2.5 cm). Three pellets of cowpea frass were placed at the base of the target as a volatile attractant for the parasitoid. An unparasitized *H. zea* larvae was attached to the center of the target with an insect pin pushed trough the last abdominal segment. Targets were positioned downwind on a leaflet of the upper leaf at a height of 25-30 cm. This target position assured maximum accessibility and visibility to the flying parasitoid. The purpose of the target was threefold:

i. The white colour, known to stand out most prominently against the background of the vegetation (Kevan 1972), could facilitate visual host location and visual location-learning by the parasitoid.
ii. The paper prevented larval feeding on the plant tissue, assuring odour uniformity among targets.
iii. By replacing targets as a whole, possible chemical markers on either the host, the frass or their immediate vicinity could be removed/transferred, without removing the plants.

Pre-flight exposure. Before being used in the experiments, naive females were allowed to antennate host frass for a period of one minute. This exposure increases the parasitoid's responsiveness to frass volatiles and initiates host oriented flight behaviour (Lewis & Jones 1971). Following this exposure, females were kept individually in a shell vial for 5 minutes before they were introduced into the flight chamber.

Test procedure. The parasitoid was released by placing the shell vial on a release point 35 cm downwind from the first plant pair at a height of 20 cm. After take-off, the parasitoid was allowed to forage freely among the eight plants containing the equivalent number of unparasitized hosts. The maximum foraging period was set at 60 minutes. Experiments were ended prematurely when all hosts had been parasitized, or when the parasitoid showed no further interest in the plant patch by leaving the arena (phototactic flight; resting on a flight-chamber wall) for two minutes. During the foraging period the parasitoid's behaviour was recorded continuously on a Tandy TRS 80 portable computer using software for behavioural research (Noldus, 1991). Host discrimination was determined by comparing the parasitoids behaviour towards unparasitized hosts, versus hosts parasitized once in the course of the experiment (from now on referred to as parasitized). Data from superparasitized hosts were excluded, since host acceptance by ***M. croceipes*** decreases with the number of times a host has been parasitized (Vinson & Guillot 1972).

Petri dish experiment (experiment 4)

In order to test for individualized chemical marking we carried out an additional experiment using Petri dishes. The methodology in this experiment was similar to the methodology used in the previous reports (Lewis & Snow 1971; Vinson & Guillot 1972; Eller *et al.* 1989; Tillman & Powell 1992). Results from this experiment could therefore also help explain the earlier reported lack of host discrimination by *M. croceipes*.

Petri dishes. We used four small Petri dishes (5 cm in diameter), each containing a single unparasitized *H. zea* larvae.

Pre-test procedure. In order to ensure that the time between first parasitization and test was equal for both self- and conspecific-parasitized hosts, we used two parasitoids simultaneously. Both naive parasitoids were individually allowed to antennate host frass for one minute. Subsequently, they were kept in seperate shell vials for five minutes, before each was introduced into one of the four Petri dishes. Upon encounter with the unparasitized host, the parasitoids generally (93%) accepted the host for oviposition. As soon as the host was accepted the lid of the Petri dish was removed and the parasitoid had the option to leave the Petri dish or to superparasitize the host. If both parasitoids avoided superparasitization, the procedure was repeated using the two remaining Petri dishes containing the second set of unparasitized hosts. Only parasitoids that avoided superparasitization in both the first and the second host were used in the actual test.

Test procedure. The first two hosts were transferred into new Petri dishes. Parasitoids were subsequently introduced into these Petri dishes containing either the host previously parasitized by the female herself, or the host parasitized by the conspecific female. The lid of the Petri dish was removed at the moment the parasitoid perceived the parasitized larvae (indicated by reduction in walking speed while raising their antennae). We recorded whether the parasitoid subsequently accepted the host (superparasitization) or abandoned the Petri dish (rejection).

Statistical analysis. The percentage acceptance of unparasitized as well as once parasitized hosts was calculated for each individual parasitoid and subsequently averaged over the parasitoids tested in each experiment. We tested fifteen parasitoids in experiments 1 & 2, twenty in experiment 3 and ten individuals in experiment 5. The overall data from experiments 1, 4 and 5 were analyzed in a Wilcoxon signed-rank test. For the multiple-comparisons in experiments 3 and 4 we used Wilcoxon signed-rank test with the Bonferroni adaptation.

EXPERIMENTS AND RESULTS

Experiment 1. To determine whether *M. croceipes* can distinguish between unparasitized hosts and self-parasitized hosts, we tested females individually in the flight chamber set-up. Host discrimination was examined at two stages in the parasitoid's foraging sequence: i. Host discrimination by the flying parasitoid.
ii. Host discrimination after landing on the target.
As a parameter for discrimination in flight we determined the percentage of target approaches (defined as casting within two cm from the target) resulting in alightment on the target leaflet (target landings). To test for discrimination in flight we compared the percentage of target landings, both for targets containing unparasitized and parasitized hosts. As a parameter for discrimination after landing, we determined whether alightments on the target leaflet resulted in host acceptance. To test for discrimination on the target we compared the percentage of ovipositions for both parasitized and unparasitized hosts.

At the beginning of the experiment the flight performance of the parasitoid was often unsteady, and target oriented flights would usually result in landing attempts. After the parasitoid's flight improved with experience, however, flight became more steady, alightments more accurate and the parasitoid became more selective in its acceptance of landing sites. Many approaches (mainly of targets containing parasitized hosts) were cut short in the hovering stage. Over the whole experiment, we found a significantly lower percentage of alightments on targets containing parasititized hosts (Fig. 1). This shows that the flying parasitoids distinguishes between parasitized and unparasitized hosts. This in-flight discrimination will be addressed further in experiment 5.

Host discrimination was also clearly manifested in the acceptance of hosts following target alightment: 89.6% of the unparasitized hosts were accepted, whereas the acceptance of parasitized hosts was only 22.5% (Fig. 1). When perceiving a host (approximately at 1 cm), the parasitoid would assume an attack posture, by raising both its thorax and its antennae. In the case of unparasitized hosts this was generally followed by oviposition. When the host had been previously parasitized, however, the parasitoid frequently held back before actual host contact, moved backwards or even leaped away from the host and eventually left the target. This avoidance behaviour suggests that the parasitoid perceives a (repellent) volatile marker.

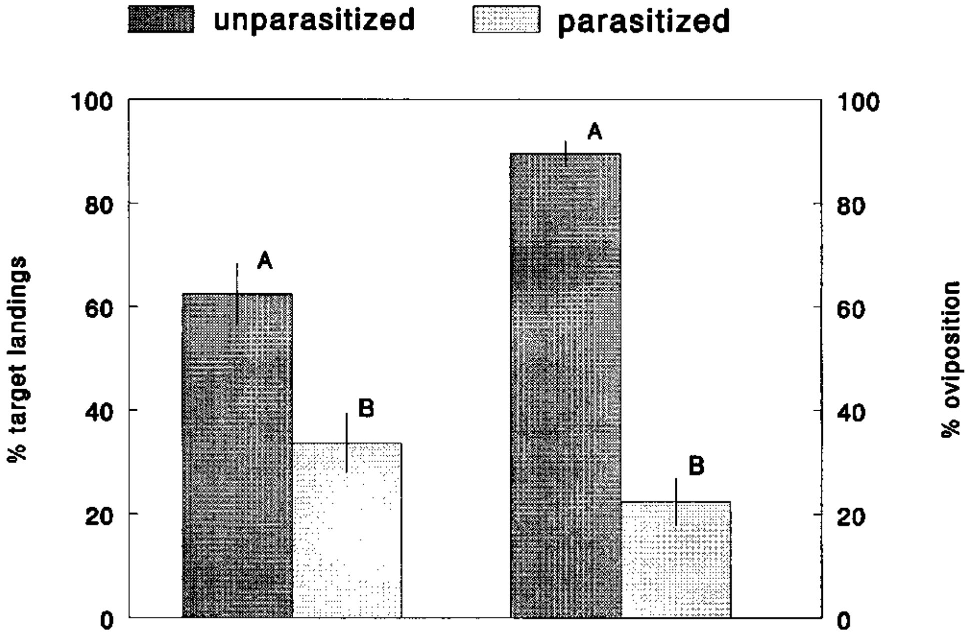

Fig 1. Host discrimination by individually foraging parasitoids.
Left half: Percentage of total plant-landings made on the actual host-target, both for targets containing unparasitized hosts or hosts being parasitized once during the course of the observation. One way analysis of variance (Scheffe test; $P < 0.002$).
Right half: Percentage target-landings resulting in acceptance of the host for oviposition, both for unparasitized hosts and hosts being parasitized once. One way analysis of variance (Scheffe test; $P < 0.0001$; $N=15$). Different letters above the bars denote significantly different means.

Experiment 2. To determine if host discrimination on the target indeed involves chemical marking, or whether it is due to visual recognition, we introduced two parasitoids simultaneously into the flight chamber. During this experiment we consequently could distinguish three host categories: 1. unparasitized hosts, 2. hosts previously parasitized by the female herself and 3. hosts parasitized by the conspecific female. The percentage of the target landings resulting in oviposition for any of these three host categories was recorded. To standardize the experiment, we only used data from females that had their first oviposition on an unparasitized host, and that, in the course of the experiment, encountered all three host categories.

Table I shows the expected acceptance of the three target categories if host discrimination were based on either species-wide chemical marking (marker not differentiated below the species level), individualized marking or visual learning. If host discrimination were based on a species-wide chemical marker, both self-parasitized and conspecific-parasitized hosts should be equally accepted. If, on the other hand, the chemical marker were individualized, conspecific-parasitized hosts should be more readily accepted than self-parasitized hosts. Lastly, if visual learning were the main host discrimination mechanism, only the self-parasitized hosts should be rejected. Thus, the acceptance of conspecific-parasitized hosts should be the key to the host discrimination mechanism (table I).

Table I: The pattern of acceptance in experiment 2 as expected for different host discrimination mechanisms

	Expected acceptance in case of		
	species wide marker	individualized marking	visual learning
Unparasitized	high	high	high
Conspecific-parasitized	low	intermediate	high
Self-parasitized	low	low	low

If we compare these predictions with the actual results (Fig. 2), we see the expected high acceptance of unparasitized hosts and low acceptance in case of self-parasitized hosts. Conspecific-parasitized hosts, however, are accepted at an intermediate level. This level is higher than expected in case of a species-wide chemical marker, and lower than expected in case of only visual learning. This ranked host acceptance leaves two hypotheses, which will be tested in the subsequent experiments:

1. Host discrimination is based on a combination of chemical marking and visual learning (experiment 3).
2. The chemical marker is individualized (experiment 4).

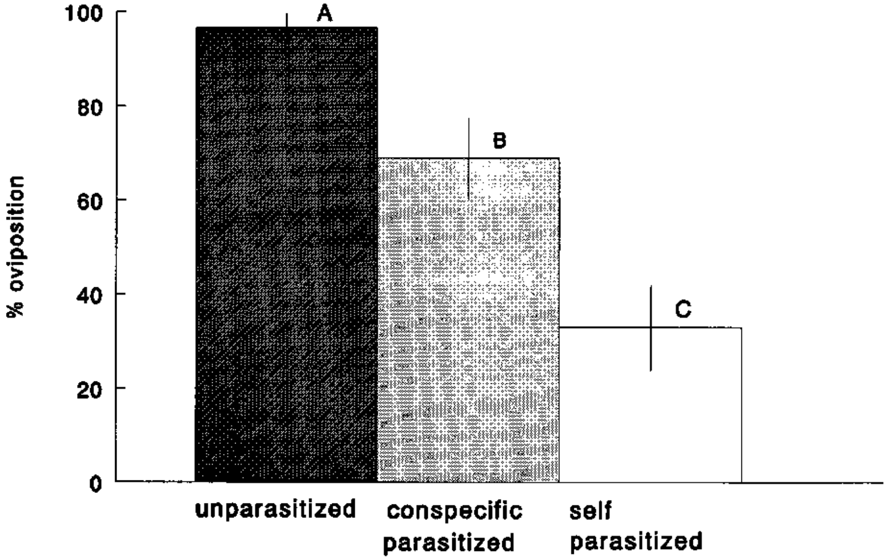

Fig 2. Host discrimination by two conspecific parasitoids, foraging concurrently. Percentage target-landings resulting in host acceptance, compared for targets containing the following host categories:

- unparasitized hosts;
- hosts parasitized once by the conspecific female;
- hosts parasitized once by the female herself.

Different letters above the bars denote significantly different means (Scheffe test; N=15).

Experiment 3. To test whether host discrimination on the target is based on a combination of chemical marking and visual learning, we allowed a single parasitoid to parasitize four out of the eight hosts available in the flight chamber. Thereafter, the parasitoid was removed briefly, in order to switch two parasitized targets with two of the unparasitized targets. This resulted in four different host categories, each represented by two targets: 1. unparasitized hosts in an unparasitized location; 2. parasitized hosts in a parasitized location; 3. unparasitized hosts switched to a parasitized location; 4. parasitized hosts switched to an unparasitized location.

Following reintroduction of the parasitoid, we recorded the fraction of target landings resulting in oviposition for any of these host categories. Table II shows the expected acceptance of the four target categories if host discrimination were based on either visual learning, species-wide chemical marking, or a combination of both mechanisms.

Table II: The pattern of acceptance as expected in experiment 3 for different host discrimination mechanisms

		Expected acceptance in case of		
status host	status location	visual learning	species-wide chemical marking	visual learning + chemical marking
unparasitized	unparasitized	high	high	high
parasitized	parasitized	low	low	low
unparasitized	parasitized	low	high	intermediate
parasitized	unparasitized	high	low	intermediate

In case of species-wide chemical marking, only the status of the host is relevant: unparasitized hosts should be accepted and parasitized hosts rejected, irrespective of their location. If host discrimination on the target were based solely on visual learning, the status of the location is paramount: unparasitized locations should be accepted and parasitized locations rejected, irrespective whether the host is parasitized or not. If, however, host discrimination were based on a combination of visual learning and chemical marking, one would expect an intermediate acceptance level in both of the switched categories.

The results from this experiment show that irrespective of the location, the acceptance of parasitized hosts is significantly lower than the acceptance of the unparasitized hosts (Fig. 3). Since target location had no significant effect, we can conclude that discrimination on the target is mainly based on chemical marking.

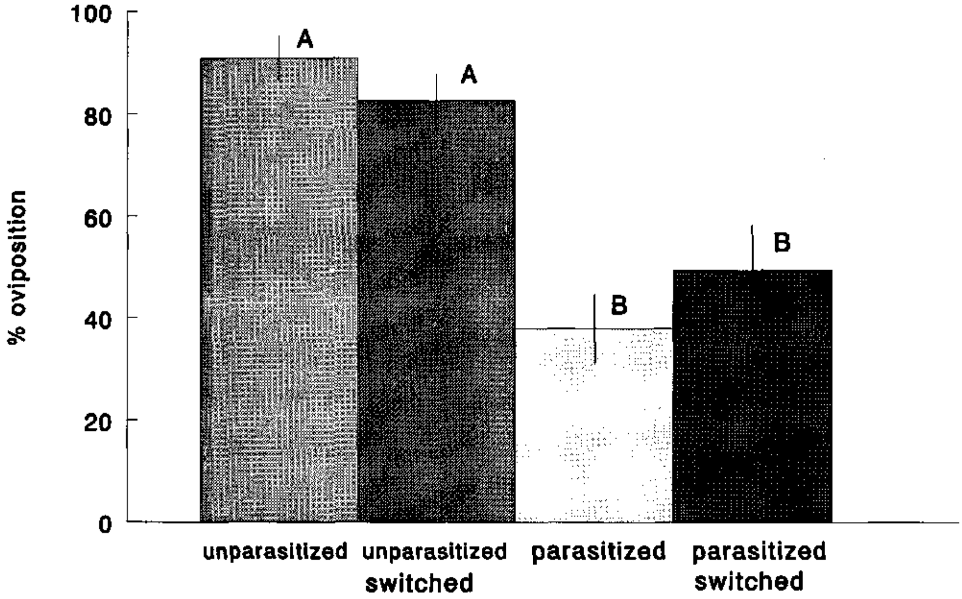

Fig 3. The role of visual and chemical cues in host discrimination by a single parasitoid. Percentage target-landings resulting in host acceptance, compared for the following target categories: an unparasitized host on an unmoved target; an unparasitized host on a relocated target; a parasitized host on an unmoved target; a parasitized host on a relocated target
Different letters above the bars denote significantly different means (Scheffe test; N=20).

Experiment 4. To test whether the results from experiment 2 were a consequence of individualized chemical marking, we compared the parasitoid's behaviour towards self- and conspecific-parasitized hosts in a Petri dish experiment.

Parasitoids that were offered self-parasitized hosts accepted them in only 45% of the cases (Fig. 4). This figure was almost twice as high (88%) for parasitoids that were offered hosts parasitized by conspecifics. The significantly higher acceptance of conspecific-parasitized hosts proves that *M. croceipes* can distinguish between her own chemical marker and the marker left by conspecifics.

Experiment 5. The following experiment was designed to further investigate the conclusion from experiment 1 that *M. croceipes* can recognize parasitized hosts in flight. Since the previous experiments showed that host discrimination by *M. croceipes* on the target is principally based on chemical marking, we specifically wanted to determine whether this volatile marker could also be perceived by the flying parasitoid. For this experiment we used the basic flight chamber set-up. However, in addition to the usual eight targets, four extra targets were prepared and kept aside. A single parasitoid was introduced into the flight chamber. As soon as all eight hosts had been parasitized, four randomly selected targets were replaced by the four additional targets containing unparasitized hosts. We subsequently determined the percentage of target-oriented flights (here defined as casting within 5 cm from the target) resulting in target landings both for targets containing parasitized and unparasitized hosts.

Target oriented flights towards unparasitized hosts were generally completed by alightment (Fig. 5). In case of parasitized hosts, however, almost half of the target-oriented flights were cut short, followed by continued in-flight search. This, to our knowledge, is the first demonstration of in-flight perception of a chemical marker.

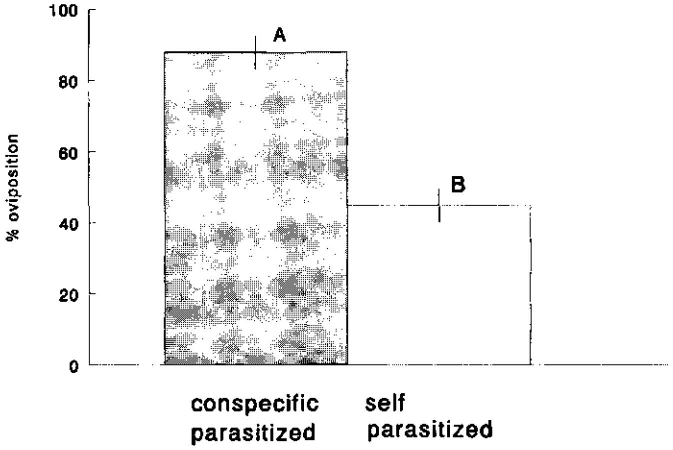

Fig 4. Individualized discrimination.
Percentage host acceptance when parasitoids were offered either self-parasitized or conspecific-parasitized hosts. Different letters above the bars denote significantly different means (Chi-square; $p=3.2 \times 10^{-6}$; N=50).

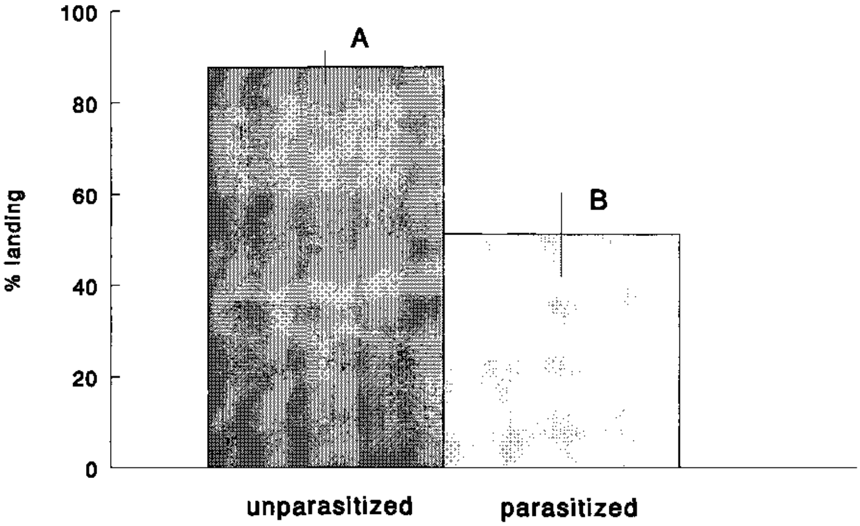

Fig 5. Olfactory discrimination by the flying parasitoid.
Percentage of cases in which hovering in front of the target was followed by alightment, compared for self-parasitized and unparasitized hosts, both in locations of previous parasitization. Different letters above the bars denote significantly different means (Scheffe test; N=10).

DISCUSSION

Host discrimination is generally determined by comparing the parasitoid's acceptance of parasitized versus unparasitized hosts. It is important to realize that these "host discrimination experiments" actually determine the parasitoids avoidance of superparasitism (Salt 1934). This means that although host discrimination can be demonstrated by proving avoidance of superparasitism, the reverse reasoning does not hold: Since superparasitism can be an adaptive strategy selectively applied by parasitoids (Visser *et al.* 1992), equal acceptance of unparasitized and parasitized hosts can not prove the disability to discriminate.

The previous studies of host discrimination in *M. croceipes* clearly confirm that the ability to discriminate is not necessarily manifested in avoidance of superparasitism. This underlines that the choice of experimental conditions can be crucial to demonstrate host discrimination. Two differences in experimental conditions (host type and experimental set-up) between previous studies and our experiments can account for their converse conclusions on host discrimination by *M. croceipes*.

1. Host type. Previous studies tested host discrimination by offering conspecific-parasitized hosts, while parasitoids in our experiments re-encountered self-parasitized larvae. Hubbard *et al.* (1985) demonstrated that *Nemeritis canescens* (Grav.) can distinguish a chemical mark left by herself from a mark left by a conspecific. Such an individualized chemical marker allows parasitoids to superparasitize selectively, according to the reproductive value of the host. Self-parasitized hosts usually represent a low reproductive value, since self-superparasitism will put the parasitoid at competition with her own genes. Conspecific-parasitized hosts, on the other hand, can be quite remunerative, depending on the relative competitiveness of the added eggs (Eller *et al.* 1990). This makes self-parasitized hosts the more likely category to be rejected and thereby more suitable candidates for demonstrating host discrimination.

Our studies confirm that conspecific-parasitized hosts are indeed more readily accepted than self-parasitized hosts. Their acceptance is especially high in the Petri dish experiments (89%). This figure corresponds with the high acceptance of (conspecific) parasitized hosts in former studies, thus explaining their conclusion that *M. croceipes* does not discriminate.

Nevertheless, the fact that the acceptance of conspecific-parasitized hosts in the flightchamber experiment was intermediate between unparasitized and self-parasitized hosts, shows that parasitoids obviously recognize and respond to the chemical marker of conspecifics as well. Moreover, it shows that the experimental set-up can be crucial if host discrimination is to be demonstrated.

2. Experimental set-up. Previous studies were done in Petri dishes, while we tested free-flying parasitoids. Avoidance of superparasitism can be based on (a combination of) different mechanisms: (i) Recognition of parasitized hosts, either by chemical marking or by detection of physiological or physical changes in the parasitized host. (ii) Recognition of previously visited locations. Parasitoids can leave a chemical mark while searching the substrate, or following oviposition (Sheehan *et al.*, 1993). In addition, parasitoids may avoid previously visited sites on the basis of visual recognition (van Giessen *et al.* 1992; Sheehan *et al.* 1993). This site-discrimination enables them to avoid duplication of searched area.

Under field conditions, both site- and host discrimination will be entangled in determining a parasitoid's decisions on host acceptance. Most host discrimination experiments, however, only test for avoidance of parasitized hosts. This can lead to an underestimation of a parasitoid's ability to discriminate. In order to get a realistic impression of the discriminating ability, it is important to study parasitoids in an experimental set-up that accounts for both avoidance mechanisms.

Because of the differences in reproductive value between unparasitized, conspecific-parasitized and self-parasitized hosts, it should be functional for parasitoids to recognize these different host categories. Literature on host discrimination names various functions of a parasitoid's ability to discriminate (van Lenteren, 1981): (i) avoidance of superparasitism may prevent waste of parasitoid eggs; (ii) it may prevent waste of hosts; (iii) it may prevent waste of time; (iiii) it may initiate migration.

We propose that the adaptive value of recognizing parasitized hosts can be reduced to the following three basic functions:

1. Preventing waste of eggs. Recognition of (self-/conspecific-) parasitized hosts allows a parasitoid to optimize its foraging decisions. When egg supply is the limiting factor, parasitoids should optimize the fitness per egg (Charnov and Skinner, 1984). Since parasitized hosts generally represent a lower return in offspring fitness, recognition of parasitized hosts will be essential for egg-limited parasitoids in order to optimize their egg allocation. For those parasitoids for which time rather than egg supply is limiting, wastage of eggs will be of only minor importance.

2. Preventing waste of time. Host discrimination can be a time-optimizing strategy when rejection time (counted from the point at which the host is perceived) is shorter than the sum of host location time and handling time. The savings in time are only substantial if handling time is extensive or if parasitized hosts can be recognized and rejected early-on in the foraging sequence. Time efficiency is especially relevant for time-limited parasitoids.

3. Reducing the risk to the parasitoid. This has not previously been considered as a function of host discrimination. Still, defensive actions of aggressive hosts can lower the fitness of foraging parasitoids considerably (Stamp 1986; Allen 1990; Potting *et al*, 1993). Since parasitized hosts can be as aggressive as unparasitized ones, or -temporarily- even more aggressive (Gardner *et al.* 1986), avoiding superparasitization can be an effective way of risk-minimalization. Host discrimination, however, will only result in risk reduction if rejection of parasitized host evokes less aggression than superparasitization.

Whether or not superparasitization is actually worth the parasitoid's eggs, time, and risk is a function of these three factors weighed against the relative reproductive success of accepting parasitized versus unparasitized hosts (Iwasa *et al.* 1984; Charnov & Skinner 1985). The ability to recognize parasitized hosts is not necessarily manifested in avoidance of superparasitism (Salt 1934; Visser *et al.*

1992). It does, however, allow the parasitoid to appraise its environment and to adjust its foraging decisions accordingly.

Previously included additional functions ("wastage of hosts" and "initiation of migration") can be considered a product of these three basic functions, rather than being independent factors.

Preventing wastage of hosts. It was proposed that in those situations where multiple parasitism increases host mortality, superparasitism would not only be a waste of offspring, but also a waste of hosts. If, however, superparasitization will kill the host, than once-parasitized hosts represent no value for future ovipositions and consequently cannot be wasted. If, under these premises, a parasitoid superparasitizes, the waste can be expressed solely in terms of invested time, eggs, and fitness: self-superparasitizing parasitoids waste both previous- and current investments, while conspecifics only waste their current investment.

Initiation of migration. Recognition of previously parasitized hosts allows parasitoids to obtain information about different levels of their environment. Besides assessing the quality of individual hosts, it also enables parasitoids to estimate local patch depletion and overall habitat exploitation. On these different foraging levels, the ability to recognize previously parasitized hosts is manifested in different forms of evasive behaviour: on the level of the individual host this ability can be expressed in host rejection, while on the patch or habitat level it can be manifested in migration. Consequently, migration, when initiated by encountering parasitized hosts should be considered an expression of the ability to recognize parasitized hosts, rather than a function of host discrimination.

From the previously known functions of host discrimination it could be argued that host discrimination would be of little value to *M. croceipes*. Since the parasitoid has an average egg supply of over 300 oocytes (Lewis & Snow 1971), the egg load is unlikely to be a limiting factor at the host densities as they usually occur in the field (Hopper & King 1986). Neither does host discrimination seem to be a time-saving strategy, since the parasitoid, once it has located its host, will oviposit within a fraction of a second.

The picture changes, however, when we include risk avoidance as a function of host discrimination and consider the here reported ability of *M. croceipes* to discriminate in flight. Although egg-limitation remains an unlikely impetus to avoid superparasitism, in-flight discrimination enables the parasitoid to optimize both its time and risks. Since the foraging parasitoid does not need to land, locate, and contact its hosts in order to recognize previous parasitizations, in-flight avoidance of parasitized hosts represents a considerable saving in time. Besides the brief oviposition time, in-flight rejection also saves the time spent hovering in front of the host-site; the landing time; and the time required to locate the (frequently hidden) host.

In-flight discrimination has also important implications for the functionality of risk avoidance. The defensive behaviour of *Helicoverpa* spp. represents a serious risk to *M. croceipes*. Upon contact by the parasitoid the larvae respond with head thrashing, biting, and regurgitation of gut content. Fourth and fifth instars can clip off parasitoid antennae and damage their wings. Third and later instars especially can be successful in wiping regurgitant onto their attacker, evoking a strong aversive response followed by extensive, time consuming cleaning.

Previously it was assumed that recognition of parasitized hosts always requires direct host contact and thereby exposure to host defenses. Our work, however, shows that *M. croceipes* can recognize and reject parasitized hosts without actual host contact. Avoidance of superparasitism, could therefore be an effective means of risk minimalization for *M. croceipes*.

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Chapter 8

Discrimination of previously searched, host-free sites by *Microplitis croceipes.*

SUMMARY

The ability of free-ranging parasitoids to discriminate between previously visited and unvisited sites containing host kairomone (caterpillar frass) but not hosts was tested. Females of *Microplitis croceipes*, a host specialist and plant generalist larval parasitoid of *Helicoverpa* (*Heliothis*) *zea*, were allowed to fly freely in a simulated plant patch in a flight chamber. Wasps spent less time searching frass sites previously searched by themselves or by conspecifics than unsearched frass sites. In addition to chemical marking, spatial memory of visual cues was implicated as a mechanism for discriminating against self-visited, host-free sites.

INTRODUCTION

Efficient foragers must be able to differentiate profitable from unprofitable foraging sites. For foraging primary parasitoids, depositing eggs in parasitized hosts is generally less profitable because those eggs typically have a low probability of survival (Bakker *et al.*, 1985). Many insect parasitoids discriminate parasitized from unparasitized hosts and thus avoid superparasitism (van Lenteren, 1981). Wäckers and Lewis (1994) showed that *Microplitis croceipes* females, the subject of the present study, avoid previously stung hosts and discriminate in flight between self-parasitized and conspecific-parasitized hosts.

Discrimination against previously visited sites ('site discrimination') has received less attention than discrimination against previously stung hosts ('host discrimination'). It is well known that parasitoids are attracted to or arrested by host-produced kairomones (see review by Vinson, 1984). However, few studies have examined parasitoid searching behavior at sites that contain host kairomones but not hosts ('host-free sites'). Since mobile hosts often move away from feeding sites (Heinrich, 1979) and any host can be removed by predators, searching parasitoids may frequently encounter host-free sites in nature. It should also be adaptive to recognize previously searched sites, if hosts are at all concealed, to avoid wasting time refinding them (Roitberg and Mangel, 1988).

Several authors have demonstrated that parasitoids can discriminate between unvisited host feeding sites and host-free sites searched previously by themselves (Price, 1970; Sugimoto *et al.*, 1986), by conspecifics (Salt, 1937; DeBach, 1944; Greany and Oatman, 1972; Waage, 1979; Galis and van Alphen, 1981; Sugimoto *et al.*, 1986), or by congenerics (Price, 1970), and that time spent searching a site increases with increasing concentration of host kairomone (Waage, 1979; Galis and van Alphen, 1981; Dicke *et al.*, 1985). To date, studies have examined only behavior of walking parasitoids searching for concealed hosts in small, artificial arenas where successful parasitism had occurred, and none has examined visual discrimination of host-free sites. Van Giessen *et al.* (1992) showed that recent (within 10 min) oviposition experience affects the propensity of *M. croceipes* to fly to a previously visited point odor source (hexane frass extract on filter paper) in a flight chamber. They found that wasps were less likely to fly to a fresh odor source if they had oviposited at that site on a prior visit than if they had only contacted frass at that site on a prior visit.

In this paper we examine the role of both visual and olfactory cues in discriminating previously searched sites by *M. croceipes* females foraging freely in an experimental patch. Sites contained a natural host kairomone (frass of larval *Helicoverpa* [*Heliothis*] *zea*) but no host. Both frass and feeding damage are known to contain semiochemicals that act as strong attractants or arrestants for *M. croceipes* (Drost *et al.*, 1986; Lewis and Tumlinson, 1988; Eller *et al.*, 1988; W. Sheehan, unpublished data; P. McCall, unpublished data). We observed wasps searching in a patch of cotton plants in a flight chamber and recorded searching duration at frass sites. We used protocols developed by Wäckers and Lewis (1994) to answer the following questions: (1) Do wasps discriminate against previously visited frass sites in the absence of hosts ('self site discrimination')? (2) Do wasps discriminate against frass sites previously visited by conspecifics ('conspecific site discrimination')? (3) Is site discrimination based on visual cues, olfactory cues, or both ('discrimination cues')?

MATERIALS AND METHODS

Microplitis croceipes were reared from diet-fed *H. zea* larvae as described by Lewis and Burton (1970). Parasitoids were kept, with honey and water, in Plexiglas cages (30 x 30 x 17 cm) at 28√C, 50-70% RH and a 16:8 L:D photoperiod. Three day old, mated females, inexperienced with plants, hosts or frass, were used in all experiments. Frass was collected before experiments from fifth instar *H. zea* fed on cotton leaves. Cotton plants (*Gossypium hirsutum* var McNair 235) used for both frass production and experiments were grown in plastic pots (10 cm diameter) in a greenhouse. Plants about 30 cm high (5 to 7 leaves) were used in experiments.

The **flight chamber** used was similar to, but larger than, that described by Drost *et al.* (1986). Air was pushed at 31 (2) cm/sec through a chamber 2 m in length and 0.75 x 0.75 m in cross section. Temperature and RH were ambient (24-28√C and 30-70%, respectively).

A **plant patch** was created in the flight chamber by placing 8 or 11 plants in the test section. Foraging sites on plants were simulated by placing three pellets of frass (approx. 25 mg) on small (4 x 2.5 cm) squares of paper. The targets were pinned to leaves. In all experiments eight plants each had a single frass target placed on an upper leaf. In two experiments we included three additional plants, each with a target having frass and a third instar larva pinned to the upper portion of the paper, to prolong wasp searching time.

Parasitoids were allowed to antennate three pellets of frass until they started walking away (usually 2-5 min) before being released in the flight chamber.

Exposure to frass puts the parasitoid in a searching mode (Drost *et al.*, 1986; Lewis and Tumlinson, 1988).

Data recording. Behavior observed in the flight chamber was recorded on a TRS 80 Model 100 portable computer, using The Observer software (Noldus, 1991). Responses measured and logged in real time included time spent searching on individual plants and targets, frequency of hovering downwind of targets, and ovipositions.

'Search time' by *M. croceipes* included all time spent on a plant between first landing and first departure. To avoid ambiguity in determining a single searching bout, return visits to the same plant were not counted until a different plant had been visited. Hovering was defined as relatively stationary flight within 2 cm of a target. A hover not followed by a landing was considered a 'rejection.' We recorded behavior until all plants had been visited, or until the wasp left the plant patch for 2 min (usually by flying to the ceiling).

Self site discrimination. To test the hypothesis that wasps spend less time searching previously self-searched than unsearched frass sites we observed individual wasps foraging in an 8-plant patch. After being exposed to frass, wasps were released individually into the flight chamber at the downwind end. In both this and the following experiment, only searches that occurred on plants after the first 3 consecutive plants encountered were considered for analysis, since initial searches were highly variable and often lengthy (2 ⫫ 15 min) as wasps gained experience searching. All targets were renewed before each of 10 wasps were tested. Search times were compared with a t-test.

Conspecific site discrimination. The experimental design used to test the hypothesis that wasps discriminate against frass sites visited by conspecifics was similar to that used in the self site discrimination study. Here, however, two wasps were released simultaneously, and individual observers recorded the behavior of each wasp (the wasps almost never encountered each other). We also added 3 plants, each with frass plus a larva on a target, for a total of 11 plants, in an attempt to increase foraging time, and we recorded ovipositions. A total of 167 search times were recorded from 14 pairs of wasps.

Data analysis. The data were analyzed first by analysis of variance, using PROC GLM in the SAS statistical package (SAS Institute, 1985). We hypothesized that search times might vary as a function of patch time and oviposition history. We therefore modelled cumulative patch time, time since oviposition, and number of previous ovipositions as covariates with visitation category (self-visited, conspecific-visited and unvisited) to check for bias and to reduce variance. For each covariate the analysis was centered at the mean. Since variances associated with treatment means were unequal, and transformation did not stabilize variances, final comparisons were made with t-tests.

Discrimination cues. To determine whether discrimination is based on vision or olfaction we again used 8 plants with frass-only sites, and 3 plants with frass-plus-larva sites to increase total searching time. In this experiment a parasitoid was allowed to search half (4) of the frass-only targets, after which she was recaptured and 2 visited targets were switched randomly with 2 unvisited targets (plants were not moved) (cf. Wäckers and Lewis, 1994). This resulted in 2 unvisited targets on visited plants, 2 visited targets on unvisited plants, 2 unvisited targets on unvisited plants, and 2 visited targets on visited plants. We then re-released the wasp (N=14) in the patch and recorded behavior as above (except that we excluded cleaning and inactive behaviors from 'searching time'). We predicted that greater reliance on olfactory than on visual information in discrimination would result in short search times on visited targets on unvisited plants (assuming that targets were visually uniform), and that greater reliance on visual information would result in shorter search times on visited plants, regardless of target. Data were analyzed by analysis of variance on log-transformed search times. Means were separated with Fisher's protected least significant difference test.

RESULTS

Self site discrimination. The 10 wasps tested made 38 first visits to frass targets and 38 repeat visits. Wasps discriminated against previously visited targets, since repeat visits were significantly shorter than first visits (11.5 sec 1.8 SE and 32.8 sec 4.4 SE, respectively; $P < 0.0001$). Previously visited targets were also rejected in flight (i.e., wasps hovered within 2 cm but did not land on targets) more often (27 times) than newly encountered targets (once). Seven of the 10 wasps rejected such targets at least once.

Conspecific site discrimination. Search times on previously unvisited targets (59.4 sec 8.8 SE; $N = 66$) were longer than searches on conspecific-visited targets (19.2 sec 3.4 SE; $N = 39$; $P < 0.0001$), and searches on conspecific-visited targets were longer than on self-visited targets (8.5 sec 1.4 SE; $N = 62$; $P < 0.001$) (Fig 1). Patch time was insignificant as a covariate with search time within treatments ($F = 0.12$,; df = 3, 161; $P > 0.95$), meaning that wasps did not significantly reduce or increase site searching time the longer they foraged in the flight chamber. Likewise, search time was independent of time since oviposition ($F = 0.25$,; df = 3, 161; $P > 0.85$) and number of ovipositions ($F = 0.03$,; df = 2, 161; $P > 0.95$).

Discrimination cues. Olfactory and to a lesser extent visual cues were used by *M. croceipes* females to discriminate between visited and unvisited sites (Fig 2). Visited targets were searched less than unvisited targets both on unvisited plants (VU < UU; $P < 0.02$) as well as on visited plants (VV < UV; $P < 0.001$), thus suggesting responses to chemical marks. Visited targets were searched less on visited than on unvisited plants (VV < VU; $P < 0.01$), thus suggesting visual discrimination by parasitoids. There was also a nonsignificant trend for unvisited targets to be searched less on visited than on unvisited plants (UV < UU; $P < 0.07$). If rejections are included as null times, the latter difference is significant ($P < 0.05$). Wasps rejected visited targets on visited plants nine times, visited targets on unvisited plants once, and did not reject the other two target-plant combinations.

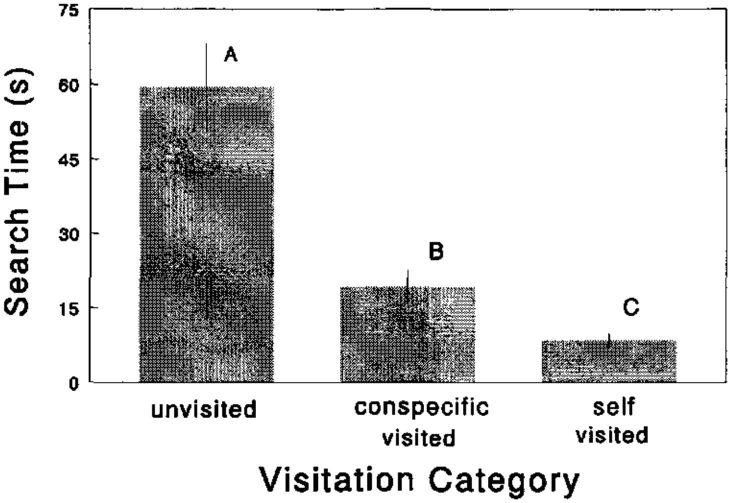

Fig 1. Average search time by *M. croceipes* females at sites with frass from fifth instar *H. zea* caterpillars. Sites had not been visited previously ('Unvisited'), had recently been visited by a conspecific wasp ('Conspecific-Visited'), or had recently been visited by the same or a conspecific wasp (Self-Visited'). Means were separated with t-tests (denoted by different letters above bars; $P < 0.05$) since transformation did not equalize variances.

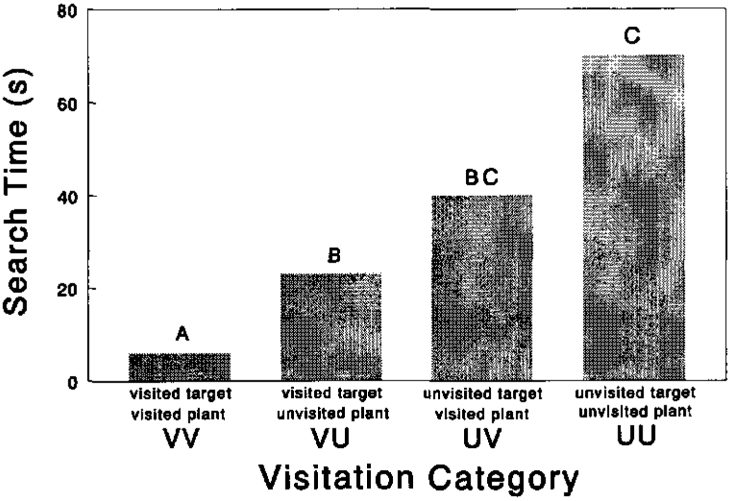

Fig 2. Average search time by *M. croceipes* females on *H. zea* frass sites. Sites were visited and switched to an unvisited plant (VU; N = 20), unvisited and switched to an visited plant (UV; N = 17), visited and not switched (VV; N = 20), or unvisited and not switched (UU; N = 9). Bars with different letters are significantly different at $P < 0.05$, based on an analysis of log transformed values.

DISCUSSION

We have shown that free flying wasps can discriminate between visited and unvisited sites in a simulated plant patch even in the absence of hosts. Such behavior will be escpecially relevant for time limited parasitoids like *M. croceipes*, since it could prevent wasps from wasting time and energy while searching less profitable, already-searched sites (Price, 1970; Sugimoto *et al.*, 1986). The time saved is not restricted to the decrease in search time on previously visited sites (. Since the foraging parasitoid does not need to land in order to recognize previously searched sites, in-flight avoidance of parasitized hosts represents an even more substantial saving in time.

Odor marking appears to be involved in foraging site discrimination by *M. croceipes*. Reduction in time spent by conspecifics searching sites previously searched by other wasps (Fig 1) strongly implies an olfactory mechanism, since wasps arriving later would not have seen the first wasps. Furthermore, reduced search time of visited targets on unvisited plants (Fig 2) also implicates olfactory cues. Odor marking has been shown to be used in host discrimination by *M. croceipes* (Wäckers and Lewis, 1994); whether the same chemicals and means of detection are used in site discrimination remains to be tested.

Visual cues also appear to be used by *M. croceipes* in site discrimination, since wasps spent less time searching visited targets on visited than on unvisited plants (Fig 2). We cannot entirely rule out chemical marking of the leaf surface beyond the target, but this cannot explain cases where wasps only searched the target during a first visit and that target was subsequently replaced with a fresh target (N = 5). In all such cases, searching time was significantly less compared with search time on unsearched targets on unsearched plants. This suggests that spatial memory of visual cues may be involved. Furthermore, probing (reflexing the abdomen toward the substrate), which may be the act of chemical marking, was restricted to frass placed on the paper targets. However, not all wasps probed, and those that did not still elicited a reduction in subsequent search time by conspecifics (suggesting chemical marking). Tarsal contact may be implicated, as in *Trichogramma* (Salt, 1937).

The role of vision has been examined in the context of learning different kinds of foraging sites (Arthur, 1967; Wardle and Borden, 1990), but visual aspects of discrimination have seldom been examined for parasitoid wasps other than *M. croceipes* (see also van Giessen *et al.*, 1992; Wäckers and Lewis, 1992). Sugimoto *et al.*, (1986) dismissed a possible role for memory in site discrimination by *Dapsilarthra rufiventris*, a parasitoid of leaf miners, because wasps discriminated equally against self-visited and conspecific-visited sites. However, in this study wasps discriminated more against self-visited targets left in place than self-visited targets switched to an unvisited plant (VV and VU, respectively, in Figure 2). One difference with the experiments of Sugimoto *et al.* (1986) is that they put wasps directly on leaves (previously infested), whereas we allowed wasps to forage freely among whole plants. Odor cues may well be of primary importance for many parasitoids at some levels of host finding, but other sensory modalities cannot be dismissed without testing in environments, such as flight chambers, where insects can move about freely.

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Summary

The use of parasitoids as biological control agents is gaining interest as an environmentally sound alternative to the use of chemical insecticides. In order to extend and optimize biological control systems, it is essential to gain insight in the stimuli and mechanisms by which natural enemies locate their hosts, as well as their food sources. Only then will it be possible to select the particular parasitoid or predator that constitutes the optimal fit for a given crop-pest combination, or (vice versa) to adjust the culturing practice in such a way that it maximizes the effectiveness of natural enemies.

This thesis represents the results of four years of research on several aspects of chemical and visual orientation in hymenopterous parasitoids. The work was conducted within the collaboration between the Department of Entomology, of the Wageningen Agricultural University and the United States Department of Agriculture, Tifton, USA. In the United States, sensory orientation in *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a solitairy larval parasitoïd of the cotton bollworm (*Helicoverpa* spp.), was studied. The research in Wageningen concerned *Cotesia rubecula* (Hymenoptera: Braconidae), a solitairy parasitoïd of the small cabbage white (*Pieris rapae*).

The foraging process of these parasitoids consists of two independent elements. Not only do they have to locate larval hosts in order to produce offspring, they also need to find nectar as an energy source. Since these two resources are not usually associated, they require seperate foraging processes. Both foraging processes have been investigated in this study. The first part (chapter 2 and 3) addresses how parasitoids employ olfactory and visual information to locate nectar sources. The remainder deals with the respective role of these sensory cues in host foraging.

The role of sugars in increasing the longevity of *C. rubecula* was reported in **chapter 2**. The fact that provision of sugarwater prolonged the life span of males and females by a factor 9 and 14 respectively, shows that finding food can be an essential element of a parasitoid's fitness.

The response of both hungry and satiated parasitoids to flower odors (floral nectar) and odors from aphid infested leaves (honeydew) was tested in a y-tube olfactometer. Irrespective of their state of hunger, parasitoids were attracted to flower odours. Parasitoids did not respond to odors from aphid infested leaf material. This underlines that in order to assess the role of natural food sources, not only their availability should be considered, but also their detectability.

In **chapter 3** the response of *C. rubecula* to flower stimuli was investigated in further detail. It was demonstrated that parasitoids can use both flower-odors and -colors during food foraging. The response of parasitoids to these food-indicating stimuli depended on the hunger state of the individual. Given a choice in a y-tube olfactometer between flower odors and odors from host-infested leaves, hungry individuals chose flower odors, while satiated individuals preferred host associated odors. Free ranging parasitoids were observed in flight chamber experiments to determine their response to visual stimuli. Hungry parasitoids sought out yellow targets and searched more actively on this color, while satiated individuals displayed a higher overall foraging activity, without reacting differentially to yellow.

The remainder of this study concentrates on the role of sensory information during host foraging. Chapter 4, 5, and 6 show how parasitoids use both olfactory and visual cues to locate their (hidden) hosts. These chapters furthermore demonstrate how parasitoids can increase the efficiency of their sensory orientation through associative learning.

In **chapter 4** aspects of olfactory and visual learning were investigated in *M. croceipes*. By using two alternative types of host sites (assemblages of hosts and associated cues) in a flight-chamber plant patch, associative learning of both odor and visual cues could be studied in free ranging parasitoids. During training sessions only one type of host site was associated with the host. To study odor learning, frass from *H. zea*, feeding on either cotton flowers or cotton leaves was offered as volatile alternatives. Subsequent choice evaluations revealed that parasitoids preferred whichever frass odor had been associated with the host during training sessions. In the same manner it was shown that parasitoids can be conditioned to visual stimuli. Thus, it was shown that female parasitoids can learn odor cues as well as visual information to distinguish between hosts feeding on different parts of the plant. This multisensory learning may enable them to concentrate their search on the plant structures that are most profitable in terms of host encounters.

Visual and olfactory learning proved to be additive: parasitoids conditioned to a combination of visual and olfactory stimuli displayed a stronger preference than individuals conditioned to either sensory component alone. When conditioned to a combination of stimuli, olfactory learning was demonstrated to be dominant over visual learning.

Learning of the individual visual elements (color, shape, and pattern) by the parasitoid *M. croceipes* was investigated in **chapter 5**. Again, two visual alternatives were offered to free ranging parasitoids, only one of which was associated with a host larva. By using alternatives that differed in either color, shape or pattern, it was shown that parasitoids can learn to distinguish host sites on the basis of each of these visual elements. When parasitoids were conditioned to a combination of shape and color, the latter was learned dominantly. The relative rate at which *M. croceipes* learns color, shape and pattern was compared with the visual learning rates in honey bees. Species specific learning predispositions were discussed in relation to the ecological context in which these species operate.

The prominent role played by the chemical component of herbivore damage in parasitoid foraging has been generally recognized. Based on data from chapter 4, it was speculated that the reported use of color, shape, and pattern information could enable parasitoids to employ the visual image of feeding damage as an additional foraging cue. **Chapter 6** deals with the question of whether host foraging *C. rubecula* do actually respond to the visual component of feeding damage. Their innate response to the visual component of leaf damage was examined in flight chamber experiments. Parasitoids were observed while foraging among plants containing different types of artificial leaf damage.

Parasitoids displayed an innate preference for leaves containing small punch holes, as opposed to leaves with big punch holes, while visible leaf damage was preferred over invisible damage (peripherally damaged leaves). This preference for the visual image of herbivore feeding was manifested in a significantly higher number of landings. The subsequent searching time, however, did not differ between the four leaf categories. The innate visual preference was no longer found when the odor component of natural feeding damage was added to the four leaf categories. This indicates that, under the given experimental conditions, visual orientation was overruled by herbivore induced volatiles. When free ranging parasitoids were given repeated oviposition experience on leaves with small punch holes, they subsequently displayed a preference for this leaf-category even in the presence of host-induced volatiles.

The final third of this study focused on the last phase of host foraging: host detection and acceptance. It was investigated how parasitoids employ both olfactory and visual information either to recognize hosts which have been previously parasitized, or sites which have been previously searched. Both studies (chapter 7 & 8) were conducted with *M. croceipes*, of which several reports state that it lacks the ability to discriminate between parasitized and unparasitized hosts.

In **chapter 7** it was reported that parasitoids foraging individually in a plant patch, *do* distinguish between unparasitized and parasitized *H. zea*. This host discrimination was manifested in a reduced number of alightments on parasitized host targets (in-flight discrimination), as well as in increased host rejection on the target. On the target chemical marking was shown to be the main mechanism underlying host discrimination. It was shown that chemical marking is also involved in discrimination by the flying female. Furthermore, *M. croceipes* was found to distinguish between self-parasitized hosts and hosts parasitized by conspecifics, indicating that the chemical marker is individualized. The seeming discrepancies between these results and previous reports could be explained by the presented data.

In **chapter 8** it was tested whether free-ranging parasitoids are also able to discriminate between previously visited and unvisited sites containing host kairomone (caterpillar frass) only. *M. croceipes* spent less time searching frass sites previously searched by themselves or by conspecifics than unsearched frass sites. Analogous to the individualized host discrimination, the parasitoids could distinguish between self-visited sites and sites visited by conspecifics. In addition to chemical marking, spatial memory of visual cues was implicated as a mechanism for discriminating against self-visited, host-free sites.

Samenvatting en conclusies

Het inzetten van sluipwespen in de bestrijding van plaaginsekten staat in toenemende mate in de belangstelling als milieuvriendelijk alternatief voor chemische bestrijdingsmiddelen.

Voor de verdere ontwikkeling en optimalisering van biologische bestrijding is het essentieel dat we inzicht hebben in de wijze waarop natuurlijke vijanden hun prooi en hun voedsel weten te vinden. Alleen dan zal het mogelijk zijn om *die* natuurlijke vijand te selecteren die het beste bij een bepaalde teelt past, of (vice versa) de teeltcondities zodanig aan te passen dat de natuurlijke vijand de plaag optimaal bestrijdt.

In dit kader heb ik 4 jaar onderzoek gedaan naar de rol van chemische en visuele stimuli bij het foerageren van sluipwespen. Dit onderzoek vond plaats binnen het samenwerkingsverband tussen de Vakgroep Entomologie van de Landbouwuniversiteit en de "United States Department of Agriculture", Tifton, GA. Tijdens mijn verblijf in de Verenigde Staten heb ik onderzoek gedaan aan *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a solitaire parasitoïde van *Helicoverpa* spp., terwijl het onderzoek in Wageningen gedaan is met *Cotesia rubecula* (Hymenoptera: Braconidae), een solitaire parasitoïde van *Pieris* spp.

Het foerageerproces van deze parasitoïden is in het bijzonder interessant, aangezien hierin twee afzonderlijke delen te onderscheiden zijn. Enerzijds zoeken zij met het oog op reproduktie naar gastheren. Anderzijds moeten deze parasitoïden in een afzonderlijk foerageerproces voedsel zien te vinden. Beide kanten zijn in dit onderzoek aan de orde gekomen. Het eerste deel van dit proefschrift behandelt hoe sluipwespen geurstoffen en kleurinformatie gebruiken om nectar te localiseren. Vervolgens wordt de rol van sensorische stimuli tijdens het gastheer-foerageren besproken.

In **hoofdstuk 2** werd in eerste instantie het effect van voeding op de overleving van *C. rubecula* onderzocht. Uit het feit dat de levensduur van mannetjes en vrouwtjes door voeding met suikerwater gemiddeld met respectievelijk een factor 9 en 14 werd verlengd, kan worden geconcludeerd dat voor deze parasitoïde het zoeken naar voedsel een onontbeerlijk element van het foerageerproces vormt.

De respons van zowel gehongerde, als gevoede parasitoïden op bloemgeuren (nectar) en geuren van bladluis geïnfecteerde bladeren (honingdauw) werd bestudeerd met behulp van een Y-buis olfactometer. Onafhankelijk van hun hongertoestand bleken parasitoïden door bloemgeuren te worden aangetrokken. Ze reageerden echter niet op de geuren van bladluis-geïnfecteerd plantmateriaal. Dit toont aan dat bij de beoordeling van natuurlijke voedselsoorten niet alleen rekening gehouden dient te worden met de aanwezigheid, maar tevens met de waarneembaarheid van de verschillende voedselbronnen.

In **hoofdstuk 3** werd verder ingegaan op de rol van verschillende bloemstimuli in het voedselfoerageerproces van *C. rubecula.* Aangetoond werd dat zowel bloemgeuren alsook kleurinformatie door parasitoïden gebruikt worden om voedselbronnen te localiseren. De sensorische voorkeur van parasitoïden bleek afhankelijk van hun hongertoestand. Wanneer parasitoïden in een olfactometer de keuze gegeven werd tussen bloemgeuren en geuren van bladmateriaal met daarop vretende gastheren, bleken hongerige sluipwespen de bloemgeur te prefereren, terwijl gevoede individuen de voorkeur gaven aan gastheer geassocieerde geuren. De respons op visuele stimuli werd onderzocht aan vrijvliegende parasitoïden in windtunnel experimenten. Gele kaartjes, aangebracht op spruitkoolplanten, werden door gehongerde individuen actief opgezocht. Bovendien vertoonden zij intensief zoekgedrag op de gele ondergrond. Gevoede individuen, daarentegen, reageerden niet specifiek op de gele kleur, maar vertoonden wel algemeen een hogere foerageeractiviteit.

In het resterende deel van dit onderzoek stond het foerageren naar gastheren centraal. De drie volgende hoofdstukken laten zien hoe visuele- en geur stimuli door sluipwespen kunnen worden gebruikt om hun (verborgen) gastheren te localiseren.

Bovendien wordt aangetoond hoe parasitoïden door het associatief leren van deze stimuli hun foerageerefficiëntie kunnen verhogen.
In **hoofdstuk 4** werden aspecten van visueel en olfactorisch leren onderzocht in *M. croceipes*. Hiertoe werden in een windtunnel-opstelling twee typen kaartjes aan vrijvliegende parasitoïden aangeboden. De kaartjes bevatten zowel visuele alsook geur stimuli. Tijdens trainingen werd slechts op één van beide kaartjes een gastheer anngeboden. In de geur-conditioneringsexperimenten bevatten de kaartjes als geuralternatieven uitwerpselen van gastheren die zich hetzij op katoenbladeren, hetzij op bloemen van de katoen hadden gevoed. Parasitoïden die met deze alternatieven geconditioneerd waren, bleken steeds *die* geur te prefereren waarop tijdens de trainingen gastheren ontmoet werden. Op dezelfde manier kon worden aangetoond dat sluipwespen visuele kenmerken van gastheerlocaties kunnen leren. Dit betekent dat sluipwespen verschillende delen van één bepaalde plant kunnen leren onderscheiden. Dit multisensorisch leren zal ze in staat stellen om zich tijdens het foerageren op de meest rendabele delen van de plant te richten.

Visueel- en olfactorisch leren bleken het geleerde onderscheidingsvermogen additief te verhogen. Individuen die geconditioneerd werden met kaartjes die zowel qua geur alsook visueel verschilden, bleken een sterkere geconditioneerde preferentie te vertonen dan individuen die slechts op één sensorische component geconditioneerd waren. Bovendien werd aangetoond dat in het proces van multisensorische conditionering het leren van de geurcomponent dominant was over het leren van de visuele kenmerken.

Het visuele leervermogen van *M. croceipes* werd nader onderzocht in **hoofdstuk 5**. Net als in het vorige experiment werd dit gedaan door twee visuele alternatieven aan te bieden, waarvan slechts één geassocieerd was met gastheerlarven. Door alternatieven te gebruiken die respectievelijk verschilden in hetzij kleur, vorm, of patroon, kon worden aangetoond dat sluipwespen gastheerlocaties kunnen leren onderscheiden op basis van ieder van deze visuele elementen afzonderlijk. Wanneer parasitoïden geconditioneerd werden op een combinatie van kleur en vorm, bleek kleur dominant geleerd te worden. De relatieve leervermogen van *M. croceipes* voor deze visuele parameters werd vergeleken met het relatieve leerpredispositie zoals dat bekend is voor honing bijen. Soort-specifieke leervermogens werden besproken in relatie met de ecologische context waarbinnen deze organismen functioneren.

Het is algemeen bekend dat chemische signaalstoffen, afgegeven door herbivoor-beschadigd plantmateriaal een belangrijke rol spelen in het gastheer-foerageren van sluipwepsen. De resultaten uit hoofdstuk 5 geven aan dat sluipwespen daarnaast ook gebruik zouden kunnen maken van de visuele kenmerken van bladschade. In **hoofdstuk 6** werd nagegaan in hoeverre *C. rubecula* inderdaad reageert op de visuele component van bladschade. De respons van vrijvliegende parasitoïden op verschillende typen kunstmatig beschadigd blad werd geobserveerd in windtunnel experimenten. Sluipwespen vertoonden een aangeboren preferentie voor bladeren met kleine ponsgaatjes over blad waaruit grotere gaten geponst waren, terwijl de beide zichtbare schadecategorieën samen geprefereerd werden boven blad zonder zichtbare beschadiging. Deze preferentie voor de visuele component van bladschade bleek uit een verhoogd aantal landingen, maar had geen effect op de verblijfsduur van de parasitoïd op het blad.

De aangeboren visuele preferentie werd niet meer gevonden wanneer de geurcomponent van gastheerschade aan de verschillende schadetypen werd toegevoegd. Dit wijst erop dat onder de experimentele condities de visuele stimuli overstemt werden door de herbivoor-geïnduceerde signaalstoffen.

Nadat vrijvliegende sluipwespen herhaaldelijke ovipositie-ervaring hadden opgedaan op de bladeren met de kleine ponsgaatjes vertoonden zij een preferentie voor deze vorm van bladschade in aanwezigheid van de signaalstoffen.

Het laatste deel van dit proefschrift houdt zich bezig met de laatste fase van het gastheer-fourageerproces, de acceptatie van gastheren. Er is onderzocht hoe olfactorische en visuele informatie door de parasitoïd gebruikt kan worden, enerzijds om reeds eerder geparasiteerde gastheren te herkennen, anderzijds om eerder bezochte locaties te vermijden. In beide studies (hoofdstukken 7 & 8) werd gewerkt met *M. croceipes*, waarvan eerder gepubliceerd was dat deze parasitoïd niet in staat is om ongeparasiteerde van geparasiteerde gastheren te onderscheiden.

In **hoofdstuk 7** wordt aangetoond dat individueel foeragerende parasitoïden wel degelijk onderscheid blijken te maken tussen geparasiteerde en ongeparasiteerde *H. zea*. Deze gastheer-discriminatie bleek zowel uit een verminderd aantal landingen in het geval van geparasiteerde gastheren, alsmede uit een verhoogd aantal afwijzingen van geparasiteerde gastheren na de landing. Er werd aangetoond dat deze afwijzing na de landing voornamelijk berust op de herkenning van een door de sluipwesp achtergelaten chemische markering. Deze chemische markering blijkt reeds door de vliegende parasitoïde waargenomen te worden, waardoor ook de discriminatie in vlucht verklaard kon worden. Tenslotte kon worden aangetoond dat *M. croceipes* haar eigen geurmerk kan onderscheiden van het geurmerk achtergelaten door soortgenoten. De ogenschijnlijke tegenstrijdigheden tussen deze studie en de eerder gepubliceerde gegevens konden aan de hand van de voorgelegde resultaten worden verklaard.

In **hoofdstuk 8** werd onderzocht of *M. croceipes* tevens in staat is om onderscheid te maken tussen eerder bezochte locaties en locaties die nog niet door haarzelf of door soortgenoten zijn bezocht. Sluipwespen bleken inderdaad minder lang te zoeken op kairomone locaties wanneer deze reeds eerder bezocht waren. Analoog aan de gastheerdiscriminatie in het vorige hoofdstuk kon worden aangetoond dat parasitoïden onderscheid kunnen maken tussen locaties die zij zelf bezocht hebben en locaties die door een soortgenote afgezocht zijn. Naast chemische markering kon aangetoond worden dat visuele herkenning een rol speelt bij de herkenning en vermijding van locaties die de sluipwesp zelf reeds bezocht heeft.

Zusammenfassung

Der Einsatz von Schlupfwespen zur Bekämpfung von Schadinsekten findet als umweltfreundliche Alternative zum chemischen Pflanzenschutz in steigendem Maße Interesse.

Zur Weiterentwicklung und Optimierung der biologischen Schädlingsbekämpfung ist es unumgänglich daß wir Kenntnisse darüber erwerben, in welcher Weise Nützlinge ihre Beute und Nahrung lokalisieren. Dann erst wird es möglich sein die Nützlinge zu selektieren, die am besten zu einer bestimmten Kulturpflanze oder deren Anbauform passen oder (umgekehrt) die Anbaubedingungen so zu variieren, daß die Nützlinge das betreffende Schadinsekt optimal bekämpfen.

In diesem Zusammenhang wurde in den vergangenen vier Jahren die Rolle chemischer und visueller Stimuli bei der Orientierung von Schlupfwespen untersucht. Diese Arbeit fand statt im Rahmen des Zusammenarbeitsverbandes zwischen der Fachgruppe Entomologie der Landwirtschaftlichen Universität Wageningen und dem United States Department of Agriculture. Während meines Aufenthalts in den Vereinigten Staaten arbeitete ich mit *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), ein solitärer Parasitoid von *Helicoverpa* spp. Demgegenüber beschäftigte die Arbeit in Wageningen sich mit *Cotesia rubecula* (Hymenoptera: Braconidae), ein solitärer Parasitoid von *Pieris* spp.

Das Furagierverhalten dieser Parasitoiden ist insbesondere deshalb interessant, weil hierbei zwei unterschiedliche Aspekte zu trennen sind. Diese Parasitoiden müssen nämlich neben Wirte zur Reproduktion, ebenfalls Nahrungsquellen finden. Beide Seiten dieses Furagierens wurden in dieser Studie berücksichtigt. Der erste Teil beschäftigt sich mit der Frage, in welcher Weise Schlupfwespen chemische und visuelle Informationen nutzen um Nektar zu lokalisieren. Weiterhin wird dann die Rolle dieser sensorischen Reize während der Wirtssuche besprochen.

Im **zweiten Kapitel** wird insbesondere die Bedeutung des Nahrungsangebotes auf das Überleben von *C. rubecula* untersucht. Aus der Tatsache, daß die Lebensdauer von Männchen und Weibchen durch Fütterung mit Zuckerwasser durchschnittlich um einen Faktor 9 bzw 14 verlängert wird, kann geschlossen werden, daß für diesen Parasitoiden die Nahrungssuche ein essentielles Element darstelt. Die Reaktion sowohl hungriger wie auch gefütterter Parasitoide auf Blütendüfte (Nektar) und auf den Duft von mit Blattläusen befallenen Blättern (Honigtau) wurde mit Hilfe eines zweiarmigen Olfaktometers untersucht. Unabhängig vom Sättigungsgrad ergab sich, daß Parasitoide durch Blütenduft angelockt werden. Demgegenüber reagierten sie jedoch nicht auf den Geruch des mit Blattläusen infizierten Planzenmaterials. Hieraus ergibt sich, daß bei dem Vergleich natürlicher Nahrungsquellen nicht nur das Vorhandensein sondern auch die Wahrnehmbarkeit berücksichtigt werden sollte.

Im **dritten Kapitel** wird näher auf die Rolle verschiedener Blütenreize bei der Futtersuche von *C. rubecula* eingegangen. Es wird gezeigt, daß sowohl Blütenduft wie auch visuelle Information vom Parasitoiden dazu benutzt werden, Nahrungsquellen zu lokalisieren. Die sensorische Präferenz des Parasitoiden war dabei abhängig von ihrem Sättigungsgrad. Wenn Parasitoiden in einem Olfaktometer die Wahl gelassen wird zwischen Blütenduft und Duft von wirtinfiziertem Blattmaterials, wählen hungrige Schlupfwespen den Blütenduft, während gefütterte Individuen den Wirtduft vorziehen. Die Reaktion auf visuelle Reize wurde bei freifliegenden Tieren im Windkanal untersucht. Hungrige Parasitoide suchten gelbe Kärtchen, die auf Kohlpflanzen angebracht waren, aktiv auf und zeigten auf der gelben Unterlage auch häufiger Suchverhalten. Gesättigte Tiere dagegen, reagierten auf die gelbe Farbe nicht spezifisch, aber zeigten algemein eine weitaus höhere Aktivität.

Im restlichen Teil dieser Arbeiten wurde insbesondere die Wirtssuche studiert. Die drei folgenden Kapitel zeigen wie visuelle und chemische Reize von Parasitoiden dazu benutzt werden, ihre (verborgenen) Wirte zu lokalisieren. Zudem wird gezeigt, daß Parasitoide in der Lage sind, durch assoziatives Erlernen dieser Stimuli die Effizienz ihrer Wirtssuche zu steigern.

Im **vierten Kapitel** werden Aspekte des visuellen und olfaktorischen Lernens bei *M. croceipes* dargestellt. Bei dieser Studie wurden den freifliegenden Parasitoiden zwei unterschiedliche Markierungen angeboten. Die Markierungskärtchen enthielten hierbei sowohl visuelle als auch olfaktorische Reize. Während des Trainings wurde nur auf einem der beiden Kärtchen ein Wirt angeboten. In den Duftkonditionierungsversuchen enthielten die Kärtchen Kot von Wirten die sich entweder auf Baumwollblättern, oder aber auf Baumwollblüten ernährt hatten.

Parasitoide, die mit diesen Alternativen konditioniert waren, bevorzugten stets den Geruch bei dem sie während des Trainings Wirte angetroffen hatten. In gleicher Weise konnte gezeigt werden, daß Schlupfwespen sich auch visuelle Merkmale der Fundstellen ihrer Wirte einprägen können. Das bedeutet, daß Parasitoide erlernen können, verschiedene Teile einer Pflanze zu unterscheiden und sich damit während der Wirtssuche auf die ergiebigsten Teile der Pflanze konzentrieren können.

Es zeigte sich, daß visuelles und olfactorisches Lernen das erlernte Unterscheidungsvermögen additiv zu steigern vermag. Individuen, die mit Kärtchen konditioniert waren, die sich sowohl olfaktorisch wie auch visuell unterschieden, zeigten eine stärkere konditionierte Präferenz als die, welche nur auf eine sensorische Komponente konditioniert waren. Zudem wurde nachgewiesen, daß im Prozess der multisensorischen Konditionierung das Erlernen der olfaktorischen Komponente dem Erlernen der visuellen Komponente gegenüber dominant war.

Das visuelle Lernvermögen von *M. croceipes* wurde im **fünften Kapitel** näher untersucht. Wie auch in den vorigen Versuchen geschah dies dadurch, daß zwei visuelle Alternativen (Kärtchen) angeboten wurden. Nur eines dieser Kärtchen war mit Wirtslarven assoziiert. Durch den Einsatz von Alternativen, die sich nach Farbe, Form oder Muster unterschieden, konnte gezeigt werden, daß Schlupfwespen in der Lage sind diese visuellen Merkmale getrennt zu erlernen.

Wenn Parasitoide auf eine Kombination von Farbe und Form konditioniert wurden, dann wurde die Farbe dominant erlernt. Das relative Lernvermögen von *M. croceipes* für diese visuellen Parameter wurde mit der relativen Lernprädisposition, wie diese von Honigbienen bekannt ist, verglichen. Das artenspezifische Lernvermögen wird im Verhältnis zum ökologischen Kontext, in dem diese Organismen stehen, diskutiert.

Es ist algemein bekannt, daß chemische Signalstoffe, die von herbivor-beschädigtem Pflanzenmaterial abgegeben werden, eine wichtige Rolle spielen beim Aufspüren der Wirte durch Schlupfwespen. Die Ergebnisse dieses Kapittels zeigen, daß Schlupfwespen zudem auch visuelle Merkmale des Blattschadens benutzen könnten.

Im **sechsten Kapitel** ist zu klären versucht worden, in wie weit *C. rubecula* auf visuelle Komponenten des Blattschadens reagiert. Die Reaktion freifliegender Parasitoide auf unterschiedliche Typen künstlich beschädigter Blätter wurde im Windkanal beobachtet. Schlupfwespen zeigten eine angeborene Präferenz für Blätter mit kleinen Lochungen gegenüber Blättern die größer gelocht waren. Diese beiden sichtbaren Schadenkategoriem wurden gegenüber unsichtbaren Beschädigungen (Blattmaterial entlang des Blattrandes entfernt) präferiert. Diese Präferenz für die visuelle Komponente des Blattschadens zeigte sich in einer erhöhten Landungsfrequenz, hatte aber keine Auswirkung auf die Verweildauer der Parasitoiden.

Diese Präferenz konnte nicht mehr nachgewiesen werden, wenn die Geruchskomponente den unterschiedlichen Schadenstypen zugesetzt wurde. Dies weist darauf hin, daß unter den Versuchsbedingungen die visuellen Reize durch herbivor-induzierte Signalstoffe überdeckt werden.

Nachdem freifliegenden Schlupfwespen wiederholt Parasitierungserfahrungen auf klein-gelochten Blätter gegeben waren, zeigten sie eine Präferenz für diese Form des Blattschadens bei gleichzeitiger Anwesenheit von herbivor-induzierten Signalstoffen.

Abschließend wurden Studien ausgeführt, um zu klären wie Parasitoide sensorische Informationen während der letzten Phase der Wirtsuche nutzen. Es wurde untersucht wie der Parasitoid olfaktorische und visuelle Informationen einsetzen kann, einerseits um bereits parasitierte Wirte zu erkennen, anderseits um bereits früher besuchte Stellen zu meiden. In beiden Studien (Kapitel 6&7) wurde mit *M. croceipes* gearbeitet, von dem veröffentlicht worden war, daß dieser nicht in der Lage sei, parasitierte von unparasitierten Wirten zu unterscheiden.

Im siebten Kapitel wird gezeigt, daß der individuelle Parasitoid während der Wirtssuche sehr wohl zuvor parasitierte von unparasitierten Wirten unterscheidet. Diese Wirtsdiskriminierung zeigte sich sowohl in einer verminderten Anzahl von Anflügen bei parasitierten Wirten, wie auch in einer erhöhten Zahl von Abweisungen parasitierter Wirte nach der Landung. Es wurde nachgewiesen, daß diese Abweisung nach der Landung insbesondere auf dem Erkennen einer von der Schlupfwespe hinterlassenen chemischen Markierung beruht. Diese chemische Markierung wird, wie sich zeigte, bereits vom fliegendem Parasitoiden wahrgenommen.

Schließlich konnte gezeigt werden daß *M. croceipes* eine eigene Geruchsmarke von den Geruchsmarkierungen der Artgenossen unterscheiden kann. Der augenscheinliche Widerspruch zwischen diesen Ergebnissen und den Aussagen der vorhergehenden Publikationen konnte an Hand der vorliegenden Ergebnisse erklärt werden.

Im achten Kapitel wurde geprüft, ob *M. croceipes* in der Lage ist, früher besuchte Stellen von noch nicht besuchten Stellen (eigener Besuch oder Besuch durch Artgenossen) zu unterscheiden. Es zeigte sich, daß Schlupfwespen tatsächlich kürzere Zeit auf Stellen verbleiben, wenn diese bereits früher besucht worden waren. Analog zu der im vorigem Kapitel beschriebenen Wirtsdiskriminierung konnte gezeigt werden, daß Parasitoide Stellen, die von ihnen selbst besucht wurden, von Plätzen, die von Artgenossen besucht worden waren, zu unterscheiden wissen.

Es wurde festgestellt, daß neben chemischer Markierung auch ein visuelles Erkennen bei der Vermeidung von Stellen, die die Schlupfwespe selbst besucht hat, eine Rolle spielt.

NAWOORD

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CURRICULUM VITAE

Felix Wäckers was born on October 6th 1961 in Arnhem, where he graduated from the "Katholiek Gelders Lyceum" in 1980. In the same year, he began his studies at the Wageningen Agricultural University.

In the period of September 1985 to April 1986, he completed his practical training in Plant Protection at the University Pertanian, in Serdang, Malaysia. During his graduate studies, he was involved in research projects at the departments of Phytopathology, Extension, and Entomology. The Entomology project was partly conducted at the 'Biologische Bundesanstalt', Darmstadt, Germany and was awarded the 1989 "scriptie prijs" by the "Stichting Wageningen Fonds". He obtained his MSc degree in August 1988.

Subsequently, he spent the periods of June 1988 to September 1989 and May 1990 to January 1991 as a Research Entomologist at the USDA-ARS (Tifton, USA) under the supervision of Dr. W.J. Lewis. During this time, he conducted research on the foraging behavior of insect parasitoids. Between stints at the USDA-ARS he wrote an investigative report on the status of biological and integrated pest management in the Netherlands.

In the period from Januari 1991 untill June 1992 he worked for the Dutch Plant Protection Service, concentrating on improving the dutch guidelines for registration of biological control agents, while supporting applications for registration. Concurrently he worked as a Research Entomologist at the Department of Entomology, Wageningen Agricultural University. Here he conducted research on sensory orientation in insect parasitoids under the supervision of Prof. Dr. J.C. van Lenteren and Dr. L.E.M. Vet.

The work reported in this thesis represents a combination of the research conducted in Tifton and Wageningen.

Since October 1st 1993, he has been employed as an Assistant Professor at the Department of Applied Entomology of the Swiss Federal Institute of Technology in Zürich Switzerland.