

Learning of Host-Finding Cues by Hymenopterous Parasitoids

*Ted C. J. Turlings, Felix L. Wäckers,
Louise E. M. Vet, W. Joseph Lewis, and
James H. Tumlinson*

Introduction

Interactions between insect parasitoids and their arthropod hosts characteristically result in the premature death of the hosts, and are obligatory for the development of the parasitic insects. This obviously places strong pressure on the hosts to avoid detection by parasitoids, and on the parasitoids themselves to improve encounter rates with suitable hosts. To confront the challenge of finding the often-inconspicuous, well-hidden hosts, parasitoids have developed various sophisticated searching strategies that depend on a vast array of environmental cues.

A wealth of information generated over the last few decades has enabled several experts to categorize the different elements of the searching behavior of parasitoids (Doutt, 1959; Vinson, 1975, 1976, 1981, 1984; Lewis et al., 1976; Weseloh, 1981; van Alphen and Vet, 1987). Although a great variety of specialized lifestyles have been described, the ability of parasitoids to modify their responses to foraging cues based on experience seems to be characteristic of many species. The ability to learn profitable cues has now been demonstrated for almost 20 different species. This chapter first presents a selective review of learning in parasitoid foraging behavior and then discusses some intriguing ecological aspects of this phenomenon.

Several studies suggest that learning may take place during the immature stage as well as during the adult stage. We will argue that adult learning generally contributes more to the foraging success of the insects than pre-adult learning. Recent evidence indicates that the adult parasitoids' responses are mainly modified through the process of associative learning: the wasps innately recognize host-derived stimuli (unconditioned stimuli) upon contact, and they associate these stimuli with surrounding stimuli (conditioned stimuli) to which they originally show no or limited respon-

siveness. Subsequently, the wasps become responsive to the newly learned stimuli and use them in their search for hosts.

While work has focused on the olfactory sense, parasitoids use other sensory modalities (visual and mechanosensory) as well to find hosts. Several studies now show that all three modalities can be affected by learning. We will point out that parasitoids appear to be particularly effective at using and learning a combination of olfactory and visual cues.

Next we will argue that associative learning is most evident in responses to cues from the environment of the host, especially its food, while responses to cues that are directly host-derived tend to be more congenitally fixed. Flexible responses to cues from the host's food and environment should be favored over fixed responses because of the enormous variability in time and space of such cues and their relatively low reliability (over evolutionary time) in indicating host presence and suitability. Several behavioral studies will be reviewed that show how wasps deal with this variability.

We will also discuss the observation that learning generally requires only very brief contact with specific innately recognized stimuli. However, learned responses are strongest and wane the least if such unconditioned stimuli are contacted in conjunction with an actual oviposition in a suitable host. Additional positive experiences reinforce and strengthen the learned responses, while lack of reinforcement (no encounters with suitable hosts) or perhaps even negative experiences (encounters with unsuitable hosts) may depress responses to certain learned cues. We will argue that these observations agree with our concept of optimal foraging.

In making our arguments, we will focus mainly on our own work involving the solitary larval endoparasitoids *Cotesia marginiventris* (Cresson), which attacks a wide range of lepidopterous species (Turlings, 1990), and *Microplitis croceipes* (Cresson), which specializes on *Heliothis* spp. (Eller, 1990). We will summarize by speculating on how learning may assist a single parasitoid female to overcome foraging problems with which she will be confronted in nature.

Onset of Parasitoid Learning

Parasitoids' searching behavior can be affected by experience at various stages of their life cycle. Sometimes cues appear to be learned by the immature insect (i.e., preimaginal conditioning), which are subsequently manifested in their responses as adults. Generally, however, experiences during the adult stage have a greater effect on the insects' responses. These experiences, as we will see, involve contacts with specific host-derived stimuli that the wasps recognize innately. In addition to this general in-

crease in responsiveness, which we refer to as "priming," the wasps may also learn to respond to previously unrecognized stimuli by linking these new stimuli to the contact stimuli. Such associative learning can significantly alter the parasitoids' preferences for specific stimuli.

Learning by Immatures

Thorpe and Jones (1937) conducted one of the earliest detailed studies on parasitoid learning. They reared the ichneumonid parasitoid *Venturia (Nemeritis) canescens* (Grav.) on its regular host, the meal moth *Ephestia kühniella* (Zell.), but also on *Meliphora grisella* (F.), the small wax moth, which the wasp does not attack under natural conditions. Wasps reared on *E. kühniella* showed a strong preference for the odor of meal moth larvae. However, attraction to the odor of wax moth larvae was induced by rearing the wasps on the unusual host. Thorpe and Jones termed this "pre-imaginal conditioning," implying that the female parasitoids acquired a preference for a specific host species (or associated cues) during the immature stage. A similar idea had been put forth by Hopkins (1917; Craighead, 1921), who observed that herbivorous beetles selected oviposition sites on plant species similar to the ones on which they had been reared. This explanation has been used to explain host preference or selection in various insect groups (Thorpe, 1939; Hershberger and Smith, 1967; Jermy et al., 1968; Jaenike, 1982, 1983), and is often referred to as Hopkins' host-selection principle. Pre-imaginal learning has also been implicated in olfactory kin recognition in social Hymenoptera (Isingrini et al., 1985).

Various theories have been brought forward to explain how experiences of immatures may cause internal changes that increase sensitivity to certain stimuli as adults. Hopkins' host-selection principle was the starting point for other studies (Jermy et al., 1968; Jaenike, 1982, 1983). In 1985, Corbet put forth the chemical legacy hypothesis, which states an alternative explanation for the above phenomena. It suggests that actual traces of chemical cues inside or outside the immature parasitoid are carried over into the adult stage, where they directly affect the sensitivity of the insect to these chemicals.

Hérard et al. (1988) shed some light on a mechanism by which chemical cues from the host in which the immatures developed may be carried over to condition the adult parasitoid. *Microplitis demolitor* were reared from *Helicoverpa zea* (Boddie) larvae fed either artificial diet or a diet of cowpea seedlings. In flight-tunnel tests, wasps reared from plant-fed hosts were readily attracted to semiochemicals emitted from host larvae feeding on cowpea. Similar responses could only be obtained from the wasps reared from hosts fed on artificial diet if the adult wasps first were given a contact

experience with the cowpea-host complex. Hérard et al. (1988) went on to demonstrate that experience with the cocoon increased the wasps' responsiveness to the semiochemicals. When plant-reared wasps were excised from their cocoons shortly before their emergence, the adult wasps responded poorly in flight tunnel bioassays. When the excised wasps were allowed to contact the cocoons prior to introduction into the flight tunnel, however, their responsiveness increased significantly. Apparently, chemicals emanating from the cocoons provide emerging wasps with information that they use in host-searching as adults. This seems to be also the case for the leafminer parasitoid *Opius dissitus* Muesebeck (Petitt et al., unpublished data). When reared on leafminers in lima bean, adults were more attracted to the odors of leafminer-infested lima bean than to those of leafminer-infested eggplant. This preference is not apparent if the parasitoids are excised from their cocoons before eclosion (Petitt et al., unpublished data). The environment in which the immature insect grows up can often have an apparent effect on the responses of the adult wasp. However, no study has shown that learning actually takes place in the immature. As Corbet (1985) suggested and Hérard et al. (1988) and Petitt et al. (unpublished data) demonstrate, cues carried by the immature may only affect the insect's responsiveness after adult emergence.

All of the above examples may be the result of early adult learning rather than learning by the immatures. Early adult learning was demonstrated by Kester and Barbosa (1991) who found that the gregarious parasitoid *Cotesia congregata* is only sensitive to learning plant odors for a few hours after emergence. They suggest that postemergence learning not only retains wasps in the habitat of potential hosts but keeps them in the vicinity of potential mates as well.

For some parasitoids, rearing environment seems to have no effect on their subsequent responses. McAuslane et al. (1990a), for example, reared *Campoletis sonorensis* (Cameron) on *Heliothis virescens* F. larvae feeding on either artificial diet or cotton foliage or sesame foliage. Insects of all three treatments responded equally well to host-damaged cotton or sesame plants. Likewise, Mueller (1983) found that the plants on which hosts were fed had no effect on preference for plants exhibited by *Microplitis croceipes* that emerged from these hosts. Adult experience, on the other hand, has a significant effect on responsiveness to semiochemicals by both *C. sonorensis* (McAuslane et al., 1990b, 1991) and *M. croceipes* (e.g., Eller et al., 1992, see below). Although the effects of both immature and adult learning are seldom studied, it appears that preadult experience generally has only a minor effect on adult host-searching behavior, compared to adult experience (e.g., Vet, 1983; Drost et al., 1988; Mandeville and Mullens, 1990; Petitt et al., 1992).-

Learning by Adults

In their studies on *V. canescens*, Thorpe and Jones (1937) also presented some of the earliest evidence for learning by adults. They showed that responses to odors from wax moth larvae (which the wasps normally do not attack) could be induced by allowing adults to contact such larvae for a period of time upon emergence.

Likewise, Vet (1983) demonstrated that the responses by the parasitoid *Leptopilina clavipes* (Hartig) are affected by both rearing environment and adult experience. This wasp normally attacks fungivorous Drosophilidae and is attracted to the odor of decaying mushrooms (a potential habitat of its hosts). Wasps emerging from hosts that were reared on a yeast medium were significantly more attracted to yeast odors than mushroom-reared wasps, but they still preferred the odor of decaying mushrooms. Obviously, the rearing environment had only a limited effect. By contrast, an adult oviposition experience in hosts feeding on the yeast medium altered their preference significantly in favor of yeast odors.

Numerous studies have now shown that adult learning can strongly modify the responses to host-related cues in many parasitoid species. Experiences can influence responses in two different ways. It can cause a general increase in the responsiveness of a female (i.e., priming), but it can also alter a female's preference for specific cues. In both cases the same or a similar mechanism may be at work, but the effects are distinct enough to discuss them separately.

Priming vs. Preference Learning

Definitions to describe the various effects that experiences may have on insect responses continue to be the cause of confusion (e.g., McGuire, 1984; Tully, 1984; Papaj and Prokopy, 1989). Without trying to add to this confusion we would like to emphasize a distinction between what we regard as two separate phenomena, priming and preference learning. The former refers to the observation that certain experiences merely make the parasitoids more responsive to foraging cues, while the latter includes those cases where the increase in responsiveness is specific for the cues that the insects encounter during the experience.

McAuslane et al. (1991b) found that the ichneumonid *Campoletis sonorensis* (Cameron) is more responsive to plant odors after contacting host larvae in the absence of plants. Hence, wasps did not need to experience plant odors in order to become more responsive to these odors. A general increase in responsiveness to odors of host feces was observed by Eller et al. (1992) for *Microplitis croceipes* after these wasps were allowed to oviposit in larvae on different plants. Even contact with hosts on artificial diet increased responsiveness to feces from plant-fed hosts. We think these are

examples of priming whereby the insects come in contact with an innately recognized stimulus (unconditioned stimulus = US) and become more receptive to other cues (not necessarily present during the experience) to which they already show some degree of responsiveness. Turlings et al. (1989) and McAuslane et al. (1991a) termed this sensitization. However, the term sensitization has been used by others (McGuire, 1984; Tully, 1984) in a very different way (see Smith, this volume). The term "priming" may be more suitable since it has been used to describe similar interactions (Birch, 1974).

Cotesia marginiventris was also considerably more responsive to odors associated with its hosts following contact with a host or its feces in the host's microhabitat (Turlings et al., 1989). Wasps became most receptive to the odor that they encountered during an experience, but the experience also caused a less dramatic increase in responsiveness to the odor of an alternative host microhabitat. The odors may have had something in common, but they were distinct enough for the wasps to differentiate between them. That the experience did not merely cause a general increase in responsiveness was also shown in olfactometer choice tests, where *C. marginiventris* exhibited minor shifts in preference in favor of the odor of the plant-host combination it had experienced (Turlings et al., 1990a).

The preference shifts, however, were not very strong. Eller et al. (1992) suggested that experience may affect preferences more strongly. They offered *M. croceipes* females choices between the odors of feces of hosts that were fed on different diets. The wasps showed no changes in preference after one experience with a complete plant-host complex (including oviposition). However a single experience did increase their overall responsiveness drastically. When the experience was repeated two or four times, the wasps exhibited a significant preference for the odor of the feces they had experienced, even if they strongly preferred the alternative odor before experience or after only one experience (Eller et al., 1992).

In summary, these studies suggest a twofold effect of experience on the wasps. First, experience makes the insects immediately more alert and responsive to odor (priming) and, second, the insects learn to respond to the specific odors that they encounter during the experience (preference learning). We will discuss now how preference, and perhaps aversion, for specific cues can be brought about through associative learning.

Associative Learning

Arthur (1971) was perhaps the first to demonstrate learning of novel odors by a parasitoid. He demonstrated that the ichneumonid *V. canescens* could be conditioned to search for hosts in a medium impregnated with

geraniol by presenting hosts to the wasps in the presence of this odorous chemical. The wasps apparently associated this novel odor with the presence of hosts.

Similar associations were found by Vinson et al. (1977) and Wardle and Borden (1989). Vinson et al. (1977) found that the parasitoid *Bracon mellitor* Say exhibited ovipositor probing in response to an antimicrobial additive (methyl parahydroxy-benzoate) in artificial diet used to rear their hosts, the boll weevil (*Anthonomus grandis* Boheman). They demonstrated that *B. mellitor* had learned this novel chemical cue and suggested that this was a "classical" form of associative learning (*sensu* Pavlov, 1941). Wardle and Borden (1989) found that the polyphagous ectoparasitoid *Exeristes roborator* (F.) learned apple odors after the wasps had experienced the odor during encounters with hosts.

We found that contacting host feces was sufficient experience for *C. marginiventris* wasps to increase dramatically their responses to odors experienced during contact (Turlings et al., 1989, 1990a). It was suggested that, upon contact, wasps recognized specific semiochemicals in the feces of suitable hosts. The parasitoids associated surrounding odors with the possible presence of hosts and subsequently used these odors as cues in host searching. That parasitoids indeed are capable of learning specific cues through such an association was demonstrated by Lewis and Tumlinson (1988) and Vet and Groenewold (1990).

Lewis and Tumlinson (1988) found that a water-soluble nonvolatile contact kairomone in the frass of host larvae served as the key stimulus in learning by *M. croceipes*. After contact with this kairomone the wasp was found to be attracted to odors that were present during the contact experience, even if these odors are not normally associated with hosts. Vet and Groenewold (1990) found that a similar mechanism triggered learning in *Leptopilina heterotoma* (Thomson), a parasitoid of *Drosophila* species. A kairomone was extracted from yeast media in which *Drosophila* larvae had been crawling. Wasps were found to be attracted to a synthetic odor (*Z*)-3-hexen-1-ol after contacting yeast containing kairomone in presence of that odor. Recently, de Jong and Kaiser (1991) demonstrated that a related specialist parasitoid, *L. bouvardi*, is also capable of learning a novel odor (perfume) in association with a successful oviposition in a host larva.

With these experiments Lewis and Tumlinson (1988) and Vet and Groenewold (1990) presented direct evidence for unconditioned stimuli (US) in host by-products, which the parasitoids recognize upon contact. During a contact, the wasps associate the US with surrounding conditioned stimuli (CS). As a result of the association the wasps will be responsive to the CS and use them as cues in subsequent host-seeking efforts. It has been suggested that, after this association, the CS may serve as US during subse-

quent experiences, a phenomenon termed “second-order learning” (Vet et al., 1990a; see also Menzel et al., this volume).

The physiological processes behind associative learning have not yet been elucidated. Evidence indicates that experience actually causes sensitivity changes in the olfactory receptors on the insects’ antennae (Vet et al., 1990b), but, unlike behavioral studies, research on physiological aspects of learning in parasitoids has only just begun. Many similarities with other Hymenoptera (e.g., Gould, this volume; Menzel et al., this volume; Smith, this volume) can be expected. Associative learning is not limited to olfactory stimuli; parasitoids are also able to link visual (next section) and mechanosensory stimuli (Monteith, 1963) with an US that indicates the presence of hosts.

Visual Learning

A Neglected Aspect of Learning in Parasitoids

As indicated above, research into sensory orientation in insect parasitoids has long focused on the capacity of parasitoids to detect and learn chemical information. This emphasis on olfaction has sometimes overshadowed the role of other sensory modalities in parasitoid foraging. The use of visual stimuli by parasitoids, for example, has received only limited attention (Wäckers and Lewis, 1992, and references within). This one-sided approach to sensory orientation in parasitoids is remarkable, considering that most of our knowledge of insect visual ecology is based on work done with Hymenoptera. Besides the prominent work on vision in honey bees (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 limited attention paid to visual orientation in parasitoids as compared to other Hymenoptera. First, bees, ants, and digger wasps, being central-place foragers, have to commute between a home base and foraging sites. This immediately raised in the minds of investigators the question of how these insects are able to find their way back to the nest and to profitable foraging locations. Subsequent research has revealed such intriguing visual mechanisms as landmark learning, and orientation to the sun, moon, and polarized light (Gould and Towne, 1988; Hölldobler, 1976; van Iersel, 1975; Gould, this volume). Parasitoids, on the other hand, are expected to abandon host sites when prolonged searching no longer contributes to fitness optimization (MacArthur and Pianka, 1966). Landmark learning or navigation by a sun-compass could enable parasitoids to search more systematically at the habitat level, allowing them

to avoid previously exploited areas. At specific locations, visual recognition of previously visited sites could enable parasitoids to avoid repeated parasitization of the same host (visual discrimination). Evidence for such visual discrimination has recently been reported by van Giessen et al. (unpublished data) and Sheehan et al. (1992).

The second aspect in which parasitoids differ from pollinators is in their interaction with resources. Interest in visual learning by flower pollinators such as honey bees was aroused by the striking visual display of insect-pollinated flowers. Darwin (1876) proposed that this visual display was a consequence of coevolution between plants and pollinators (see Lewis, this volume). Von Frisch (1915) showed long ago that bees indeed use visual information to locate nectar sites, and that associative learning of visual stimuli enables them to specialize on the most rewarding nectar sites.

Parasitoids and predators, on the other hand, might well put selection pressure on their host resource to minimize chances of being detected. Therefore, in contrast to the mutualistic interaction between plants and their insect pollinators, parasitoids and their hosts are involved in an evolutionary game of hide-and-seek. This game, in combination with the small size of hosts should restrict the role of host-derived stimuli in parasitoid foraging including visual stimuli. Parasitoids, however, appear to have adopted a different strategy to employ visual information while foraging for hidden hosts. Responses to visual stimuli have been found to be modifiable by experience analogous to olfactory learning. Visual cues from the hosts are limited, but associative learning of visual stimuli from the hosts' environment (e.g., the plants they feed on) enables parasitoids to exploit visual information during their search.

What Visual Cues Do Parasitoids Employ?

The ability of hymenopteran parasitoids to learn visual stimuli has been known since Arthur (1966, 1967) showed that the ichneumonid parasitoid *Itopectis conquisitor* (Say) could learn to discriminate visually between rewarded and unrewarded microhabitats. Although learning of colors was the likely basis of this conditioned preference, his experiments did not exclude the possibility that the parasitoids were distinguishing microhabitats on the basis of their brightness (i.e., difference in light intensity). More recently, Wardle (1990) provided direct evidence for color learning. She demonstrated unambiguously that the parasitoid *Exeristes roborator* (F.) could be conditioned to the color of rewarding microhabitats, using differently colored microhabitats of equal intensity within the range of insect visible wavelengths. In subsequent experiments, Wardle and Borden (1990) showed that the parasitoid was also able to learn to distinguish microhabitats on the basis of their form. Form and pattern learning have

also been demonstrated in work with *Microplitis croceipes* (Wäckers and Lewis, 1992).

Visual stimuli can be used by parasitoids for various purposes. For instance, *Campoletis sonorensis* females navigate by visual orientation to plants in search of hosts while visual plant stimuli also enhance mate location by males (McAuslane et al., 1990a). Wäckers and Lewis (unpublished data) reported that visual stimuli are involved in several stages of the host location sequence in *M. croceipes*. During target-oriented flight, parasitoids distinguish among targets on the basis of their visual characteristics. Conspicuous visual targets improve the accuracy of the landing. After alighting, parasitoids respond to moving objects and surface vibrations by assuming an "attack posture" often leading to an oviposition attempt in the moving subject.

Visual vs. Olfactory Cues

The ease with which an insect can visually detect an item is a function of the item's dimensions, pattern, and contrast against background, as well as the distance between the insect and the item, and the intensity of illumination (Prokopy and Owens, 1983). Visual detection is independent of air currents, and detection of visual stimuli is not altered by small changes in distance to the source (Miller and Strickler, 1984). On the other hand, detection of olfactory stimuli is influenced by the rate of emission of odor molecules, the release area, the distance between insect and odor source, wind speed, turbulence, and contrast against background odors. Since odors are transmitted as meandering plumes in moving air, an insect downwind from an odor source will encounter odor stimuli in bursts, which makes olfactory detection variable in time and in distance from the source.

In short, visual signals supply more reliable information on the direction of and the distance to the source, independent of wind direction (Prokopy, 1986). However, since physical barriers obstruct visual signals more than odor plumes, olfactory orientation would be more useful in situations where vision is hindered, such as in dense plant canopy.

The results obtained with *M. croceipes* demonstrate how parasitoids may enhance search efficiency by learning both olfactory and visual cues. In this wasp, host-finding success could be increased when information from the two sensory modalities was combined. When *M. croceipes* females experienced odor cues in association with specific visual information during encounters with hosts, they exhibited a stronger conditioned response than when they had experience with only one of the two stimuli (Wäckers and Lewis, unpublished data).

How May Learning Increase Parasitoid Foraging Success?

Sources and Reliability of Cues

The current consensus is that many wasps should learn because cues that may guide wasps to their hosts at a certain time in a certain place are unpredictable (Vet et al., 1990a; Lewis et al., 1990; Vet and Dicke, 1992). Over evolutionary time, associations between hosts and cues should be minimized by selection and most cues should be unreliable indicators of host presence and host suitability over multiple generations (Vet et al., 1991; Wäckers and Lewis, unpublished data). Yet within the life span of an individual wasp, a few cues could be highly reliable, and learning of these cues through experiences would allow many parasitoids to more effectively exploit the diversity of potential cues that may lead them to hosts (Tumlinson et al., 1992). Learning will enable wasps to adjust their responses to changes in host quality and abundance. Vet et al. (1990a) argue that responses to cues that are directly host-derived (such as kairomones) should be congenitally fixed, as such cues are intimately and reliably linked with the material presence of the host. Responses to such cues are expected to be conservative to change in both an ontogenetic and an evolutionary sense. Although host cues may be highly reliable, they will not be readily available to foraging parasitoids. After all, selection will place constant pressure on hosts to minimize the production and/or release of signals that may give away their presence (Tumlinson et al., 1991; Vet et al., 1991). Many parasitoids therefore rely on cues that are furnished not by the hosts, but by the hosts' environment. Cues from the hosts' environment such as host plant volatiles, however, are presumably less reliable because each host species can generally feed on more than one plant species and different plant structures. Until recently, evidence of variability in cues has been lacking. We can now substantiate the variability of plant and host cues with some of our own work on *Cotesia marginiventris* and *Microplitis croceipes*.

Variability in Volatile Cues

Cotesia marginiventris is a generalist that attacks the larvae of many Lepidoptera (Turlings, 1990). *Microplitis croceipes* is more specialized in that it can only successfully develop in *Heliothis* and *Helicoverpa* larvae (Eller, 1990). Despite its specialization this wasp, like *C. marginiventris*, finds its hosts in many different habitats. To locate hosts in these habitats, both *C. marginiventris* (Turlings et al., 1990b, 1991a,b) and *M. croceipes* (Drost et al., 1986, 1988; Elzen et al., 1987; Eller et al., 1988a,b; Zanen and Cardé, 1991; McCall and Turlings, unpublished data) rely principally

on volatile cues emitted by plants that have been damaged by the hosts. The chromatograms in Figure 3.1 illustrate the enormous variety in volatile blends that several of these plants release when damaged by hosts. Each of the depicted volatile blends was the result of feeding-damage inflicted by one particular host, the beet armyworm, *Spodoptera exigua*. *C. marginiventris* is capable of learning to distinguish between odors released by the same host feeding on different plants (Turlings et al., 1990a). The differences in the volatile blends released by different plants also explain why a specialist parasitoid like *M. croceipes* is capable of odor learning

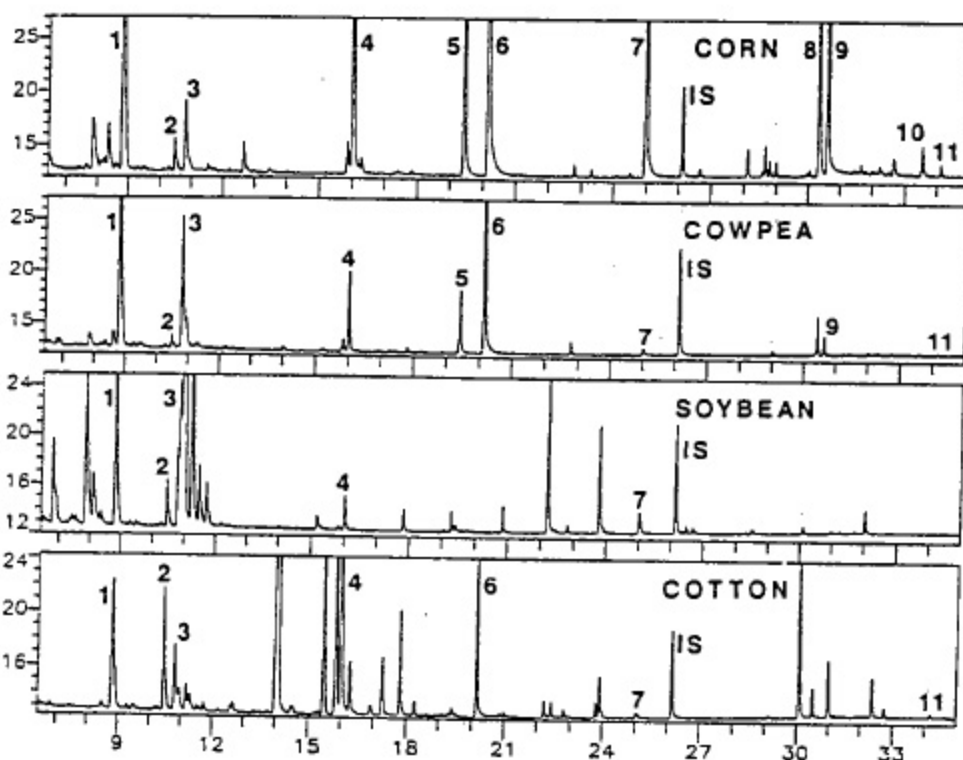


Figure 3.1. Chromatographic profiles that illustrate the differences and similarities in volatiles released by various plants damaged by beet armyworm caterpillars. The volatiles released by corn, cowpea, soybean, and cotton seedlings were collected for 2 hours while the plants were fed upon by 15 caterpillars. The volatiles were trapped on Super Q adsorbent and analyzed by means of gas chromatography. For details on collection and analysis techniques see Turlings et al. (1991b). An internal standard (IS = nonyl-acetate) was added to the samples for reference. The numbered compounds are: 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexen-1-ol; 4, (*Z*)-3-hexen-1-yl acetate; 5, linalool; 6, (*3E*)-4,8-dimethyl-1,3,7-nonatriene; 7, indole; 8, α -*trans*-bergamotene; 9, (*E*)-(β)-farnesene; 10, (*E*)-nerolidol; 11, (*3E,7E*)-4,8,12-trimethyl-1-3-7-11-tri-decatetraene. Volatiles were collected and analyzed by P.J. McCall and T.C.J. Turlings.

(Drost et al., 1986, 1988; Eller et al., 1988b; Lewis and Tumlinson, 1988; Kaas et al., 1990; Zanen and Cardé, 1991; Wäckers and Lewis, unpublished data).

Odors released by different larvae feeding on the same plant species can be quite different as well. When we collected the volatiles released by several lepidopterous pests feeding on corn seedlings we observed consistent differences in ratios in several of the released compounds (Turlings and Tumlinson, unpublished data). *C. marginiventris* is able to distinguish between the odors released by the two closely related host species *Spodoptera frugiperda* J.E. Smith (fall armyworm = FAW) and *S. exigua* Hübner (beet armyworm = BAW). When given a choice in a flight tunnel, female wasps fly more often to corn seedlings with hosts that they had contacted previously than to corn seedlings with the other host (Fig. 3.2). *Microplitis croceipes* also can distinguish between the different odors that

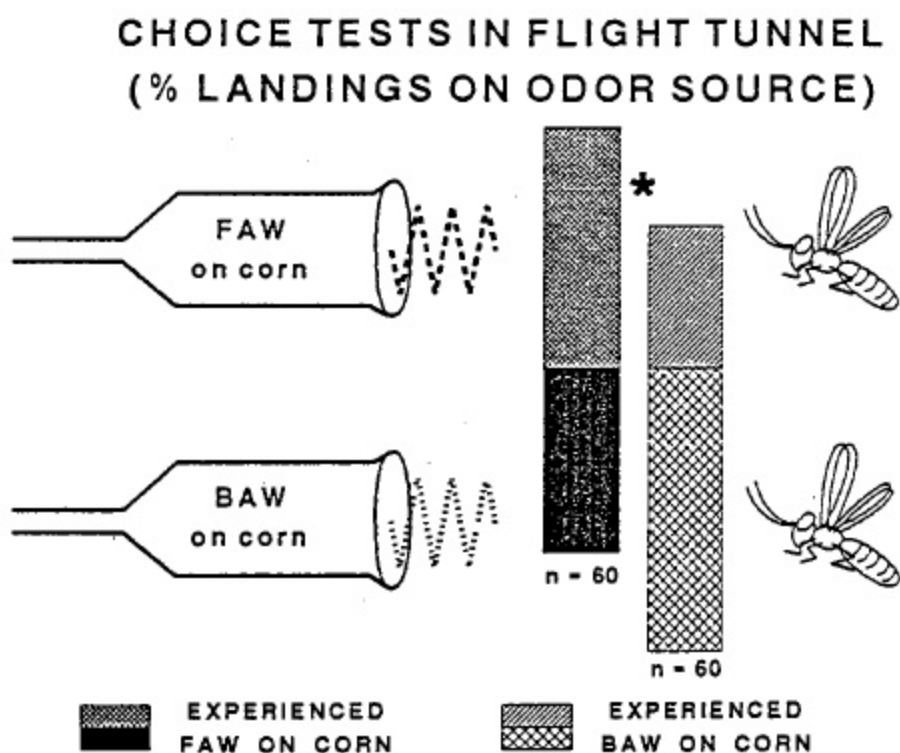


Figure 3.2. Effect of learning on preferences for odor cues exhibited by *C. marginiventris* in a two-choice flight-tunnel bioassay. Females received a 30-second contact experience with either fall armyworm (FAW) or beet armyworm (BAW) on corn. They were then offered a choice between odor sources with the two host species feeding on corn placed next to each other in a flight tunnel. The asterisk indicates a significant shift in odor preference (chi-square = 5.69, $p < 0.02$).

are released when hosts and nonhosts are feeding on the same species of plant (Zanen and Cardé, 1991).

When caterpillars feed on different structures of the same plant the odors emitted vary also. Figure 3.3 shows the odor blends that can be obtained when *H. zea* feeds on different parts of cotton plants. Wäckers and Lewis (unpublished data) were able to demonstrate that *M. croceipes* can actually learn to distinguish odors emitted by feces from hosts that feed on these different plant structures.

From these examples it is clear that, at least in those cases where the hosts are larval herbivores, the plants are essential contributors of host location cues. In fact, as we will see next, plants may actively provide the wasps with reliable chemical information, thereby significantly adding to the maze of information with which the wasps will have to deal.

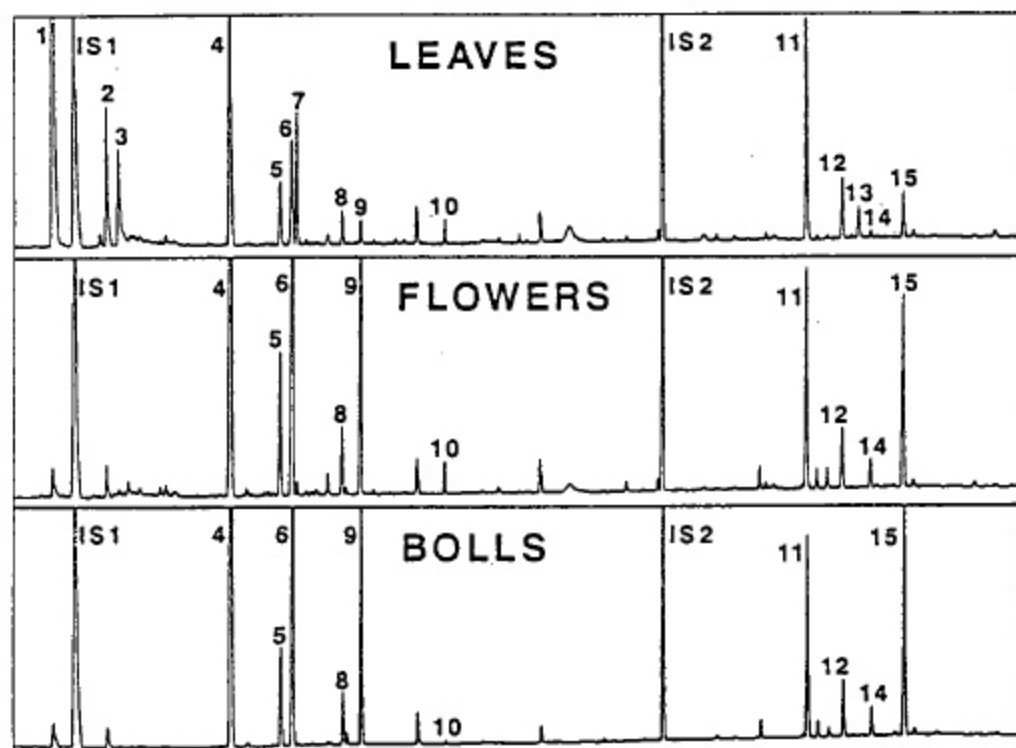


Figure 3.3. Volatiles released by various parts of cotton plants when fed upon by corn earworm caterpillars. For more details on procedure see legend with Figure 1 and Turlings et al. (1991b). Tentative analysis of the volatiles by mass spectrometry indicate the following identities: 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexen-1-ol; 4, α -pinene; 5, β -pinene; 6, myrcene, 7, (*Z*)-3-hexen-1-yl acetate; 8, limonene; 9, ocimene; 10, (*3E*)-4,8-dimethyl-1-3,7-nonatriene; 11, caryophyllene; 12–15, various sesquiterpenes. IS1 and IS2 are internal standards (*n*-octane and nonyl-acetate). Volatiles were collected and analyzed by P.J. McCall and T.C.J. Turlings.

Active Role of Plants

Many parasitoids are attracted to the odors from plants on which their hosts feed (Vinson, 1975, 1981; Vinson et al., 1987; Nordlund et al., 1988; Whitman, 1988; Whitman and Eller, 1990; Williams et al., 1988). Recent research shows that such plants are actively involved in attracting natural enemies of their herbivores. For example, when spider mites feed on lima bean, leaves of this plant release a blend of volatiles that attracts predatory mites (Dicke and Sabelis, 1988; Dicke et al., 1990a). The release of this specific blend of volatiles cannot be induced by artificially damaging the leaves (Dicke and Sabelis, 1988).

Similarly, corn leaves initiated the release of relatively large amounts of terpenoids in response to damage inflicted upon them by caterpillars (Turlings et al., 1990b). Several observations suggest that this is an active response by the plants: (1) Only a minor response can be induced by artificial damage. However, a strong volatile release can be induced when artificially damaged sites are treated with regurgitate of the caterpillars (Turlings et al., 1990b). (2) The plant's response is not instantaneous. Terpenoid release reaches significant amounts only several hours after damage (Turlings et al., 1990b). (3) Undamaged leaves of herbivore-damaged corn plants will release terpenoids in unusually large amounts as well (Turlings and Tumlinson, 1992).

Cotesia marginiventris females are strongly attracted to herbivore-damaged corn leaves (Turlings et al., 1990b, 1991a). Again, prior experience of the wasps was a very important factor in their responses. When the wasps were experienced on freshly damaged seedlings (which did not release significant amounts of terpenoids) they would fly to freshly damaged leaves just as readily as to leaves with 15-hour-old damage (which released large amounts of terpenoids) (Fig. 3-4). On the other hand, wasps that had experienced seedlings with old damage showed a strong preference for the terpenoid-releasing plants over plants with fresh damage. Apparently, the wasps were able to learn the odors that the plants with old damage were emitting.

In the earlier discussion on visual learning, we mentioned the mutually beneficial interactions between pollinators and their nectar sources (i.e., plants) and pointed out that selection would work in a different direction for interactions between parasitoids and their resources (i.e., hosts). Yet here is a case in which plants and parasitoids too may have evolved an interaction from which both profit (earlier suggested by Price, 1981; Price et al., 1980, 1986; Vinson, 1975; Vinson et al., 1987; Dicke and Sabelis, 1989; Dicke et al., 1990b; Turlings et al., 1990b; Turlings and Tumlinson, 1991). It is premature to conclude that plants are purposely signaling the presence of herbivores to natural enemies, since other functions could form

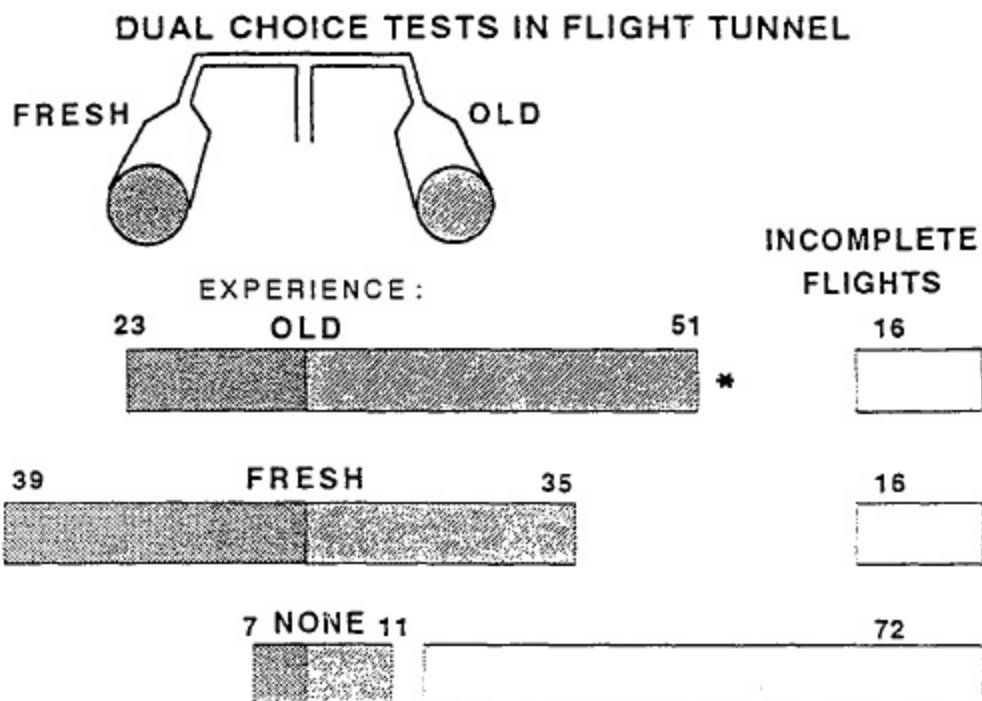


Figure 3.4. Effect of experience on preferences for odor cues exhibited by *C. marginiventris* in a two-choice flight-tunnel bioassay. Females received a 30-second contact experience with beet armyworm on either corn that had been damaged overnight (OLD) or on corn with fresh beet armyworm damage (FRESH). A third group of wasps received no experience (NONE). They were then offered a choice between two odor sources placed next to each other in a flight tunnel. The sources consisted of beet armyworm larvae feeding on corn with fresh or old damage. The asterisk indicates a significant preference for odors from corn with old damage (Chi-square test; $p < 0.05$).

the basis for the observed volatile release (Turlings and Tumlinson, 1991). It is clear, however, that the induced responses in plants add a new dimension to variation in host-location cues. Odors will vary depending upon the degree and age of the inflicted damage, and induced odor emissions will again differ for different parts of the plants. It is even possible, as speculated by D.R. Papaj (personal communication), that plants not only emit chemical "signals," but change visually (color and/or shape) as well, thereby providing the wasps with even more cues.

Increasing Search Efficiency Through Learning

The above examples illustrate the variability in cues that may guide wasps to hosts and the way in which learning assists parasitic wasps in coping with this variability. Two recent field studies demonstrate that learning

aids the wasps in their foraging efforts. Papaj and Vet (1990) released *Leptopilina heterotoma* (Thomson) in a forest with systematically arranged apple-yeast and mushroom baits, both infested with hosts (*Drosophila melanogaster* or *D. phalerata*). They were able to show that a 2-hour experience on the artificial apple-yeast or mushroom microhabitats had increased the wasps' foraging success threefold. First, experienced wasps found baits more often than naive ones. Second, experienced wasps found baits faster than naive ones. Third, females were more likely to find the habitat that they had experienced than the alternative habitat (Papaj and Vet, 1990).

Lewis and Martin (1990) demonstrated the importance of host density and associated cues, as well as experience, for the foraging efficiency of *M. croceipes*. They showed that in a soybean plot artificially infested with variable host and host feces densities, both a more complete experience and high host density increased foraging efficiency (Lewis and Martin, 1990; Martin and Lewis, unpublished data).

These are encouraging results for the planned application of prerelease conditioning of beneficial insects for biological control purposes (Prokopy and Lewis, this volume). These field studies present convincing evidence that odor learning increases parasitoid foraging success in nature.

Retention of Learned Responses Depends on Reward

Learning can occur very rapidly (Vet, 1988; Vet and Groenewold, 1990; Turlings et al., 1989, 1990a; Martin and Lewis, 1992; Poolman-Simons et al., 1992). Effects of experience, however, may be short lived (Martin and Lewis, 1992) and may have only limited consequences for the long-term odor preferences exhibited by insects (Eller et al., 1992). Repeated experience will strengthen the effects and influence the wasps' foraging strategies in a more permanent way.

Cotesia marginiventris' response was significantly altered after a brief (20-second) contact experience with a plant-host complex (Turlings et al., 1989). In fact, contact with the host was not even necessary; simply contacting host frass in the presence of host-damaged leaves was sufficient to increase the responses to odors from the plant-host complex dramatically (Turlings et al., 1989). These single brief experiences may have an immediate strong effect on the responsiveness, but do not necessarily influence a female's preference significantly. In experiments with *M. croceipes* females, wasps required more than one experience before the odor of the feces they had experienced was preferred over an alternative odor (Eller et al., 1992). Poolman-Simons et al. (1992) showed that, in parasitoids of *Drosophila* larvae, brief experiences have an immediate effect on preference, but not on acceptance of host microhabitats. A single oviposition

experience was sufficient to alter the insects' preferences; repeated experiences increased the time that wasps would spend in the habitat they had experienced.

Repeated experience should increase responsiveness and acceptance of certain cues and the wasps then should focus more strongly on a particular resource after repeated successful encounters with it. One-time experiences with either frass or an oviposition may not provide the wasps with adequate information about what is available within their foraging area. Repeated experience should allow them to determine where and which host species are available and adjust their foraging strategy accordingly.

The most positive experience for a female parasitoid would seem to be successful oviposition in a suitable host. If that were the only measure used to assess host presence, encounters with host feces without an actual contact with the host would not alter a female's responsiveness. In fact, contact with feces or plant damage without oviposition actually reduces the responsiveness of *C. sonorensis* (McAuslane et al., 1990b). On the other hand, the chances that a female contacting feces will actually find a host might sometimes be high. Therefore, it is not surprising that, in some instances mere contact with host products increases responses significantly (Lewis and Tumlinson, 1988; Vet and Groenewold, 1990; Eller, 1990; Turlings et al., 1989; van Giessen et al., unpublished data). Several studies show that actual oviposition does increase responsiveness, and particularly preference more than contact with host products. *Leptopilina heterotoma* females are strongly attracted to (*Z*)-3-hexen-1-ol after smelling this volatile when contacting a *Drosophila*-produced kairomone, but responsiveness was stronger if, in addition to contacting the kairomone, they were allowed to oviposit in hosts (Vet and Groenewold, 1990). Likewise, Martin and Lewis (unpublished data) found that an oviposition plus contact with host feces had a stronger effect on the responsiveness of *M. croceipes* than just contact with feces. Those wasps that contacted only feces showed an increase in responsiveness that decreased within an hour, while responsiveness for those that had experienced feces with an oviposition remained high for more than 48 hours (Martin and Lewis, unpublished data). Drost et al. (1986) also showed a relatively long-lasting effect of a complete experience for *M. croceipes*.

In the absence of continued experience, parasitoids tend to "forget" what they learned within a few days (e.g., Sheehan and Shelton, 1989; Papaj and Vet, 1990; McAuslane et al., 1991a). Intuitively, this makes sense. Cues that are associated with hosts should be learned by parasitoids but, when the wasps' subsequent efforts to locate hosts by tracking the same or similar cues are in vain, they may profit by orienting to other cues that are more reliable indicators of host presence.

Van Giessen et al. (unpublished data) showed that wasps will not give up easily. In fact, if *M. croceipes* encountered only frass at an odor source

to which they had flown in a flight tunnel, they would readily fly to the same source a second time. Yet if wasps oviposited in a host after landing at the odor source, they appeared to avoid that particular source during additional flights. Van Giessen et al. (unpublished data) went on to show that, through visual learning, wasps were able to recognize sites at which they successfully parasitized a host which they would avoid subsequently. This avoidance through learning is likely to reduce self-superparasitism.

It seems that the wasps are capable of exploiting the most profitable cues. They will use those cues that repeatedly led them to successful encounters with suitable hosts. The wasps' tendency to "forget" may enable them to resort to different cues when the cues that they have learned are no longer rewarded. This will only be true if the drop in response to the learned cues increases the chances of encountering new hosts or host sites that are not associated with those cues. Thus far, no evidence on this point is available.

To Summarize: a Short Story

The following paragraphs present a conjectural scenario that illustrates how a female parasitoid might use her learning abilities to most effectively exploit various chemical and visual cues in the field. It is a step-by-step description of a parasitoid's host-searching efforts and emphasizes the complexity of cues that a parasitoid may encounter during her lifetime. The story may be removed from biological reality, but it touches on the essential aspects of how learning may assist parasitoids in locating hosts.

A female *C. marginiventris* parasitoid emerges from a fall armyworm (*S. frugiperda*) larva in a cotton field. The newly emerged wasp may initially not be ready to search for hosts; she may first require food and a mate to develop and fertilize her eggs. Mated females respond more intensely to host contact kairomones than unmated females (Loke and Ashley, 1984). To locate food and perhaps also mates, the wasp will use various cues which will be quite different from those that she will eventually use to locate hosts. The wasps associate different odor cues with different types of resources (food or hosts). The intensity of a wasp's response to these cues depends on her physiological state: a hungry wasp will respond strongly to the odor that she previously encountered during feeding, while a food-satiated wasp will respond to odors that she perceived while contacting host kairomones (Lewis and Takasu, 1990).

A satiated and mated mature female will subsequently spend most of her time searching for hosts. Although her mother successfully located the larva feeding on the leaves of a young cotton plant only 12 days earlier, suitable (early) stages of this lepidopterous host are no longer available.

However, earlier-instar *H. zea* larvae are quite abundant, as are cabbage loopers (*Trichoplusia ni*), which are far less suitable as hosts (Turlings et al., 1990a; M.R. Strand, personal communication). The *H. zea* larvae feed predominantly on the bolls of the cotton plants, while cabbage loopers feed mainly on the leaves. The chromatograms in Figure 3.3 indicate that odor blends vary when a herbivore feeds on different parts of the cotton plants. Most obvious is the absence of the typical low-molecular-weight green leaf volatiles in collections from cotton bolls and flowers. The wasp may initially follow odor cues, the traces of which she perceived on the cocoon from which she emerged. By tracking odor blends that are roughly similar to the one that led her mother to her host, she should eventually encounter corn earworm and cabbage looper larvae in the field. Contact kairomones will allow her to assess the suitability of the alternative hosts. During each of her encounters with the hosts, she will learn more and more to distinguish one host species from the other by both smell and vision. She will find the corn earworms mainly on bolls and thus learn to visually search for bolls. Furthermore, she will respond to the specific odor emanating from infested cotton bolls. Eventually she will search primarily for the most abundant suitable host present, i.e., corn earworm.

We can make the story more complicated by introducing a new generation of highly suitable fall armyworm larvae into the field a week or so after our female wasp emerges. By that time, many corn earworm larvae have been parasitized not only by *C. marginiventris* but also by the competing specialist parasitoid *M. croceipes*. The remaining corn earworm larvae have become too large for parasitization. At this time it is much more profitable for the wasp to track odors released by fall armyworm on cotton. Negative reinforcement may facilitate the transition. The wasp will encounter more and more unsuitable corn earworm larvae (already parasitized or too large) and she will therefore learn to avoid the odor blend that is associated with corn earworm and become more responsive to new blends. After several successful encounters she will search primarily for fall armyworm larvae and respond mainly to odor and visual stimuli associated with that host.

We probably exaggerated the dynamics of host populations in a cotton field but, even if such changes only occur over longer periods of time, it is clear that fixed responses to specific volatiles will not allow generalist wasps to deal with these changes. We limited the story to one plant species. Obviously, adding plant species to the searching range of the wasps would increase the complexity of the host environment and perhaps add to the benefits of associative learning.

Conclusions

Behavioral studies on parasitoid foraging behavior clearly indicate that learning is the rule rather than the exception for many species. Our theories

(*sensu* Vet et al., 1990a; and Lewis et al., 1990) on how odor learning will modify the wasps' responses require that females innately respond to a diversity of chemicals commonly associated with hosts and/or their habitat. In the immobile immature insects the sensitivity to some of these chemicals may be elevated due to their presence in the insect's local chemical milieu, but more likely chemicals carried over from the immature affect the adult directly. This heightened sensitivity is expressed in the increased responsiveness to these odors by the adult. As an adult, a female wasp will continue to adjust her responses to specific stimuli in accordance with her experience. Each time a chemical or another type of cue is encountered in conjunction with innately recognized stimuli, the wasp will increase her response levels to this cue and/or lower her responses to others.

The process of associative learning is not limited to olfaction. Several examples now show that visual learning plays a key role as well. The different sensory modalities complement each other, each adding to the efficiency with which wasps are able to locate the hosts in specific habitats.

Although responsiveness can be dramatically increased in parasitoids by simply allowing them to contact host-related kairomones, the strength and tenacity of their responsiveness may strongly depend upon the reward (oviposition) that was associated with this contact. Repeated experiences may be required before wasps lock onto specific cues. This may allow them to assess the host situation in an area and adjust their foraging strategies accordingly. Effects of experiences are not lasting; in the absence of continued experience, they will wane within days or even hours. This may enable the wasps to switch more easily to the use of more reliable and more profitable cues when the cues that they had previously learned become less lucrative.

As suggested by many investigators (e.g., Nordlund et al., 1981; Wardle and Borden, 1985; Vet and Groenewold, 1990; Lewis et al., 1990; Vet and Dicke, 1991), the phenomenon of associative learning in parasitoids may be exploited for purposes of biological control (Prokopy and Lewis, this volume). It might be possible to condition mass-reared parasitoids prior to their release in a target area. When done properly, this may increase strongly the searching efficiency of the released insect such that control will be more effective. Some field data that support the potential of this procedure have recently been published (Lewis and Martin, 1990; Papaj and Vet, 1990).

Learning in parasitoids is not only of interest for future exploitation for biological control purposes. Parasitoids are ideal for comparative ecological studies because of the enormous range of different lifestyles that are found among the numerous parasitoid species. Detailed studies on parasitoid learning may answer many questions in insect behavior. For instance, how do instincts evolve (Papaj, this volume)? At what stages of their lives are insects sensitive to learning? Which different sensory modalities are af-

ected by learning? Also questions regarding the link between dietary specialization of insects and their ability to learn can be answered by studying parasitoids. As several chapters in this volume indicate, parasitoid ecology is particularly useful for modeling the advantages and consequences of learning by insects.

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REFERENCES

- Alphen, J.J.M. van, and Vet, L.E.M. 1987. An evolutionary approach to host finding and selection. In J.K. Waage and D.J. Greathead (eds.), *Insect Parasitoids*, Academic Press, London, pp. 23–61.
- Arthur, A.P. 1966. Associative learning in *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 98:213–223.
- Arthur, A.P. 1967. Influence of position and size of host shelter on host-searching by *Itopectis conquisitor* (Hymenoptera: Ichneumonidae). *Can. Entomol.* 99:877–886.
- Arthur, A.P. 1971. Associative learning by *Nemeritis canescens* (Hymenoptera: Ichneumonidae). *Can. Entomol.* 103:1137–1141.
- Birch, M. 1974. Introduction. In A. Neuberger and E.L. Tatum (eds.), *Frontiers of Biology*, Vol. 32: Pheromones. Amsterdam, North-Holland, pp. 1–7.
- Corbet, S.A. 1985. Insect chemosensory response: A chemical legacy hypothesis. *Ecol. Entomol.* 10:143–153.
- Craighead, F.C. 1921. Hopkins host-selection principle as related to certain cerambycid beetles. *J. Agric. Res.* 64:189–220.
- Darwin, C. 1876. *The Effects of Cross- and Self-Fertilization in the Animal Kingdom*. (Murray, London).
- De Jong, R., and Kaiser, L. 1991. Odor learning by *Leptopilina boulardi*, a specialist parasitoid (Hymenoptera: Eucoilidae). *J. Insect Behav.* 4:743–750.
- Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokhoven, H., and de Groot, A.E. 1990a. Isolation and identification of a volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. *J. Chem. Ecol.* 16:381–396.
- Dicke, M., and Sabelis, M.W., 1988. How plants obtain predatory mites as bodyguards. *Neth. J. Zool.* 38:148–165.

- Dicke, M., and Sabelis, M.W., 1989. Does it pay plants to advertize for bodyguards? Towards a cost-benefit analysis of induced synomone production. In H. Lambers (ed.), *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants*. Academic, The Hague, pp. 341-358.
- Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J., and Posthumus, M.A. 1990b. Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *J. Chem. Ecol.* 16:3091-3118.
- Doutt, R.L. 1959. The biology of parasitic Hymenoptera. *Annu. Rev. Entomol.* 4:161-182.
- Drost, Y.C., Lewis, W.J., and Tumlinson, J.H. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals. V. Influence of rearing method, host-plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J. Chem. Ecol.* 14:1607-1616.
- Drost, Y.C., Lewis, W.J., Zanen, P.O., and Keller, M.A. 1986. Beneficial-insect behavior mediated by semiochemicals. I. Flight behavior and influence of pre-flight handling of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.* 12:1247-1262.
- Eller, F.J. 1990. Foraging behavior of *Microplitis croceipes*, a parasitoid of *Heliothis* species. Ph. D., dissertation, University of Florida, Gainesville, FL, 221 pp.
- Eller, F.J., Tumlinson, J.H., and Lewis, W.J. 1988a. Beneficial arthropod behavior mediated by airborne semiochemicals. II. Olfactometric studies of the host-location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *J. Chem. Ecol.* 14:425-434.
- Eller, F.J., Tumlinson, J.H., and Lewis, W.J. 1988b. Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host-location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environ. Entomol.* 17:745-753.
- Eller, F.J., Tumlinson, J.H., and Lewis, W.J. 1992. Effect of host diet and preflight experience on the flight response of *Microplitis croceipes* (Cresson). *Physiol. Entomol.* 17:in press.
- Elzen, G.W., Williams, H.J., Vinson, S.B., and Powell, J.E. 1987. Comparative flight behavior of parasitoids *Campoletis sonorensis* and *Microplitis croceipes*. *Entomol. Exp. Appl.* 45:175-180.
- Frisch, K. v. 1915. Der farbensinn und formensinn der Biene. *Zool. Jahrb. Abteil. Zool. Physiol.* 35:1-182.
- Gould, J.L. and Towne, W.F. 1988. Honey bee learning. *Adv. Insect Physiol.* 20:55-86.
- Hérard, F., Keller, M.A., Lewis, W.J., and Tumlinson, J.H. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host-diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J. Chem. Ecol.* 14:1597-1606.
- Hershberger, W.A., and Smith, M.P. 1967. Conditioning in *Drosophila melanogaster*. *Anim. Behav.* 15:259-262.

- Hölldobler, B. 1976. Recruitment behavior, home range orientation and territoriality in harvester ants, *Pogonomyrmex*. *Behav. Ecol. Sociobiol.* 1:3-44.
- Hölldobler, B., and Wilson, E.O. 1990. *The Ants*. Springer-Verlag, Berlin.
- Hopkins, A.D. 1917. [Contribution to Discussion]. *J. Econ. Entomol.* 10:92-93.
- Iersel, J.J.A. van 1975. The extension of the orientation system of *Bembix rostrata* as used in the vicinity of its nest. In G. Baerends, C. Beer, and A. Manning (eds.), *Function and Evolution in Behavior*. Clarendon, Oxford, pp. 143-157.
- Isingrini, M., Lenoir, A., and Jaisson, P. 1985. Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proc. Natl. Acad. Sci. USA* 82:8545-8547.
- Jaenike, J. 1982. Environmental modification of oviposition behavior in *Drosophila*. *Am. Nat.* 119:784-802.
- Jaenike, J. 1983. Induction of host preference in *Drosophila melanogaster*. *Oecologia* (Berlin) 58:320-325.
- Jermy, T., Hanson, F.E., and Dethier, V.G. 1968. Induction of specific food preference in lepidopterous larvae. *Entomol. Exp. Appl.* 11:211-230.
- Kaas, J.P., Elzen, G.W., and Ramaswamy, S.B. 1990. Learning in *Microplitis croceipes* Cresson (Hym., Braconidae). *J. Appl. Entmol.* 109:268-273.
- Kester, K.M., and Barbosa, P. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae). *J. Insect Behav.* 4:727-742.
- Lewis, W.J., Jones, R.L., Gross, H.R., Jr., and Nordlund, D.A. 1976. The role of kairomones and other behavioral chemicals in host finding by parasitic insects. *Behav. Biol.* 16:267-289.
- Lewis, W.J., and Martin, W.R. 1990. Semiochemicals for use with parasitoids: Status and future. *J. Chem. Ecol.* 16:3067-3089.
- Lewis, W.J., and Takasu, K. 1990. Use of learned odours by a parasitic wasp in accordance with host and food needs. *Nature* 348:635-636.
- Lewis, W.J., and Tumlinson, J.H. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331:257-259.
- Lewis, W.J., Vet, L.E.M., Tumlinson, J.H., Lenteren, J.C. van, and Papaj, D.R. 1990. Variations in parasitoid foraging behavior: Essential element of a sound biological control theory. *Environ. Entomol.* 19:1183-1193.
- Loke, W.H., and Ashley, T.R. 1984. Behavioral and biological responses of *Cotesia marginiventris* to kairomones of the fall armyworm, *Spodoptera frugiperda*. *J. Chem. Ecol.* 10:521-529.
- MacArthur, R.H. and Pianka, E.R. 1966. On optimal use of a patchy environment. *Am. Nat.* 916:603-609.
- Mandeville, J.D., and Mullens, B.A. 1990. Host preference and learning in *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *Ann. Entomol. Soc. Am.* 83:1203-1209.

- McAuslane, H.J., Vinson, S.B., and Williams, H.J. 1990a. Influence of host plant on mate location by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.* 19:26-31.
- McAuslane, H.J., Vinson, S.B., and Williams, H.J. 1990b. Effect of host diet on flight behavior of the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *J. Entomol. Sci.* 25:562-570.
- McAuslane, H.J., Vinson, S.B., and Williams, H.J. 1991a. Influence of adult experience on host micro-habitat location by the generalist parasitoid, *Campoletis sonorensis*. *J. Insect Behav.* 4:101-113.
- McAuslane, H.J., Vinson, S.B., and Williams, H.J. 1991b. Stimuli influencing host microhabitat location in the parasitoid *Campoletis sonorensis*. *Entomol. Exp. Appl.* 58:267-277.
- McGuire, T.R. 1984. Learning in three species of Diptera: The blow fly *Phormia regina*, the fruit fly *Drosophila melanogaster* and the house fly *Musca domestica*. *Behav. Genet.* 14:479-526.
- Miller, J.R., and Strickler, K.L. 1984. Finding and accepting host plants. In W.J. Bell and R.T. Cardé (eds.), *Chemical Ecology of Insects*. Sinauer, Sunderland, MA, pp. 128-157.
- Monteith, L.G. 1963. Habituation and associative learning in *Drino bohemica* Men. (Diptera: Tachinidae). *Can. Entomol.* 95:418-426.
- Mueller, T.F. 1983. The effect of plants on the host relations of a specialist parasitoid of *Heliothis* larvae. *Entomol. Exp. Appl.* 34:78-84.
- Nordlund, D.A., Jones, R.L., and Lewis, W.J. (eds.). 1981. *Semiochemicals, Their Role in Pest Control*. Plenum, New York, 306 pp.
- Nordlund, D.A., Lewis, W.J., and Altieri, M.A. 1988. Influences of plant produced allelochemicals on the host and prey selection behavior of entomophagous insects. In P. Barbosa and D.K. Letourneau (eds.), *Novel Aspects of Insect-Plant Interactions*. John Wiley, New York, pp. 65-90.
- Papaj, D.R., and Prokopy, R.J. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annu. Rev. Entomol.* 34:315-350.
- Papaj, D.R., and Vet, L.E.M. 1990. Odor learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *J. Chem. Ecol.* 3137-3150.
- Pavlov, I.P. 1941. *Lectures on Conditioned Reflexes*, 2 vols. International Publishers, New York.
- Petitt, F.L., Turlings, T.C.J., and Wolf, S.P. 1992. Adult experience modifies attraction of the leafminer parasitoid *Opius dissitus* Muesebeck to volatile semiochemicals. *J. Insect Behav.*, in press.
- Poolman-Simons, M.T.T., Suverkropp, B.P., Vet, L.E.M., and de Moed, G. 1992. Comparison of learning in related generalist and specialist eucoilid parasitoids. *Entomol. Exp. Appl.*, in press.
- Price, P.W. 1981. Semiochemicals in evolutionary time. In D.A. Nordlund, R.L. Jones, and W.J. Lewis (eds.), *Semiochemicals: Their Role in Pest Control*. John Wiley, New York, pp. 251-279.

- Price, P.W., Westoby, M., Rice, B., Atsatt, P.R., Fritz, R.S., Thompson, J.N., and Mobley, K. 1986. Parasite mediation in ecological interactions. *Annu. Rev. Ecol. Syst.* 17:487-505.
- Price, P.W., Bonton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., and Weis, A.A.E. 1980. Interactions among three trophic levels: Influence of plant interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11:41-65.
- Prokopy, R.J. 1986. Visual and olfactory stimulus interaction in resource finding by insects. In T.L. Payne, M.C. Birch, and C.E.J. Kennedy (eds.), *Mechanisms in Insect Olfaction*. Oxford University Press, Oxford, pp. 81-89.
- Prokopy, R.J., and Owens, E.D. 1983. Visual detection of plants by herbivorous insects. *Annu. Rev. Entomol.* 28:337-364.
- Rosenheim, J.A. 1987. Host location by the cleptoparasitic wasp *Argochrysis armilla*: The role of learning (Hymenoptera: Chrysididae). *Behav. Ecol. Sociobiol.* 21:401-406.
- Sheehan, W., and Shelton, A.M. 1989. The role of experience in plant foraging by the aphid parasitoid *Diaretiella rapae* (Hymenoptera: Aphidiidae). *J. Insect Behav.* 2:743-759.
- Sheehan, W., Wäckers, F.L., and Lewis, W.J. 1992. Discrimination of previously searched host sites by microplitis croceipes. *J. Insect Behav.*, in press.
- Thorpe, W.H. 1939. Further studies on pre-imaginal olfactory conditioning in insects. *Proc. R. Soc. Lond. B* 127:424-433.
- Thorpe, W.H., and Jones, F.G.W. 1937. Olfactory conditioning and its relation to the problem of host selection. *Proc. R. Soc. Lond. B* 124:56-81.
- Tinbergen, N., and Kruyt, W. 1938. Über die Orientierung des Bienenwolfes (*Philanthus triangulum* Fabr.) III. Die Bevorzugung bestimmter Wegmarken. *Zs. Vergl. Physiol.* 25:292-334.
- Tully, T. 1984. *Drosophila* learning: Behavior and biochemistry. *Behav. Genet.* 14:527-557.
- Tumlinson, J.H., Turlings, T.C.J., and Lewis, W.J. 1992. The semiochemical complexes that mediate insect parasitoid foraging. *Agric. Zool. Rev.*, in press.
- Turlings, T.C.J. 1990. Semiochemically mediated host searching behavior of the endoparasitic wasp *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). Ph.D. dissertation, University of Florida, Gainesville, FL, 178 pp.
- Turlings, T.C.J., Scheepmaker, J.W.A., Vet, L.E.M., Tumlinson, J.H., and Lewis, W.J. 1990a. How contact foraging experiences affect the preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *J. Chem. Ecol.* 16:1577-1589.
- Turlings, T.C.J., and Tumlinson, J.H. 1991. Do parasitoids use herbivore-induced plant chemical defenses to locate hosts? *Fl. Entomol.* 74:42-50.
- Turlings, T.C.J., and Tumlinson, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn: Proc. Natl. Acad. Sci. USA, in press.

- Turlings, T.C.J., Tumlinson, J.H., Eller, F.J., and Lewis, W.J. 1991a. Larval-damaged plants: Source of volatile attractants that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomol. Exp. Appl.* 58:75–82.
- Turlings, T.C.J., Tumlinson, J.H., Heath, R.R., Proveaux, A.T., and Doolittle, R.E. 1991b. Isolation and identification of allelochemicals that attract the larval parasitoid *Cotesia marginiventris* (Cresson) to the microhabitat of its hosts. *J. Chem. Ecol.* 17:2235–2251.
- Turlings, T.C.J., Tumlinson, J.H., and Lewis, W.J. 1990b. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–1253.
- Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J., and Vet, L.E.M. 1989. Beneficial arthropod behavior mediated by airborne semiochemicals. VII. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* 2:217–225.
- Vet, L.E.M. 1983. Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym.: Eucoilidae), a parasitoid of fungivorous *Drosophila*: The influence of conditioning. *Neth. J. Zool.* 33:225–248.
- Vet, L.E.M. 1988. The influence of learning on habitat location and acceptance by parasitoids. Proceedings of the Third European Workshop on Insect Parasitoids, Les Colloques de l'INRA. 48:29–34.
- Vet, L.E.M., and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37:141–172.
- Vet, L.E.M., and Groenewold, A.W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16:3119–3135.
- Vet, L.E.M., De Jong, R., Giessen, W.A. van, and Visser, J.H. 1990b. A learning-related variation in electroantennogram responses of a parasitic wasp. *Physiol. Entomol.* 15:243–247.
- Vet, L.E.M., Lewis, W.J., Papaj, D.R., and Lenteren, J.C. van, 1990a. A variable-response model for parasitoid foraging behaviour. *J. Insect Behav.* 3:471–490.
- Vet, L.E.M., Wäckers, F.L., and Dicke, M. 1991. How to hunt for hiding hosts: The reliability-detectability problem in foraging parasitoids. *Neth. J. Zool.* 41:202–213.
- Vinson, S.B. 1975. Biochemical coevolution between parasitoids and their hosts. In P.W. Price (ed.), *Evolutionary Strategies of Parasitic Insects and Mites*. Plenum, New York, pp. 14–48.
- Vinson, S.B. 1976. Host selection by insect parasitoids. *Annu. Rev. Entomol.* 21:109–133.
- Vinson, S.B. 1981. Habitat location. In D.A. Nordlund, R.L. Jones and W.J. Lewis (eds.), *Semiochemicals—Their Role in Pest Control*. John Wiley, New York, pp. 51–77.
- Vinson, S.B. 1984. Parasitoid-host relationship. In W.J. Bell, R.T. Cardé (eds.), *Chemical Ecology of Insects*. Sinauer Associates Inc., Sunderland, MA, pp. 111–124.

- Vinson, S.B., Barfield, C.S., and Henson, R.D. 1977. Oviposition behaviour of *Bracon mellitor*, a parasitoid of the boll weevil (*Anthonomus grandis*). II. Associative learning. *Physiol. Entomol.* 2:157-164.
- Vinson, S.B., Elzen, G.W., and Williams, H.J. 1987. The influence of volatile plant allelochemicals on the third trophic level (parasitoids) and their herbivorous hosts. In V. Labeyrie, G. Fabres, and D. Lachaise (eds.), *Insects-Plants*. W. Junk Publishers, Dordrecht, 109-114.
- Wardle, A.R. 1990. Learning of host microhabitat colour by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Anim. Behav.* 39:914-923.
- Wardle, A.R., and Borden, J.H. 1985. Age-dependent associative learning by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 117:605-616.
- Wardle, A.R., and Borden, J.H. 1989. Learning of an olfactory stimulus associated with a host-microhabitat by *Exeristes roborator*. *Entomol. Exp. Appl.* 52:271-279.
- Wardle, A.R., and Borden, J.H. 1990. Learning of host microhabitat form by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *J. Insect Behav.* 3:251-263.
- Weseloh, R.M. 1981. Host location by parasitoids. In D.A. Nordlund, R.L. Jones, and W.J. Lewis (eds.), *Semiochemicals: Their Role in Pest Control*. John Wiley, New York, pp. 79-95.
- Whitman, D.C. 1988. Plant natural products as parasitoid cuing agents. In H.G. Cutler (ed.), *Biologically Active Natural Products*. American Chemical Society, Washington, DC, pp. 386-396.
- Whitman, D.W., and Eller, F.J. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* 1:69-75.
- Williams, H.J., Elzen, G.W., and Vinson, S.B. 1988. Parasitoid host plant interactions, emphasizing cotton (*Gossypium*). In P. Barbosa, and D.K. Letourneau (eds.), *Novel Aspects of Insect Plant Interactions*. New York, pp. 171-200.
- Zanen, P.O., and Cardé, R.T. 1991. Learning and the role of host-specific volatiles during in-flight host finding in the specialist parasitoid *Microplitis croceipes*. *Physiol. Entomol.* 16:381-389.