

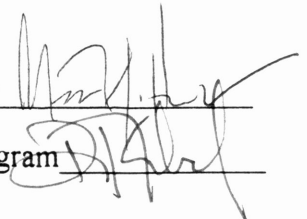
**Biological Control of *Bemisia tabaci* with
indigenous *Eretmocerus* hymenopterans of the American Southwest:
effects of tritrophic interactions**

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1. Introduction

In some systems, agricultural producers implement an augmentative biological control strategy by releasing large numbers of native hymenopteran parasitoids to reduce insect herbivore densities, which favors the grower economically. These biological control agents permit reducing pesticide use on food and fiber crops, which has many repercussions: lower costs for the grower and consumer; production of a healthier, higher quality food item; and less collateral damage on nearby organisms and ecosystems. Moreover, alternative pest management strategies avoid the serious problems associated with relying on pesticides as the sole control measure: first, target pests develop pesticide resistance, rendering chemical control more costly and less reliable, and second, minor pests surge and become economically destructive when their natural control agents are wiped out by pesticides.

However, pesticides can be easily applied in most situations, while the use of parasitoids in augmentative biological control is currently restricted to a much more limited range of situations where experience or research has proven their efficiency. While biological control has been practiced since the last century, the research community has much to learn about the underlying mechanics. Much basic biological and ecological work remains to be done to promote the use of biological control agents as reliable pest controls across a broad spectrum of agroecosystems.

Sweet potato whitefly, *Bemisia tabaci* (Homoptera:Aleyrodidae) wreaks economic damage on a wide range of field and greenhouse crops in the tropical, subtropical, and warmer temperate zones. Its geographic distribution is worldwide; in the Americas it is distributed across both continents except where limited by cool temperatures. It attacks field, vegetable, and greenhouse crops: cotton, alfalfa, peanuts, cabbage, broccoli, cauliflower, melons, squash, cucumber, tomato, pepper, and potatoes, as well as ornamental crops such as poinsettias and hibiscus. It transmits at least 20 viruses (Byrne et.al., 1990) causing over 40 diseases of vegetables, ornamentals, and field crops (Brown

& Bird, 1992). In the U.S., every state in the sunbelt is affected. In the Southwest *Bemisia* is arguably the single worst agricultural pest; in 1991, a particularly bad year, damage estimates in Texas alone reached about \$250 million and around 6500 jobs lost (Bready 1991).

Species of parasitic wasps in the genus *Eretmocerus* (Hymenoptera: Aphelinidae) occur naturally across the U.S. and show considerable promise as control agents of *Bemisia* (Powell and Bellows, 1992; Butter and Vir, 1989; Bellows and Arakawa, 1988; Gerling, 1986; Sharaf and Batta, 1985; Shoshana and Gerling, 1985; Rose and DeBach, 1982; Gerling, 1966). Scientists at several different universities and research institutes in the American Southwest, including researchers at Texas A&M, are currently studying *Eretmocerus spp.* and a related genus, *Encarsia spp.*

Understanding the foraging strategies and reproductive patterns of these two genera of parasitoids would shed light on their efficiency in different agroecosystems, indicating when and why they are likely to colonize crops and regulate pest populations. We could then better estimate what crops and under what conditions these two species would be suitable for biological control of whitefly. Field trials could verify this information and the techniques could then be disseminated to growers. *Bemisia tabaci* is a very serious pest in the southern regions of the U.S., and identifying an indigenous natural enemy whose control activities could be promoted through human intervention would be a significant gain for growers.

2. Bionomics

Whitefly spend their whole life in one environment, the underside of leaves of their host plant. Nymphs and adults feed on phloem fluids in the plant vascular system. The adult females deposit eggs which hatch into crawlers, or motile first instar nymphs. The crawlers roam about the leaf underside for a short distance before settling. They insert their mouthparts into the leaf tissue and become sedentary, going through three more

nymphal instars. The fourth instar undergoes a pupation-like process and then emerges as an adult from the nymphal cuticle. The adults mate and the female lays eggs, attaching them to the leaf underside.

Eretmocerus spp. are solitary internal parasitoids of whitefly nymphs. Eggs are deposited underneath first through fourth instar nymphs. The larval parasitoid hatches and chews a hole in the host's venter and penetrates from underneath. The parasitoid develops through at least three larval instars within the host. Pupation is completed inside the desiccated host cuticle after which the adult emerges. Adult *Eretmocerus* females also kill hosts by feeding upon them; they stab the host with their ovipositor, then turn and feed on the hemolymph exuding from the wound (Gerling, 1986; Foltyn and Gerling, 1985).

3. Statement of the problem and study objectives

The research community is in the process of classifying the *Eretmocerus* genus across the United States. A recent study crossing closely related populations found in Texas and California showed that they are reproductively incompatible (Hunter, Antolin, and Rose, unpublished manuscript), corroborating minute morphological differences found by Rose and Zolnerowich (unpublished data). These data suggest the two populations are sibling species--morphologically very similar but reproductively isolated. Field observations reveal that the Texas population frequently parasitizes *B. tabaci* on both cucurbits and cole crops. In contrast, the California population, while also found in cucurbit crops, is not found on cole crops (Roltsch & Pickett, 1994). Furthermore, in preliminary laboratory experiments, very few progeny of California *Eretmocerus* were produced on *Bemisia* on collards, relative to the Texas population (Hunter, Antolin, and Rose, unpublished manuscript). The salient question from these observations is, why do two morphologically very similar species of *Eretmocerus* perform so differently on the same host plant? This research project has addressed this central question.

We used two approaches to try to answer this question. First, we wanted to see if there was a plant effect on the reproductive capacity of the parasitoids. That is, do cole crops reduce or limit reproduction of the California wasp? Secondly, we sought to uncover whether there was a plant effect on the wasps as they search for hosts, hypothesizing that cole crops could fail to elicit recognition and colonization by the California wasp as it searched for whitefly hosts.

4. Literature review

The chapters on host finding in Boethel & Eikenbary (1986), Waage & Greathead (1986), and especially Godfray (1994) review the body of theory surrounding host finding in parasitoids. Certain aspects of this body of theory are particularly relevant to our study.

a. Tritrophic interactions

In terms of basic research in biological control, this study follows a recent trend among certain investigators (cf. Price et.al., 1980) to incorporate the third trophic level in research on plant-insect and insect-insect interactions: plant, herbivore, and parasitoid are studied conjunctively. Tritrophic interactions in nature cannot be accurately comprehended by simply adding together pair-wise interactions. Numerous studies suggest that plant morphology acutely affects the outcome of plant-herbivore interactions because of plant architecture effects on enemies of the herbivore (cf. Kareiva and Sahakian, 1990). Subtle changes in plant architecture can dramatically influence host plant resistance due to the effects at the third trophic level (Butter and Vir, 1989).

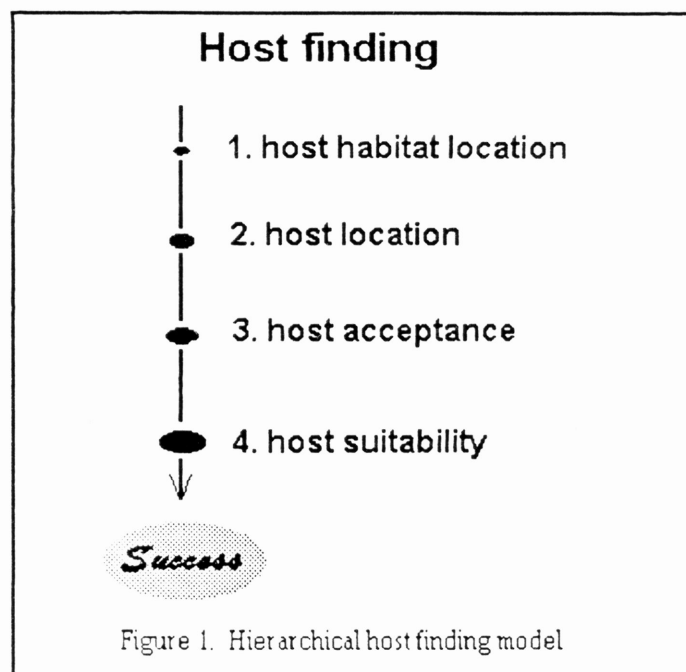
In our case we considered the influence of the two host plant's differing leaf surface anatomy on the activities of the parasitoid. Cucurbits have trichomous leaves covered with fine hairs whereas cole crops have no trichomes. While in most cases trichomous leaf surfaces are considered detrimental to the activities of parasitoids (e.g. Ruberson, 1989), anecdotal evidence suggests *Eretmocerus* females may have difficulty

sliding their ovipositor underneath a host on a smooth leaf such as is found on cole crops. Hence the lack of trichomes on cole crop leaves could inhibit wasp reproduction. Perhaps the Texas wasp had adapted a behavioral response to smooth leaves that give it a reproductive advantage relative to the California wasp, thereby explaining the field observations.

b. Host finding models

Alternately, however, it could be that California females never colonize cole crops. Host finding is the process whereby a newly emerged female wasp disperses from its site of birth and then searches for whitefly hosts on which to oviposit. If chemical cues from cole crops fail to elicit a response from the California wasps in their host-finding process, they would not alight on cole crop plants, thereby explaining their absence in the field.

Parasitoids must locate hosts in a structurally complicated and chemically heterogeneous environment. An early model for host finding developed in the eighties and generally accepted in the literature divided successful parasitism into a hierarchical process involving four stages (Figure 1)(Vinson & Iwantsch, 1980).

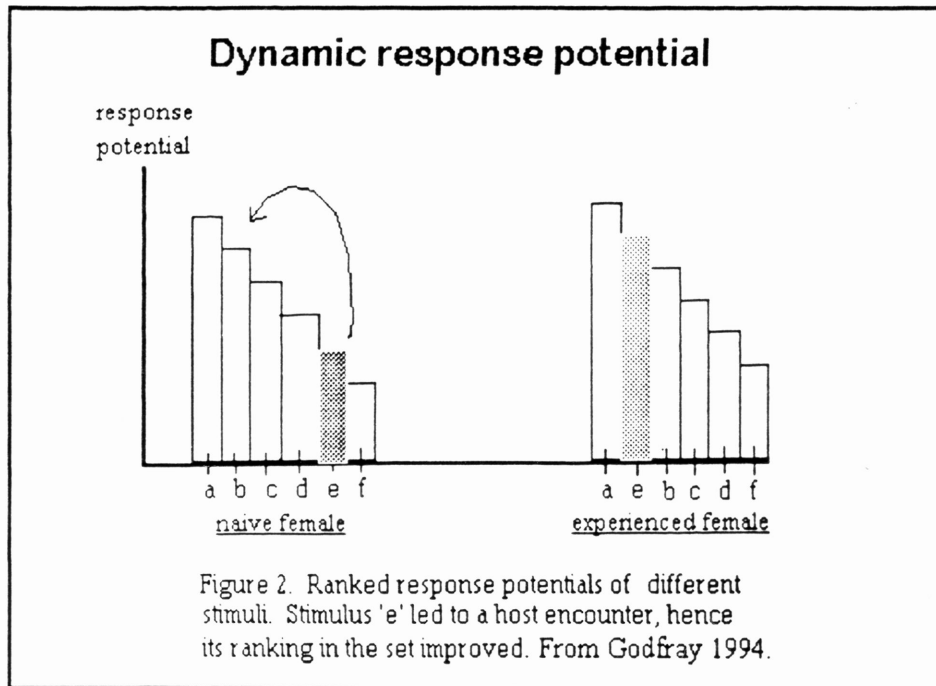


In this model the parasitoid uses a variety of sensory cues to locate the proper habitat and then the host. These cues could involve sight, sound, and perception of heat or movement within the host plant substrate. However, many researchers give priority to olfactory cues related to volatile compounds produced by the plant host, the insect host, or the interaction between them. Following the figure above,

- 1) A set of environmental cues encountered in dispersal would lead the parasitoid to the vegetational community in which it could expect to find hosts.
- 2) Having located the proper plant habitat successfully, the parasitoid then focuses on a different set of cues to lead it to its host.
- 3) Having found the host, the parasitoid would employ a variety of sensory mechanisms to determine if the host is suitable, possibly including olfactory cues, antennation, and probing with its ovipositor. If the parasitoid accepts the host and lays an egg, then
- 4) the process is completed when offspring emerge from the host.

In the nineties emerged a newer and more dynamic model, still under development (Lewis et.al. 1990, Vet et.al. 1990). This model recognizes that different stimuli vary in their information content, in the closeness of the association between a stimulus and the host. The parasitoid responds most strongly to the stimulus most closely associated with the host. Therefore one stimulus guiding parasitoid behavior could be superseded by another stimulus that the parasitoid recognizes as being superior or more closely associated with the host; host habitat location is redundant if the host can be found directly.

In this model, a naive or newly emerged parasitoid is born with an innate set of "response potentials" to different stimuli (Figure 2). A parasitoid presented with a number of different stimuli will react to the one with the greatest response potential. The ranking of different stimuli will be fine tuned by natural selection to maximize host location. The role of learning by the parasitoid is given a primary role in shifting the ranking of different stimuli over the insect's lifetime. If a certain stimulus is associated with the presence of

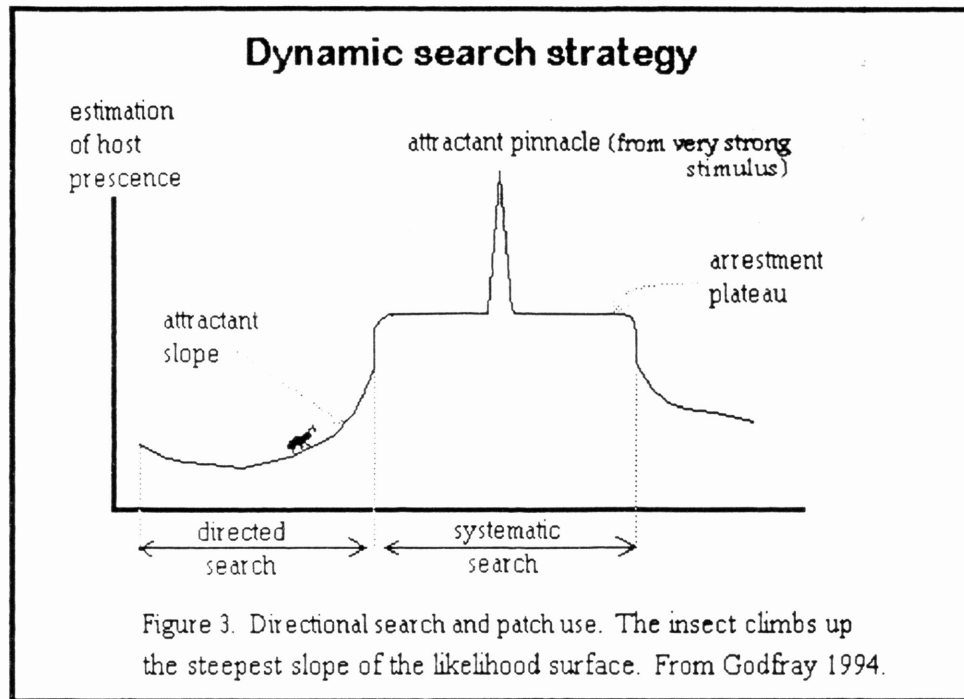


hosts, its ranking may increase. Thus a naive parasitoid, upon finding a host on a particular plant, uses volatile chemicals associated with that plant to find new hosts. This suggests both innate (genetic) responses and conditioned responses (associative learning).

Of course, the ranking of stimuli will vary between species, depending on their life history, and experience can generate variation in ranking even between individuals of one species. While researchers have known for many years that parasitoids are capable of learning from their environment, only recently has the ubiquity of learning been fully appreciated (Godfray, 1994). The spatial distribution of hosts is seldom fixed but varies both between generations and in some cases during the lifetime of a searching parasitoid. This is especially true for generalist parasitoids attacking a wide variety of hosts in different habitats and for specialist parasitoids of polyphagous hosts. Hence, parasitoids will be strongly selected to gather information on current host distribution and modify their search strategy accordingly.

Godfray argues that this model leaves out strategies that explicitly involve movement patterns in space, such as a systematic search. Hosts are not usually evenly distributed throughout a particular habitat; rather, they tend to aggregate in clusters or

"patches". Hence, the parasitoid would utilize a *directional* movement strategy when searching for a patch (between-patch searching), and would utilize a *systematic* movement strategy within a patch. A second dynamic model addresses this issue, relating the two different search modes (Figure 3).



Superimposed on the real world is a surface, the height of which represents the parasitoid's estimation of the presence of a host. The parasitoid will move toward the host most efficiently by climbing the steepest slope of the likelihood surface. Stimuli most closely associated with the host will have the steepest slopes. This model incorporates the distinction between attractant cues and arrestant cues, which provide two different kinds of information. Attractant cues impart directional information and guide the host via concentration gradients to a specific point in space. Arrestant cues, in contrast, do not impart directional orientation but indicate the presence of a host in the near vicinity, delimiting the patch area and signaling a behavioral change in searching pattern. Attractant chemicals frequently have a low molecular weight and greater volatility, while arrestment chemicals generally have a higher molecular weight and lower volatility. It

should be noted that non-olfactory cues could operate in these models; one could easily imagine a parasitoid delimiting search behavior on the basis of visual cues, for example. Further, some cues may function as repellents rather than attractants or arrestants.

Different modes of travel might be associated with certain elements in these models. For example, flight may be dominant mode of travel for the host plant finding stage (first model) or for directional searching (third model). Walking might be associated with the host finding stage or with systematic search. In terms of the second model, wasps may guide their movement with one set of ranked stimuli when traveling in one mode and use another set of ranked stimuli when traveling in a different mode. Hence, a wasp may respond very differently to the same stimuli when flying compared to walking along the leaf surface.

c. Nature and role of cues

There are further issues. Chemical cues can be categorized on the basis of source:

1) Cues from the host plant. Parasitoids might be attracted to a particular physiological stage, organ, or tissue, such as recently opened mushroom caps about to sporulate or scents produced by flowers of angiosperms. Secondary plant compounds have been shown in some instances to attract parasitoids which are plant, rather than insect, specialists (Read et al., 1970).

2) Cues from the insect host. Here, for example, parasitoids might be attracted to odors from one host stage but not another. Many parasitoids respond to their hosts' sex or aggregation pheromones.

3) Cues from the interaction of plant and insect host. Experimentally, this is often infested foliage. Examples of host plant/insect interactions would include frass or honeydew, plant tissue damaged by feeding, or a volatile released by the reaction between host labial gland secretions and the substrate. The attractant may be a mixture of plant

and insect compounds or a unique compound produced by the plant in response to insect feeding.

4) Cues from other organisms intimately related to the host habitat. In other systems the volatile may originate from yet another organism intimately associated with the host or habitat, such as peach fungus or substrate yeast.

In some systems chemical clues from the host plant itself can attract parasitoids in the absence of a host, while in other systems the parasitoid responds only to the host. Some parasitoids will ignore a stimulus if it is perceived alone, but will respond if it is perceived in combination with other stimuli. Naive parasitoids may be selected to orient towards stimuli associated with its emergence site, but this information may be superseded by information derived from subsequent searching. Additionally, a given level of stimulus can either sensitize a parasitoid and cause it to increase the response potential of the stimulus, or desensitize the insect and cause it to fail to respond to the stimulus. Is a particular clue susceptible to sensitizing or desensitizing? What is the level of stimulus that causes this effect to be expressed?

Life history matters. Some polyphagous parasitoids are closely associated with a particular habitat and opportunistically attack a variety of taxonomically unrelated hosts. These parasitoids are more likely to respond to habitat cues, while habitat generalists or host specialists will key in on host cues. Behavioral plasticity or ease of conditioning (the parasitoid's learning curve) is likely to be greater in habitat or host generalists, where successive generations are exposed to different hosts or different environments. Learning may also be more adaptive for long-lived species.

Thus, parasitoids respond in finely textured ways to a wealth of different stimuli, with many variations. The specifics for any one species would largely be determined by its natural history. One would expect taxonomically related species to have similar host finding assemblages, but their proximity may vary. For example, Heinz, working with an olfactometer, found that of two aphelinid species in the genus *Encarsia*, *E. transvena* did

not respond to whitefly infested poinsettia leaves, while *E. tabacivora* responded weakly (unpublished data). (In the same set of trials, *Delphastus pusillus*, a coccinellid beetle predator of whitefly larvae, responded strongly.) In any one species, the nuances of host finding would have to be dissected out in order to comprehensively interpret olfactory tests.

5. Reproductive capacity

The question we sought to answer was why two morphologically very similar *Eretmocerus* species perform so differently on cole crops in Texas and California. Our first study examined the reproductive capacity of the two wasps. Do cole crops inhibit reproduction of the California wasp? In order to answer this question, we designed an experiment to measure reproductive capacity of the two *Eretmocerus* spp. on *B. tabaci* infesting two species of host plant: melon (*Cucurbita melo*) and cabbage (*Brassica olearaca* cv. *capitata*). Both of these crop plants are important components of the agroecosystems of South Texas and the Imperial Valley in California.

a. Materials and methods

Field populations of the Texas wasp were originally collected from the Rio Grande Valley in June of 1994, and were maintained in laboratory culture for the duration of our study in rearing facilities at the Biological Control Laboratory at Texas A&M University. California wasps were obtained from a culture maintained by Oscar Minkenburg of the University of Arizona, originally collected from a Phoenix greenhouse, and subsequently cultured by us. In a previous study, this population could not be distinguished biologically from the California Imperial Valley population (Hunter, Antolin, & Rose, unpublished manuscript). All wasps were reared for at least one generation on hibiscus (*Hibiscus rosa-sinensis*) before being used experimentally.

Small leaf cages were attached to the foliage of whitefly-infested melon and cabbage plants. Dr. Scholl's Callous Cushions are annular foam pads with adhesive backing, 0.3 cm tall and with an internal diameter of 1.6 cm delimiting 2.0 cm². They adhere readily to leaf surfaces, and the side of the pad opposite the leaf is coated with a soft wax and covered with a square of fine nylon organdy screen, forming a cage enclosing a disk shaped space approximately equal in volume to three stacked nickels.

Wasps were used in experiments within 48 hours after emerging. Harvested females were confined with males for at least a half hour, after which the females were removed for use. Whitefly nymphal densities averaged 31 nymphs/cm² on the melons and 12.5 nymphs/cm² on the cabbage. Individual female wasps were released into the leaf cages and allowed to oviposit. Because of the disparate nymphal densities on the two host plants, we chose to use the absolute number of progeny produced for comparison rather than the more conventional measurement of percent parasitism. Additionally, we allowed a time interval of only 4 hours for wasps to oviposit to ensure they were not host-limited. The experiment was replicated in time. Cages were maintained on the live plants for three weeks, after which leaves with cages were harvested and placed in petri dishes in order to count progeny.

b. Results

For each of the leaf cages, the number of parasitoid progeny that emerged were counted. These data were analyzed using two-way analysis of variance with parasitoid species and host plant species as main effects. The null hypothesis for this analysis was:

H_0 = there is no effect of plant species or wasp species on number of progeny produced. Average number of progeny for the four treatments are given in Figure 4.

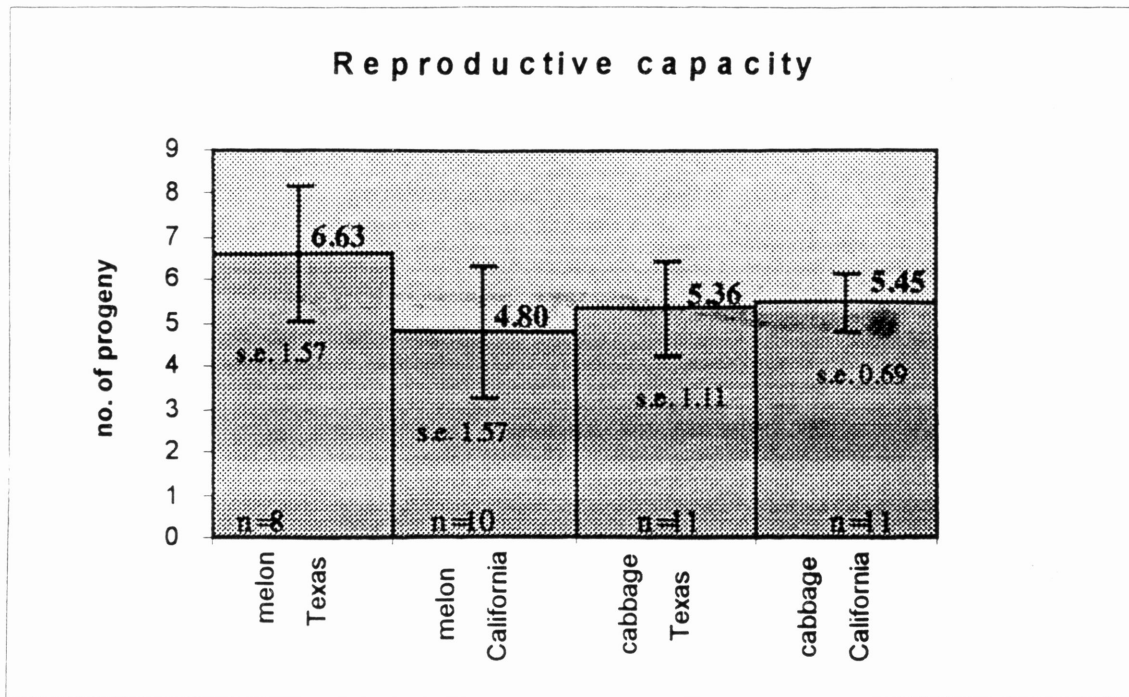


Figure 4. Texas and California wasp progeny on melon and cabbage.

A two-way analysis of variance shows no effect of wasp species or plant species on number progeny produced (Table 1).

Source	DF	sum of squares	mean square	F value	P
wasp species	1	23.34	23.34	1.6	0.2950
plant species	1	4.03	4.03	0.28	0.6352
plant*wasp	1	28.09	28.09	1.93	0.2592

Table 1. Results of ANOVA to determine the effect of wasp species and plant species on progeny produced. There is no significant effect of either wasp species, plant species, or their interaction.

Clearly, we are unable to reject the null hypothesis and conclude that based on our data there is no effect of either wasp species or plant species on number of progeny produced.

However, due to contamination by other parasitoids and problems with plant diseases, the number of utilizable replications for each treatment was low, rendering the conclusion tentative. Further trials would certainly make the conclusion more robust.

c. Conclusions

It seems, then, that the California wasp, when confined in a leaf cage, is able to reproduce on the smooth leaf surfaces of cole crops just as well as the Texas wasp. The difference in the leaf surface morphology of the two plants appears to have no effect on the ability of the wasps to reproduce. This forces us to examine further alternatives to explain the absence of the California wasp on cole crops.

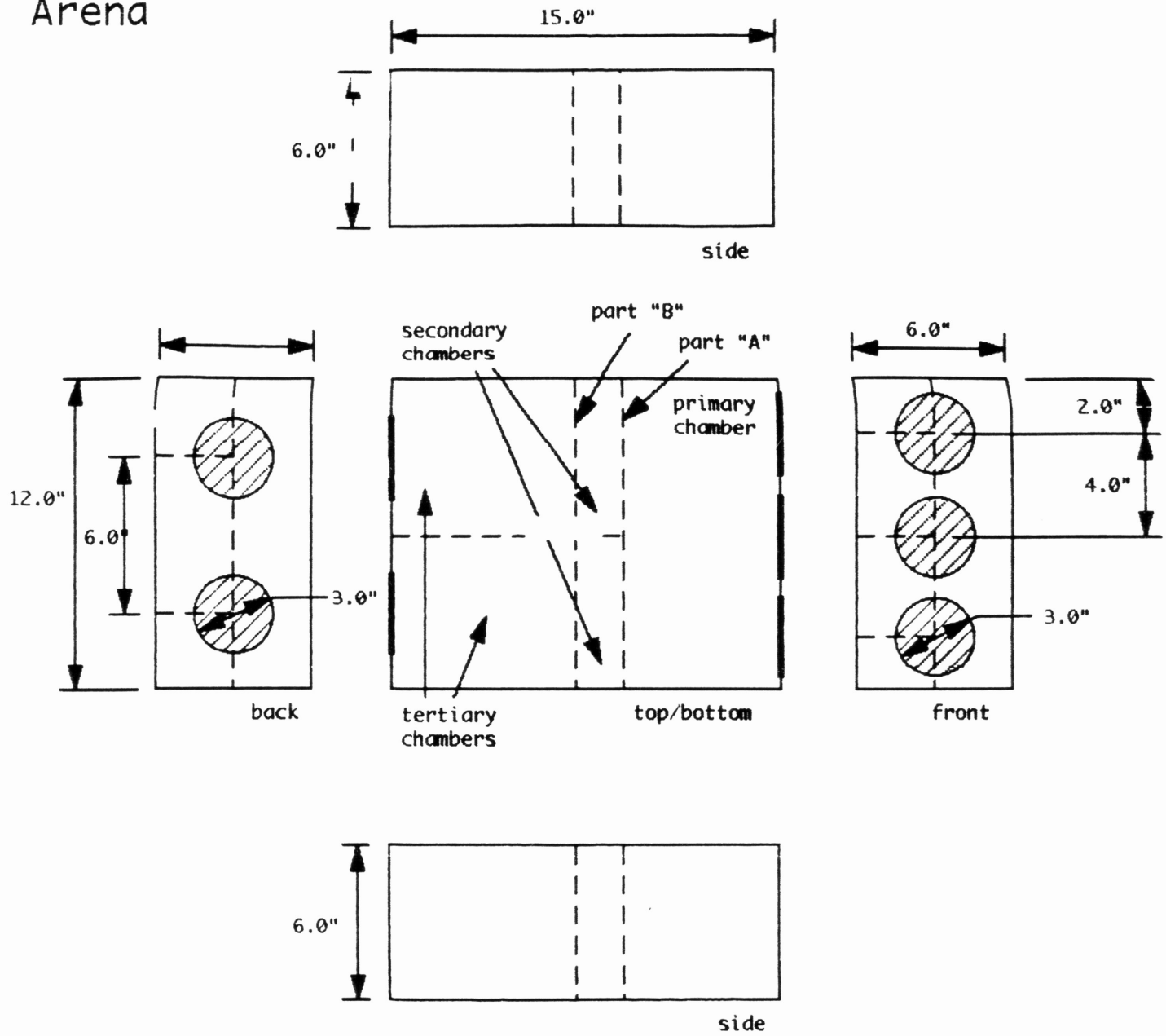
6. Plant preferences

One possibility is that the California *Eretmocerus* may not colonize the cole crop habitat to begin with. When California wasps were released into cages with whole cabbage plants, they produced much less progeny relative to the Texas wasps. If volatiles from cole crops fail to trigger a response from the California wasp, it would never alight on cole crop leaves to begin searching for hosts and ovipositing; hence the field observations of no *Eretmocerus* on cole crops in California. This chain of reasoning led us to investigate whether the wasps would show a preference for one plant or the other in a set of behavioral experiments.

a. Materials and methods

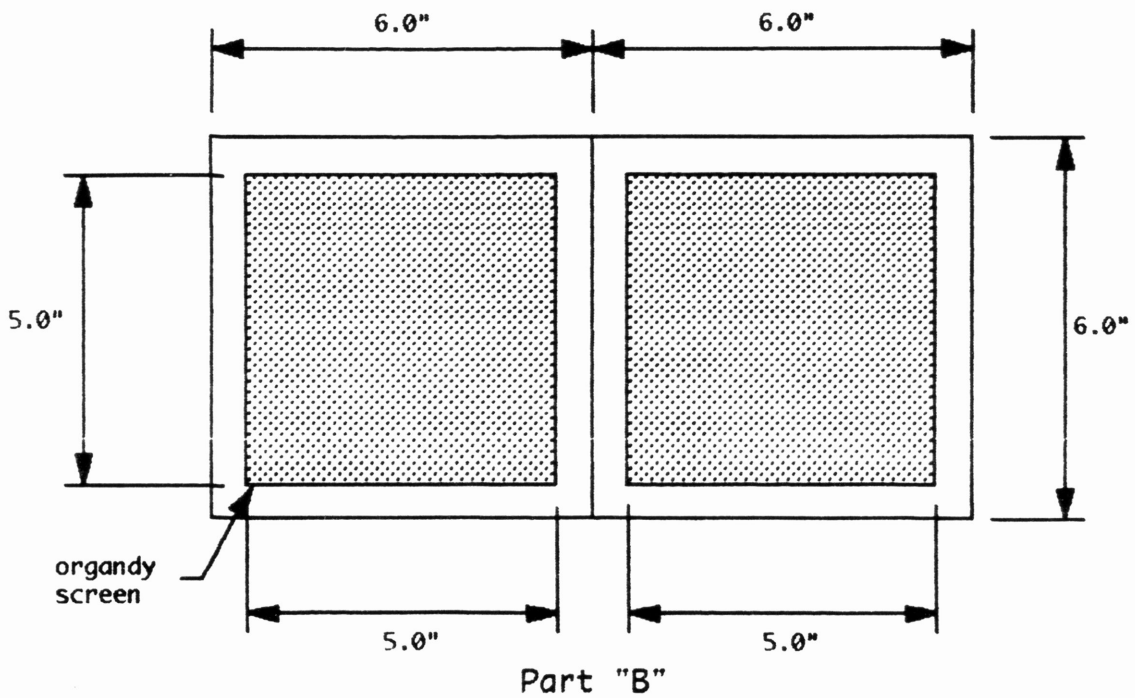
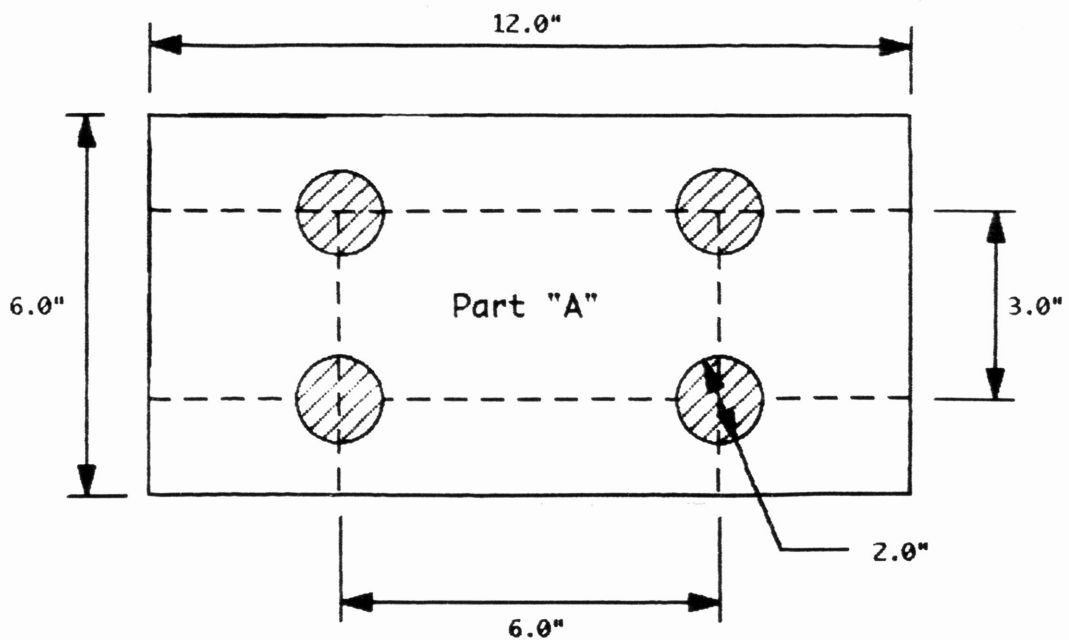
A plexiglas arena was constructed to expose female wasps to odor plumes from two plants. The arena was so constructed that the wasps, released into a primary chamber at one end of the arena, could choose to enter one of two secondary chambers (Figure 5). Each of these secondary chambers had one wall made from organdy which exposed it to air currents from one of two tertiary chambers. Infested plant foliage could be placed in

Experimental Arena



Scale 1:6

Figure 5.

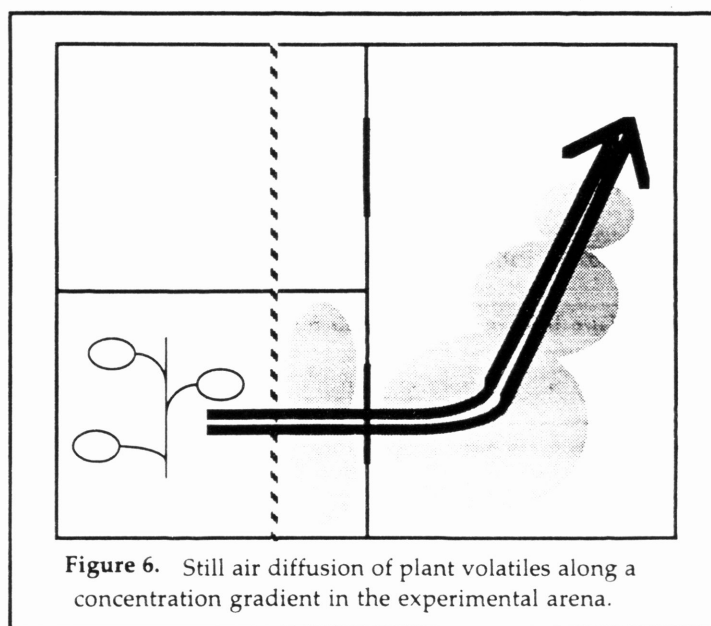


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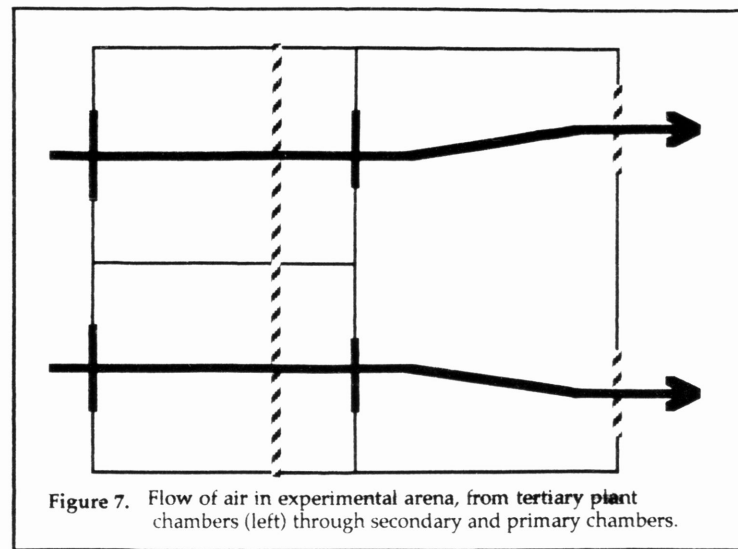
Figure 5 cont'd.

one of the tertiary chambers allowing volatiles to diffuse along a concentration gradient into the secondary chamber and then to the primary chamber. Our thinking was that the wasps, seeking hosts, would travel along the concentration gradient toward the odor source. An insect entering one of the intermediate chambers adjacent to the foliage would be scored positive for that plant type (melon or cabbage).

To verify whether volatiles would in fact diffuse through the arena in the way we expected, we used ventilation smoke tubes (Mine Safety Appliances Co., Pittsburgh, Pennsylvania). These tubes combine ethylenediamine and acetic acid to produce smoke through a non-heat generating process. We used them to observe still air diffusion with all external portals closed off (Figure 6). Despite adequate diffusion through the arena, in

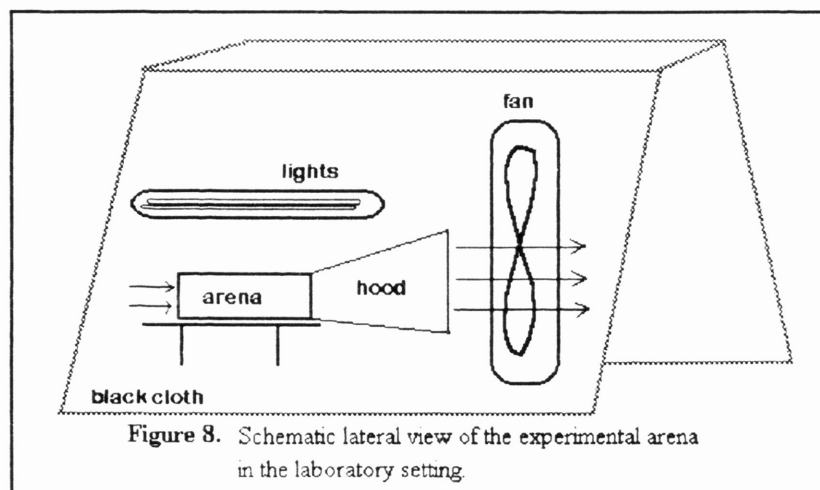


preliminary trials with whitefly infested melon and cabbage leaf material, wasps showed no movement when the air was still. For this reason air flow through the arena was achieved to draw air currents from the open ended tertiary chambers containing plants, through the secondary and primary chambers and out the arena through screened portals in the primary chamber (Figure 7).



The portals were connected by a hood to a fan that pulled air through the arena's chambers. The middle portal at the front end was closed off to air flow to keep the currents of air from each plant separated. Speed of airflow through the arena was adjusted to minimize circulation of peripheral currents within the arena. Airflow greatly increased the wasp's activity.

To minimize the possible effect of light bias on the wasp's choices, a rack of two 4 ft fluorescent light bulbs was hung longitudinally and centered laterally above the arena at a height of two feet. The entire assemblage was cloaked with a dark cloth to prevent outside sources of light from penetrating to the arena (Figure 8). Preliminary trials



without plants to measure possible light bias suggested that light was evenly distributed in the arena.

I performed other preliminary trials with an infested bouquet of hibiscus leaves in one chamber to determine the period of time in which wasps were most active. Wasps were found to be most active for an interval of fifteen minutes after being released into the primary chamber, after which most of them settled. A time interval of fifteen minutes was allowed in the actual experimental trials, after which the insects were scored according to their position in the arena. Twenty female wasps were released for each trial.

In the experimental trials bouquets from whitefly infested melon and cabbage plants with three leaves of approximately 12.5 cm² each were placed into water vials. These were then placed in the tertiary chambers of the arena. Whitefly nymphs were primarily in the first and second instars, with many eggs also present. Nymphal densities averaged about 20 nymphs/cm². Contrary to expectations, relatively few wasps entered the secondary chambers. Because each side of the primary chamber was dominated by the volatiles emanating from the plant on that side, we scored the wasps by recording what side of the arena they were on, the melon side or the cabbage side. Each side consisted of half the primary chamber and the corresponding secondary chamber. We interpreted the wasps' location in the arena (on the melon side or the cabbage side) to express their preference for one plant or the other.

b. Results

The null hypothesis for this experiment was:

H_0 = there is no effect of wasp species or plant species on the wasps' location in the arena.

For each release of 20 female wasps, the number of wasps observable on the side of the arena corresponding to each plant were recorded. No trend was observable in the results (Table 2) to indicate that either of the two wasps were choosing one plant over another.

<u>wasp</u>	<u>mean number of wasps choosing:</u>	
	<u>melon</u>	<u>cabbage</u>
Texas	5.00	4.50
California	7.00	5.50

n = 20 in all treatments

Table 2. Results of plant preference trials in experimental arena.
No trends are apparent.

c. Discussion

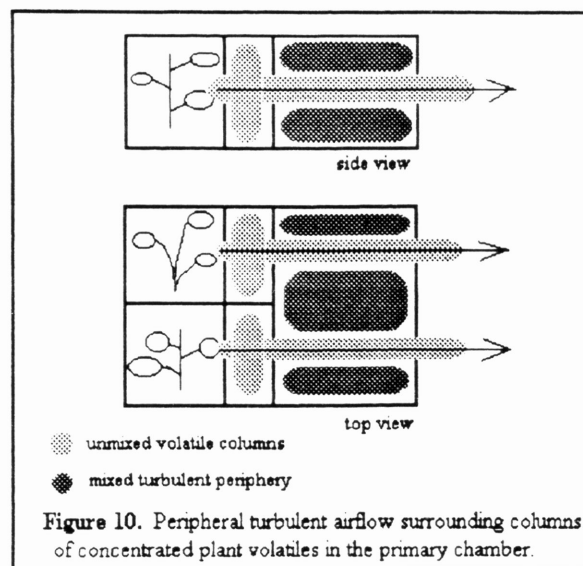
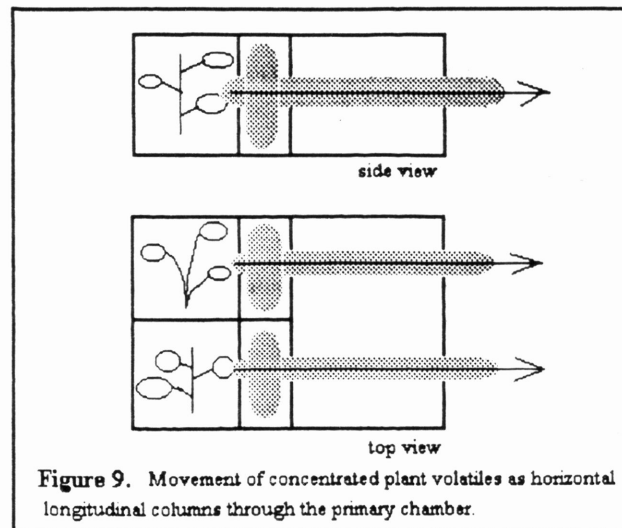
Our results clearly indicate that wasps in our experimental apparatus did not respond to volatiles released by 1st and 2nd instar whitefly nymphs feeding on melon or cabbage plant material. *Interpreting* these results in reference to the wasp's natural history is a bit more difficult. The first part of following section discusses some problems related to the experimental arena that might render it unsuitable for use to study plant preferences. Subsequent to this devil's advocate critique, we will effect a 180° rotation in sympathies and assume the results are in fact representative of the wasp's behavior in nature when exposed to these stimuli. This allows us to contemplate what the results (i.e. no response) might signify for the wasps' behavior in terms of the body of theory on host finding discussed above.

7. Critique

a. Problems with the arena

The arena was originally designed to be used under still air conditions based on simple diffusion of volatiles along a concentration gradient. However, the wasps failed to respond to still air conditions. Using portals on either end of the arena, air flow could be pulled through the arena from the plants in the tertiary chambers through the secondary chambers to the primary chambers where the wasps were released (Figure 7). This

resulted in 1) high concentrations of the corresponding plant volatiles in the secondary chambers, and also in 2) currents with relatively high concentrations of plant volatiles traveling longitudinally in two horizontal columns from the secondary chamber through the primary chamber and out the front portals (Figure 9). Surrounding these two columns of flowing air in the rest of the volume enclosed in the primary chamber were mixed peripheral currents of varying concentrations of volatiles from both plants, moving as vortexes and whorls (Figure 10).



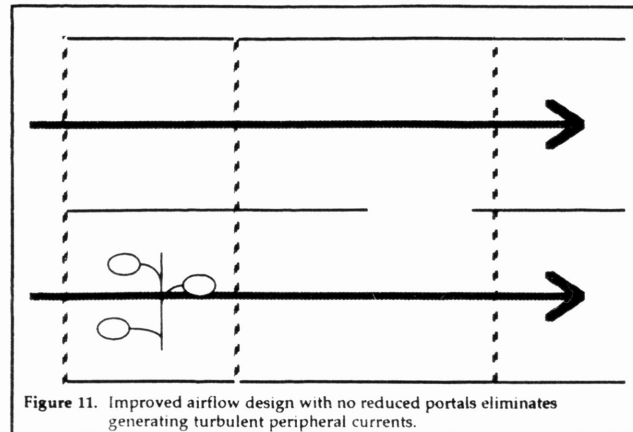
While in each half of the primary chamber there were higher *average* concentrations of volatiles from the corresponding plants, these average concentrations were not distributed evenly through the corresponding half of the primary chamber. In particular, the air moving around the periphery of the two horizontal columns of air was of mixed and varying concentrations, presenting the wasps with multiple and changing stimuli.

Furthermore, the two columns of relatively pure concentrations of volatiles in fact occupied only about a third of the total volume of the primary chamber. Most of the actual volume was occupied by mixed peripheral currents. More troubling still, these columns were suspended through the interior portion of the primary chamber whereas the internal surfaces of the primary chamber, on which the wasps spent most of their time, were continuously subject to turbulent, mixed peripheral currents (Figure 9). The wasps were released from an upright glass test tube in the center of the primary chamber. They left the test tube through the following three modes of travel, ranked in order of descending frequency: 1) jumping, 2) walking, 3) flying. After leaving the test tube they spent almost all their time walking on the chamber's internal surfaces (walls, ceiling, floor) interspersed with infrequent jumps or flight. Hence, some 99% of the wasp's residence time in the primary chamber was on the walls in areas of turbulent peripheral currents, conceivably prohibiting the wasps from making a choice based on concentration gradients of plant volatiles.

Additionally, we do not know whether these wasps behave differently when flying as opposed to walking and jumping. In further experiments it would be advisable to encourage the wasps to leave the release receptacle in a uniform mode of travel. Flying could be encouraged by a strip of honey just below the rim on the exterior surface of the release test tube, preventing the wasps from simply walking down the outside of the test tube. Walking could be encouraged by releasing the wasps from a test tube lying flat on the floor of the primary chamber. Jumping might conceivably be encouraged by using a

test tube with a ring of honey and imposing moving shadows and/or flashes of light on the wasps.

An experimental arena similar in principle to the one used but simpler, designed to work with airflow and to minimize peripheral turbulence, might look like Figure 11. The



cross sectional areas through which air flows are all equal in shape and area, eliminating the turbulence caused by forcing air flow through the smaller cross sectional area delimited by the portals. The intermediate chambers are eliminated so that the wasps have a single cavity in which to roam. Dividers in the primary chamber help keep currents from the two plant chambers distinct while permitting the wasps to travel from side of the primary chamber to the other.

b. Host finding theory applied

In this section we assume that despite the problems mentioned above, the average concentration of volatiles from the two plants in the primary chambers gave the wasps sufficient sensory stimulus to express a preference for one plant or the other. That is, the results we observed would parallel the behavior of these *Eretmocerus* wasps in nature when exposed to the same stimuli. The failure of wasps to respond to volatiles from the whitefly infested plant material could be interpreted in a number of ways.

It could be simply that the right stimuli were absent. Perhaps the wasps respond to volatiles from floral tissue rather than leaf tissue. The wasps could respond exclusively to third or fourth instar whitefly nymphs, which were virtually absent from the experimental leaf bouquets. Perhaps the wasps do not respond to nymphs at all, but only to adults and the chemicals they release: behavioral pheromones or, for example, scents released from ovipositing females. If any of these stimuli is required by the wasps in combination with the materials present, there would be no experimental response. The issue of whether these wasps respond differently to different life stages would be particularly easy to determine experimentally, and would increase our knowledge of the wasp's behavior.

Another unknown is whether the chemical cues that serve to attract the wasps are subject to sensitization or habituation by the wasps. Our results do not suggest sensitization, but given that in our arena the wasps are *constantly* exposed to volatiles from infested leaves, it is possible that wasps were habituated to the volatiles present and that is why they failed to respond. Of course, it is also possible that *Eretmocerus* become neither sensitized or habituated to these stimuli.

Whether the infested plant volatiles act as attractants or arrestants would be another fit subject for further study. Conceivably, the chemical cues provided function not as attractants along whose concentration gradient the wasps travel, but as arrestment cues that signal the wasp to stop and initiate a systematic search. While no overtly systematic search patterns were observed during the trials, the plexiglas substrate may not stimulate a natural searching behavior, and it is possible that further observations and/or manipulations could uncover them.

These factors could also interact. A less volatile arrestment cue could signal the wasp to initiate a systematic search, but the simultaneous presence of more volatile attractants at unnaturally high concentrations in the confined space of the arena could confuse the wasp and generate erratic behavior. Subsequent sensitization or partial sensitization to the attractants after a given period of time (e.g. about 15 minutes) could

trigger two overlapping behavioral responses. All of these issues merit further study to understand the foraging behavior of *Eretmocerus* wasps, and why they are likely to settle on some crops but not others.

8. Summary

My research sought to uncover why two morphologically very similar species of *Eretmocerus* wasps performed so differently in the field on cole crops. I measured the wasps' reproductive capacity on the different host plants to see if the California wasp produced lower numbers of progeny when confined to a leaf cage on cabbage, relative to melon and also to the Texas wasp on both crops. As no difference in reproduction was found, we sought to explain the field observation by testing the wasps for plant preference in behavioral experiments, thinking that cole crops may fail to elicit a response from the California wasps searching for hosts. The wasps expressed no preference, but it is unclear whether this was an artifact of the arena or whether the cues we provided the wasps in the laboratory simply did not fully correspond to the complex of clues used in nature by the wasps to locate their hosts. Further study on the nature of the cues used by these wasps to locate hosts would permit us to interpret our results more meaningfully.

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Congratulations to Molly. Get maternal, baby.

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