110
711
THS

THESIS

MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 10625 7037

This is to certify that the

thesis entitled

ROLE OF *ALLIUM* VISUAL AND CHEMICAL STIMULI IN

THE OVIPOSITION BEHAVIOR OF *DELIA ANTIQUA* (MEIGEN)

presented by

Marion Olney Harris

has been accepted towards fulfillment
of the requirements for

M.S degree in Entomology

James R. Miller
Major professor

Date 20 May 1982

O-7639

LIBRARY
Michigan State
University

MICHIGAN STATE UNIVERSITY

OVERDUE FINES:
25¢ per day per item

RETURNING LIBRARY MATERIALS:
Place in book return to remove charge from circulation records

[illegible]
~~FEB 2 5 '87~~
JAN 0 8 '88

ROLE OF *ALLIUM* VISUAL AND CHEMICAL STIMULI IN THE OVIPOSITION BEHAVIOR OF *DELIA ANTIQUA* (MEIGEN)

G 117637

by

Marion Olney Harris

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1982

ABSTRACT

ROLE OF *ALLIUM* VISUAL AND CHEMICAL STIMULI IN THE OVIPOSITION BEHAVIOR OF *DELIA ANTIQUA* (MEIGEN)

by

Marion Olney Harris

Stimuli emanating from onion stems play an important role in releasing *Delia antiqua* oviposition behavior. Surrogate stems were used to assess the role of non-chemical stimuli from stems. In the presence and absence of chemical stimuli, flies laid more eggs on yellow stems, thereby indicating a role for stem hue in oviposition behavior. When visual, structural and chemical stimuli were presented separately and in various combinations, combinations of all three stimuli had a synergistic effect on oviposition.

While non-propyl disulfides of garlic and propyl disulfides of onion elicit virtually identical EAG responses, their effects on oviposition are dissimilar. At concentrations where dipropyl disulfide is stimulatory, diallyl disulfide does not increase or decrease oviposition on a chemically neutral visual stimulus. Response to garlic chemicals is, however, synergized by the same factors which synergize onion chemicals. Larval feeding studies indicated that oviposition preferences reflect the suitability of the host for larval survival.

To my grandmother and grandfather,
Marion and Stoddard Stevens

ACKNOWLEDGEMENTS

My sincere thanks go to my major professor, Jim Miller, for his munificent and unstinting enthusiasm, for allowing me to pursue some of my wilder ideas, and for letting me learn from my own mistakes. I also am deeply indebted to Ring Cardé, who jumped ship as I was finishing my Master's Degree, but whose comments and advice were critical to my understanding of insect behavior. His incisive mind will be sorely missed. My committee members, Edward Grafius, Fumio Matsumura and Guy Bush also gave much useful advice.

My thanks also go to the proletariat of the lab. Joan Harlin put in countless hours doing work of the most tedious sort, listened to my endless complaints, and kept me amused during gargantuan field experiments. John Behm converted scribbled diagrams of experimental materials into objects of art. Beatrice Gloria helped on all fronts, both skilled and unskilled, and did so with a competence and cheerfulness that made life in the lab far more pleasant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

The onion fly, *Delia antiqua* (Meigen), is a muscoid fly in the family Anthomyiidae. The members of this family, which consists of over 550 North American species, are quite diverse in their feeding habits, some being dung breeders, others phytophagous, and a small number, aquatic and predaceous (Borror and DeLong 1971). The best known members of the Anthomyiidae are, of course, the black sheep of the family, pest species which attack numerous commercial crops and cause considerable damage. With few exceptions, these pest species fall into the genus *Delia* (revised from *Hylemya* by Hennig in 1974) and vary in their feeding habits. Some, such as the seedcorn fly, *D. platura* (Meigen), are polyphagous while others are specialists on groups of related plants, such as *D. antiqua* on the *Allium* species and the cabbage fly, *D. brassicae* (Wiedemann), on the Cruciferae.

Virtually nothing is known about *D. antiqua*'s existence outside of home gardens and commercial *Allium* fields. Inasmuch as it is an underground feeder, this lack of information may be due to its nonapparency; however, in the Netherlands where the sterile insect control technique is being used, an effort was made to determine the significance of wild hosts in maintaining *D. antiqua* populations. Still, no wild hosts were found (Loosjes 1976). *A. vineale*, which is perhaps the most common of the wild *Allium* species, was apparently not visited by onion flies and was not a suitable host, inasmuch as larvae placed on bulbs in the lab could not penetrate the outer scales of the bulb and therefore perished (Wolff 1973). Loosjes (1976) has reviewed the literature on the biology of *D. antiqua* and its association with the onion, *A. cepa*.

While remarkably little is known about *D. antiqua* pre-oviposition behavior, there is some indication that *A. cepa*'s influence begins well before oviposition occurs. When flies were released outside of onion fields, Loosjes (1976) found that whereas females recaptured in onion fields had been mated within 5 to 9 days after emergence, mating was significantly delayed in females recaptured outside of onion fields. The observation was confirmed by Ticheler (1971) who worked extensively with lab cultures of *D. antiqua*. Whether onions primarily affect the behavior of the fly or its physiology, and subsequently its behavior, is not known.

Host plant odors might affect the physiology of *D. antiqua* in several ways. Since *D. antiqua* females will only mate when the ovaries are well developed (Ticheler 1970 as cited by Loosjes 1976), it is possible that in the absence of host plant odors, ovaries do not develop and that females therefore do not become receptive to males. It is also possible that the absence of odors interferes with mating by inhibiting the release of female sex pheromone. Stimulation of pheromone release by plant odors has been reported to occur in several phytophagous insects (Riddiford and Williams 1967, Renwick and Vite 1972, Rahn 1969). While there is no reported evidence that pheromones play a role in D. antiqua mating behavior (which is rarely observed and not well understood), cuticular hydrocarbons have been implicated as sex pheromones in many Diptera, including those in the closely related Muscidae (Howard and Blomquist 1982).

Allium fields might also affect the pre-oviposition behavior of D. antiqua by serving as aggregation and mating sites for males and females. Loosjes (1976) found that 70% of the D. antiqua immigrants in an onion field arrived before the average age of mating, and that during the period when mating normally occurs, migration of males and females was reduced. Within onion fields, both male and female flies are highly responsive to synthetic onion volatiles (Vernon et al. 1981) and chopped onion (Dindonis and Miller 1980b). The use of host plants as mating sites has been well documented in the family Tephritidae (Prokopy 1977).

There is one other generally ignored, but extremely important pre-oviposition behavior. Before laying eggs, females must locate and feed on protein sources (McLeod 1964). Where females find these exogenous sources of protein is a mystery. Flies have occasionally been observed feeding on flowers, grasses, bird excrement and rotting onions (Loosjes 1976, Dindonis and Miller 1980b, Kästner 1929) and accumulate in large numbers in traps baited with enzymatic yeast hydrolysate (Miller and Haarer 1981). Given the paucity of concentrated protein sources in nature, it might be expected that if D.

antiqua were to possess a finely tuned and chemically mediated host-finding behavior, it would occur here.

Much work has been done on the role of secondary plant chemicals in the oviposition behavior of D. antiqua. In 1968, Matsumoto and Thorsteinson demonstrated that dipropyl disulfide and propanethiol, two major volatile components of onion, released oviposition behavior when applied to moist sand. Since then a number of alkyl sulfides and thiols, as well as their analogues have been assayed for activity as oviposition stimulants. The results of these studies prompted two groups (Vernon et al. 1978, Ishikawa et al. 1978) to independently conclude that oviposition stimulants must contain a sulfur atom possessing two unshared electron pairs and bonded to a saturated hydrocarbon chain three to five carbons long. In addition to the stimulants emitted by onions, several microbially produced synergists of the alkyl sulfides have been discovered, including ethyl acetate and tetramethyl pyrazine (Ikeshoji et al. 1980).

In much of the early work done on insect-plant relationships, it was implicitly assumed that the presence of a few key stimulants (Dethier 1954) or the absence of deterrents (Jermy 1958, Thorsteinson 1960) would indicate to the herbivore whether or not the site was suitable for oviposition or feeding. However, recent work has indicated that the chemical information used during host finding is considerably more diverse, and that insects respond to a summation of inputs from receptors sensitive to a range of plant constituents (Dethier 1971). Furthermore, Dethier (1982) has pointed out that if an insect is exposed to a stimulus and does not overtly "behave", the absence of a particular behavior does not negate the possibility that the stimulus has been perceived and that the information is being used in some manner.

This expanded view of insect perceptual capabilities allows one to ask further questions about the types and diversity of information used by *D. antiqua* when finding and accepting a host plant. Instead of using only the so-called "stimulatory" chemicals, might not *D. antiqua* be sensitive to the non-stimulatory volatile disulfides as well and base host acceptance on the ratio of positive to negative factors emitted by the plant? Furthermore, might not *D. antiqua* use other sensory modalities to supplement information provided by chemical stimuli? Onions differ from surrounding vegetation not only in chemical characteristics, but also in their structural and visual characteristics. Thus, while *D. antiqua* may be capable of finding submerged unsprouted onion bulbs for oviposition (Carruthers 1979), the redundancy which results from the use of several sensory modalities might facilitate rapid and accurate location of larval food resources. Visual and structural stimuli have been shown to be of consequence in the oviposition behavior of other *Delia* species (Sömme and Rygg 1972, Zohren 1968).

In the following two chapters, the role of chemical and visual stimuli in the oviposition behavior of _D_. _antiqua_ is explored. In Chapter 1, I have examined oviposition behavior to determine whether visual and structural stimuli emitted by the host plant significantly modulate responses to secondary plant chemicals. In Chapter 2, I have addressed the question of whether _D_. _antiqua_ is capable of perceiving volatile disulfides which do not fit the specifications for stimulatory disulfides, and whether the non-stimulatory disulfides constitute positive, negative or neutral oviposition stimuli.

CHAPTER 1

Synergism of Visual and Chemical Stimuli in the Oviposition Behavior of *Delia antiqua*

INTRODUCTION

The oviposition preferences of the onion fly, D. antiqua, generally follow plant taxonomic lines, with Allium species being preferred among plant genera, and the onion, A. cepa, being preferred among the Allium species. Within A. cepa, plants in certain developmental or physiological states are more commonly oviposited on than others. Within an onion field, large numbers of eggs are found on onions which are rotting (Loosjes 1976), nematode and larval-infested (Loosjes 1976, Workman 1958, Armstrong 1924) or diseased (Crête 1972). In the spring, volunteer onions and onions which have been sown early receive a disproportionate number of eggs (Gray 1924, Sleesman 1934, Treherne and Ruhmann 1922).

The proximate behaviors leading to increased oviposition on these particular onions and the stimuli which elicit these behaviors are not well understood. Some of the behaviors are undoubtably mediated by secondary plant chemicals. Loosjes (1976) found that dispersal patterns of onion flies from emergence sites to neighboring fields could be explained by random movement. Once flies had entered a field, their rate of movement slowed 2.5 fold, suggesting that flies accumulate in onion fields because of inverse klino- or orthokinetic rather than anemotactic responses to onion odors emanating from fields. Within fields, onion flies have been observed orienting anemotactically when stimulated by volatiles from chopped onion

(Dindonis and Miller 1980b, Kästner 1929); however, in both cases it was not entirely clear whether the onion odors were signalling the presence of an oviposition, feeding or mating site. Interpretation of the biological significance of results from field experiments comparing the number of flies caught in traps baited with various sulfur and non-sulfur containing chemicals (Loosjes 1976, Vernon et al. 1981, Dindonis and Miller 1980ab, 1981ab) is also difficult, being confounded by our lack of understanding of the mating and feeding behavior of adult flies. Matsumoto and Thorsteinson (1968) did report that more females landed on screens and inserted their ovipositors through mesh above dishes containing dipropyl disulfide and propanethiol. Thus, the sulfur-containing chemicals can mediate both pre- and post-alighting behaviors.

Other workers have compared D. antiqua oviposition preferences without determining whether the presented stimuli exerted their effects on pre- or post-alighting behaviors. Hough et al. (1981) showed that onion flies laid many more eggs on nonsterile seedlings than on sterile seedlings. They suggested, based on the work of King and Coley-Smith (1969), that bacteria in the soil convert sulfoxides, given off by onion roots, into the volatile sulfides which elicit oviposition. Based on extensive work analyzing volatiles from fresh onions and oviposition experiments on onion volatiles and their analogues, Ishikawa et al. (1978) and Vernon et al.

(1978) hypothesized that chemicals eliciting oviposition must contain a single sulfur atom having two unshared pairs of electrons, and bonded to a saturated hydrocarbon chain three to five carbons long. Ikeshoji et al. (1980), noting increased oviposition on larval-infested onions in the field, investigated the chemical basis for this preference and showed that microbially produced volatiles, ethyl acetate and tetramethyl pyrazine, significantly increased oviposition when presented with dipropyl disulfide. However, traps baited with these oviposition synergists in combination with dipropyl disulfide did not increase trap catch relative to dipropyl disulfide (Harris et al. unpublished). The two synergists may therefore play a role in post-alighting behaviors either by causing more females to remain and oviposit on a treatment, or by causing single females to deposit more eggs than they would on treatments containing dipropyl disulfide alone. Since none of the identified oviposition stimulants elicit a response equivalent to an onion slice (Pierce et al. 1978), it might be suggested that oviposition in D. antiqua is mediated by a complex volatile mixture rather than any single "key" volatile. The importance of complex odors in insect-plant interactions has been noted in other behavioral studies (Tichenor and Seigler 1980, Staedler and Hanson 1978) and is supported by electrophysiological studies (Staedler 1976, Dethier 1980).

While most of the behavioral work on D. antiqua has concentrated on chemical stimuli, there are indications that visual and structural stimuli play a role in releasing oviposition behavior. Gray (1924) found that cull onions planted so that leaves branched below the soil surface received more eggs than cull onions having a pronounced neck and foliage branching well above the soil surface. Though it is possible that chemical stimuli emanating from the differently planted culls differed, it is more likely that flies were responding to differences in visual and structural stimuli. Onion flies also seem to prefer plants which are neither extremely small (Workman 1958) nor plants which are healthy and fully developed (Perron 1972). Once again, this preference could be mediated by chemicals rather than plant size; however, Hough (1981) found that females laid more eggs on taller objects (toothpicks and aquarium reed) even without the presence of chemical stimuli. From experiments with onions in mono- and mixed cultures, Müller (1969) suggested that, while long-range orientation by D. antiqua is mediated by olfactory stimuli, close-range orientation is mediated by plant shape and brightness and might be hindered by concealing onion plants among other plants.

The present work addresses the role of structural and visual stimuli in the oviposition behavior of the onion fly. While no attempt was made to determine whether the stimuli being examined affected pre- or post-alighting behaviors, I have established the importance of above-ground stimuli and describe some of the visual characteristics of the onion plant which are important in eliciting oviposition behavior.

MATERIALS AND METHODS

Small sprouted onions (variety Abundance), having bulbs 2 to 3 cm in diameter and stems 5 to 7 cm long, were cut into two parts, stem and bulb. The bulb was submerged in a cup 8 cm in diameter and 5 cm deep, filled with 200 ml of washed silica sand and 50 ml of distilled water. The stem was placed in a second cup so that it rose vertically from the sand and presented a visual stimulus similar to sprouted cull onions in the field. A third cup, containing only sand and water, served as a control. The surface of every dish of sand used here and in the following experiments was imprinted with 20 holes, 5 mm deep and 2 mm wide, which are thought to facilitate oviposition (G. Ritcey, University of Guelph).

All treatments containing chopped onion were prepared as follows. A layer of sand (15 ml), 5 ml of chopped onion and a top layer of 80 ml of sand were sequentially added to plastic dishes (8 x 8 x 2.5 cm). Dishes moistened with 20 ml of distilled water were placed in a white styrofoam frame (9 x 9 x 3.5 cm). To stabilize sand moisture, the styrofoam frame was placed on top of another plastic dish containing 25 ml of distilled water. Contact between the sand in the upper dish and the water reservoir was maintained by a filter paper strip

(7 x 1 cm) inserted through a 2 cm slit in the bottom of the upper dish.

Treatments containing synthetic chemicals were prepared similarly. Dipropyl disulfide (Eastman Kodak, Rochester, NY, 98% pure by GLC) and ethyl acetate (Aldrich Chemical Co. Inc., Milwaukee, Wis., 99% pure by GLC) were released from Beem polyethylene embedding capsules (Pelco Electron Microscopy Supplies, Ted Pella Co., Tustin, CA.) submerged under 1 cm of sand in the center of the oviposition dish.

Eight cm sections, cut from 5 to 8 mm diameter onion stems, were used as above-ground stimuli in experiments comparing oviposition on real vs. surrogate stems. Surrogate stems consisted of 5 mm ID Pyrex™ glass tubes cut in 8 cm sections and heat sealed to a tapered end. The open end of the tube was sealed with a cork during experiments. When called for, 7 x 0.5 cm papers were placed inside glass tubes to provide color or brightness stimuli. Red, orange, yellow, yellow-green and blue silkscreened papers (Geller Artists, New York, NY) and neutral scale (gray) papers (N 0.5 to N 9.5, Munsell Color, Baltimore, Maryland) were printed on one side. Hence, two sheets of each color were glued back to back and then cut to size. Reflectance spectra (400 to 700 nm) of all papers used as visual stimuli in these experiments were recorded on a Bausch and Lomb 505 spectrophotometer using a polychromatic detector and magnesium carbonate as the standard. In each oviposition dish two 6.5 cm tall stems, either onion or surrogate, were placed 5 cm apart on the diagonal.

In experiments using onion as a chemical stimulus, treatments were placed in cages immediately upon preparation; however, in experiments using synthetic chemicals, treatments were first allowed to age for 8 hours. The design of all experiments was randomized complete block per cage per sampling time. Rather than completing all blocks of a given experiment and then moving on to another experiment, one block of each experiment was completed in random order and this cycle repeated over time. This precaution was taken to avoid training effects possible with flies (Roitberg and Propoky, unpublished) repeatedly exposed to the same treatments. During these experiments (July through October, 1981), cages containing several hundred flies were placed either outdoors or in the greenhouse, depending on the weather. Parental stock for the culture of flies used in the experiments was obtained from Dr. Freeman McEwen, University of Guelph, Ontario. When used in these experiments, flies were 8 to 10 generations removed from the field. Food was retained in cages during all experiments and consisted of honey, water and the artificial diet of Ticheler (1971). Treatments were generally removed after 24 hours; however, since very few eggs were laid during the first 2 days of the experiment with the range of colors presented without chemical stimuli, these treatments were kept in cages for 3 to 4 days. Eggs were collected from the sand by flotation, counted, and recorded as a percent of the total number of eggs laid in the block. Sand, onion, corks, and capsules containing chemicals were discarded, and surrogate stems were washed, rinsed with acetone and placed in a drying oven for 24 hours before being reused.

Data generated by these experiments did not fulfill the assumption of normality either with or without transformations. Therefore, results were analyzed using the nonparametric equivalent of the F test, Kruskal-Wallis one way analysis of variance by ranks (Siegel 1956).

RESULTS

Stems vs. Bulbs

Initial experiments comparing oviposition on stems vs. bulbs included an intact onion plant (i.e. stem and bulb together); however, as almost all eggs (ca. 80 to 90%) were laid on this treatment, we removed it subsequently, to force females to oviposit either on onion stems or submerged bulbs. When given only these treatments and a control, flies laid far more eggs around the stems (Table 1). While the results of this experiment did not indicate whether the stem's chemical or visual stimuli played the major role in eliciting oviposition, work by Pierce et al. (1978) discounted the possibility that the stem's chemical stimuli were solely responsible. Pentane extracts of onion bulbs elicited 16 times more oviposition than did extracts of either onion stems of leaves.

Surrogates vs. Onion Stems

The importance of the interaction between the stem's chemical and visual/structural cues was investigated by fashioning a surrogate stem and comparing oviposition around

TABLE 1. Relative importance of above-ground *vs.* below-ground stimuli for oviposition by the onion fly[1].

Treatment	Mean[2] % eggs laid/treatment[3]	
bulb submerged in sand	5.3	b
stem standing in sand	94.7	a
sand control	0.0	c

[1] Three replicates

[2] Means followed by the same letter are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 786.

it with oviposition around an onion stem of similar shape and size, both with and without chopped onion as a below-ground chemical stimulus. While there was great variability in egg counts from block to block (Table 2), there was no significant difference between onion stems and surrogate stems as long as both were presented along with chopped onion in the sand. When no chopped onion was placed beneath the sand, onion stems (which provided their own chemical stimuli) received significantly more eggs than did surrogate stems. It cannot be assumed from these results that olfactory or gustatory stimuli from onion stems do not play a role in oviposition behavior of _D_. _antiqua_. Color stimuli from onion stems and surrogate stems were not identical inasmuch as surrogates contained yellow rather than green papers. Since yellow is more stimulatory than green to many herbivores, it may have somehow compensated for the lack of appropriate chemical stimuli in the surrogate stem.

Range of Colors

Surrogate stems made it possible to manipulate color stimuli while holding odor and structural stimuli constant. The question of whether vertical objects of all colors were equally stimulatory was addressed by placing red, orange, yellow, green, blue, black and white papers in glass tubes and assaying them alongside clear tubes and dishes containing no tubes. When the range of colors was presented along with chopped onion beneath the sand (Figure 1c) significantly more eggs were laid in treatments having yellow stems. Green was

TABLE 2. Relative effectiveness of onion stems vs. surrogate stems both with and without onion odors in stimulating oviposition by the onion fly[1].

	Mean[2] % eggs laid/treatment[3]			
	Below-ground stimulus			
Above-ground stimulus	Sand		Sand + Onion	
onion stem	16.9	b	43.3	a
surrogate stem	1.0	c	36.7	a
no stem	0.0	d	2.1	c

[1] Five replicates

[2] Means followed by the same letter are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 6185.

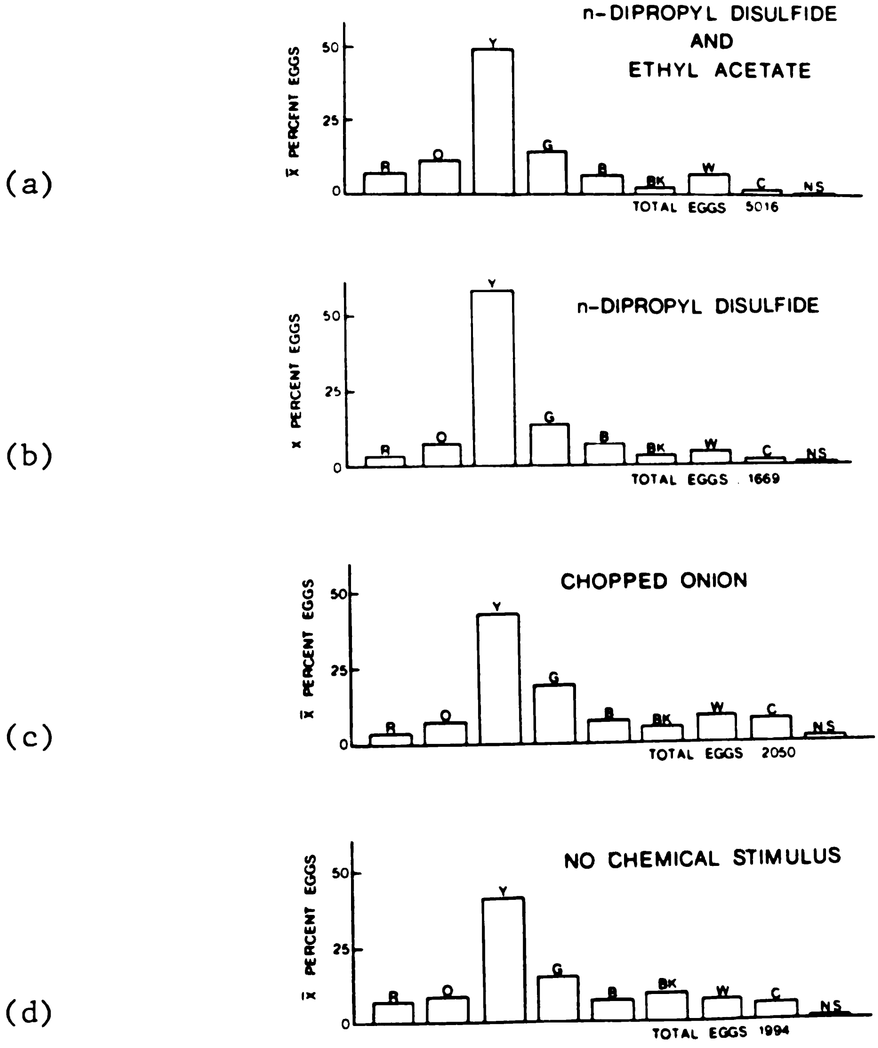

FIGURE 1. Influence of stem hue (color) on oviposition of *Delia antiqua* (Meigen) given various chemical stimuli (noted above). (R=red, O=orange, Y=yellow, G=green, B=blue, BK=black, W=white, C=clear, NS=no stems). Data from 4 replicates are expressed as mean % eggs laid/treatment.

less stimulatory than yellow, but received significantly more eggs than the remainder of the treatments. When either dipropyl disulfide or dipropyl disulfide and ethyl acetate were used, results were very similar (Figure 1a and 1b). When the range of colors was run with no chemical stimulus (i.e. plain sand), essentially no oviposition occurred during the first two days of the experiment; however, on the third and fourth days, when the threshold for release of oviposition behavior had probably been reduced, flies laid significantly more eggs on yellow stems (Figure 1d). In this experiment, oviposition on green stems did not differ statistically from the remaining stems, and the variability of oviposition response to all treatments was much greater than in previous experiments.

Neutrals vs. Yellow Stems

Wavelength discrimination (color vision) is not the only explanation for the oviposition preferences heretofore seen. Spectral reflectance of the colored papers used in the experiments (Figure 2) show that while all colors have a characteristic pattern of reflectance at certain wavelengths (hue), they can also be distinguished by how much light they reflect (brightness or value). Of the colors presented, yellow does not reflect nearly as much light as does white, but reflects far more than does red, orange, green, blue or black. It is possible, therefore, that the onion fly is not distinguishing between hues but is stimulated to oviposit by gray vertical objects which reflect as much light as the yellow used in our experiments. This hypothesis was tested by comparing

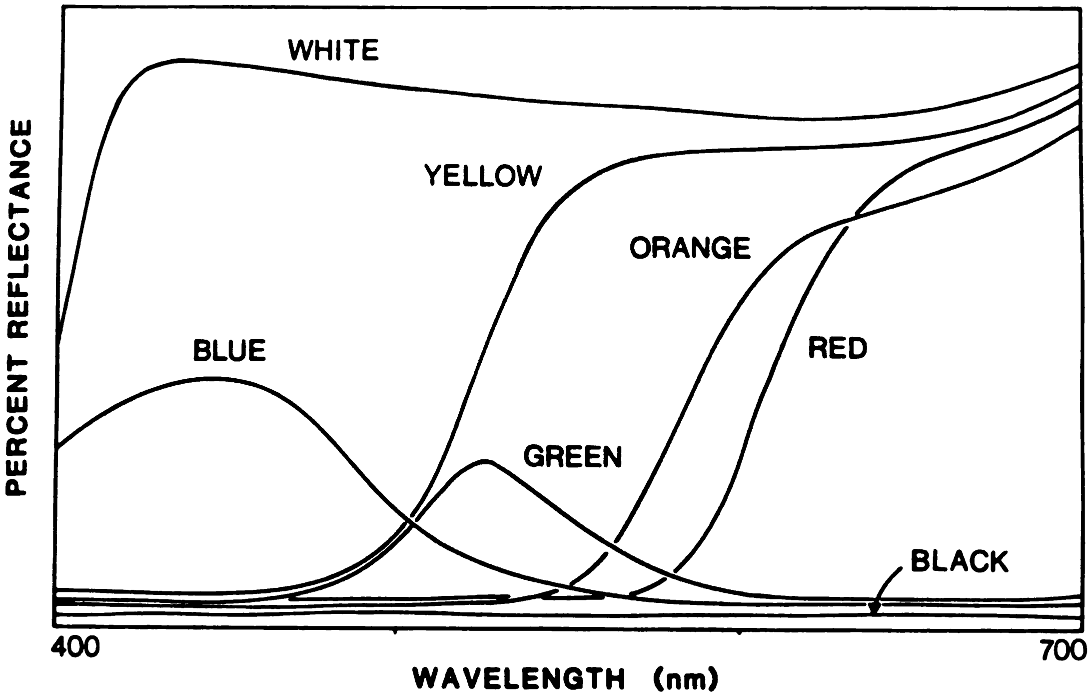

FIGURE 2. Spectral reflectance curves of colored papers used in onion fly oviposition experiments. Reflectance spectra were recorded on a Bausch and Lomb Spectronic 505.

oviposition in response to yellow, orange, and a series of gray papers (Figure 3), using dipropyl disulfide and ethyl acetate in the first experiment and chopped onion in the second experiment as chemical stimuli. Flies laid significantly more eggs (Figure 4) on yellow stems. Oviposition on the grays, which corresponded to yellow in terms of brightness, did not differ significantly from the darker and lighter grays, indicating that the increased amount of oviposition on yellow stems was due to a preference for a particular hue rather than brightness.

Relative Importance of the Three Stimuli

I tested the relative importance of color, structural and chemical cues both separately and in various combinations. In one experiment, chopped onion was used as the chemical stimulus and six different treatments were presented: sand alone, sand with chopped onion (chemical alone), clear stems in sand (structural alone), clear stems in sand with onion (structural and chemical), yellow stems in sand (structural and visual), and yellow stems in sand with onion (visual, structural and chemical). Combining the three stimuli had a synergistic, rather than additive, effect on oviposition (Table 3). Few eggs were laid on treatments containing only chemical, structural or visual plus structural stimuli. However, the addition of a clear glass tube to a treatment containing only moist sand and chopped onion caused a significant increase in the number of eggs laid (2.8% → 15.8%). This suggested that the onion stem elicits oviposition not only

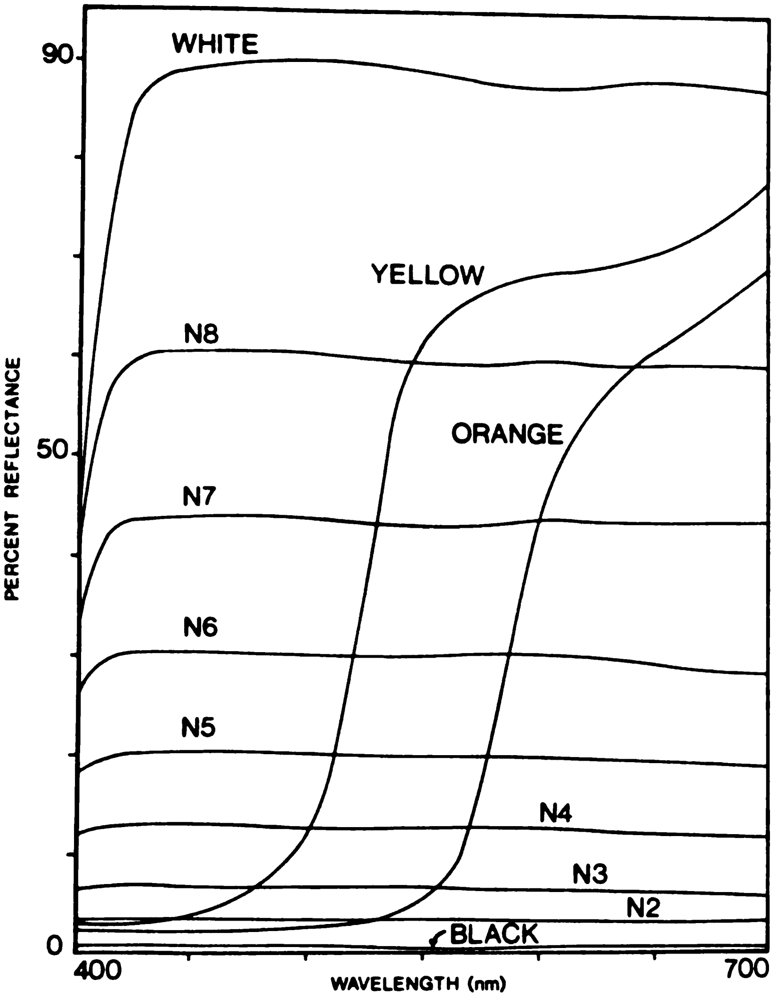

FIGURE 3. Spectral reflectance curves of neutral and colored papers used in onion fly oviposition experiments. Reflectance spectra were recorded on a Bausch and Lomb Spectronic 505.

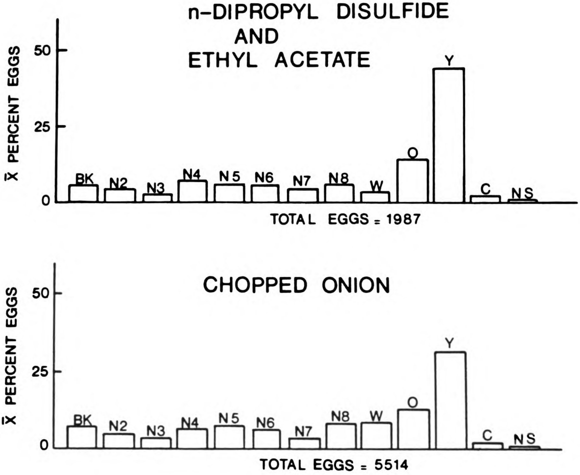

FIGURE 4. Influence of stem brightness on oviposition of *Delia antiqua* (Meigen) given either n-dipropyl disulfide and ethyl acetate or chopped onion as chemical stimuli. (BK=black, N2 - N8 = neutral grays of values 2 to 8, Munsell color notation, W=white, O=orange, Y=yellow, C=clear, NS=no stems). Data from 3 replicates are expressed as mean % of eggs laid/treatment.

TABLE 3. Relative importance of visual/structural stimuli and onion odor for oviposition by the onion fly[1].

	Mean[2] % eggs laid/treatment[3]			
	Chemical stimulus			
Visual/structural stimulus	nothing		chopped onion	
no stems	0.0	d	2.8	c
clear glass stems	0.4	dc	15.8	b
yellow glass stems	2.8	dc	78.1	a

[1] Four replicates

[2] Means followed by the same letter are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 2832.

because it reflects light of stimulatory wavelength but also because it presents a structure which plays a role in pre-oviposition behaviors. Preliminary observations in the lab and in the field indicate that flies almost invariably spend several minutes walking up and down onion and surrogate stems before probing the soil or sand with their ovipositors and eventually laying eggs. Such a stem walk has also been observed during the pre-oviposition behavior of D. brassicae (Zohren 1968). An alternative, perhaps more conservative, explanation for increased oviposition on glass stems should also be considered. Glass stems are not entirely transparent: specular reflection consisting of all wavelengths of light occurs at the outer surface of the glass (Snodderly 1979). This reflection could impart visually discernable qualities to an apparently transparent object. If this were the case, oviposition on glass stems might have occurred because of visual rather than structural characteristics.

A comparison of the relative importance of color, structure and chemical stimuli was also run using dipropyl disulfide, the major volatile component of onion, and ethyl acetate, a microbially produced synergist of oviposition. Again, the combination of color, structural and chemical stimuli elicited the most oviposition (Table 4). The best oviposition stimuli reported in the literature, dipropyl disulfide and ethyl acetate (Ikeshoji et al. 1980) performed poorly without the addition of visual and structural cues. As reported by Ikeshoji et al. (1980), ethyl acetate did not have an effect on its

TABLE 4. Relative importance of visual/structural stimuli and onion-(dipropyl disulfide) and bacteria-produced (ethyl acetate) volatiles for onion fly oviposition[1].

Visual/structural stimulus	Mean[2] % eggs laid/treatment[3]			
	Chemical stimulus[4]			
	none	ETAC	Pr_2S_2	Pr_2S_2 + ETAC
no stems	0.0 d	0.1 d	0.1 d	0.1 d
clear stems	0.4 d	1.2 d	1.2 d	7.1 c
yellow stems	9.2 c	9.9 c	22.6 b	47.4 a

[1] Three replicates

[2] Means followed by the same letter are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 3597.

[4] ETAC = ethyl acetate, Pr_2S_2 = dipropyl disulfide.

own but did synergize dipropyl disulfide, causing more than twice as much oviposition in treatments with yellow stems, and over 5 times as much oviposition with glass stems. The addition of structural cues (glass stems) to the best chemical stimulus again caused a significant increase in oviposition (0.7% → 7.1%).

DISCUSSION

Stimuli emanating from onion stems play an important role in releasing oviposition by D. antiqua. The quality (hue) rather than the quantity (brightness) of light reflected by the stem influences oviposition responses. This contradicts the work of Müller (1969) who suggested that close-range orientation in D. antiqua is mediated by plant shape and brightness rather than hue; however, Müller's conclusions concerning the importance of brightness for oviposition were based on an experiment measuring landing frequency of D. antiqua flies on colored disks mounted horizontally and baited with onion juice. The observed landing response may have been unrelated to oviposition behavior or may have been only one of a series of behaviors resulting in oviposition.

D. antiqua preference for yellow stems was expressed in the presence of several different chemical stimuli and also when no chemical stimulus was placed in the sand. Although color perception is not well understood it has been suggested that the preference for yellow (rather than green) shown by many herbivores (Kennedy, Booth and Kershaw 1961, Moericke 1969, Prokopy and Boller 1971) occurs because yellow reflects more light than green in the wavelength range (540 to 560 nm) where green foliage reflects maximally. Thus, yellow might present a "supernormal foliage-type stimulus" (Prokopy 1972). Preliminary observations comparing D. antiqua behavior on different color stems indicate that yellow stems elicited more oviposition behavior because of effects on both pre- and post-alighting behaviors.

Treatments combining chemical and visual/structural characteristics have a synergistic, rather than additive, effect on oviposition. The mechanism behind this synergistic effect is not known but could be due to the two stimuli having either a sequential or simultaneous effect on oviposition behavior. The fly might, for instance, respond anemotactically or klino- or orthokinetically to olfactory stimuli, and upon reaching the oviposition site, encounter visual/structural stimuli which, in the presence of chemotactile stimuli, cause the fly to oviposit. On the other hand, the presence of olfactory stimuli might cause the fly to "pay attention to" visual stimuli. Vaidya (1969) found that the lemon butterfly, Papilio demoleus, did not respond to isolated citrus odors or colors; however, when an odor source was placed near colored papers, butterflies responded to colors with a characteristic drumming response and did not "search" for the source of the odor. Behavioral observations of individual onion flies on treatments presenting a single stimulus or various combinations of stimuli would be very useful, and might explain how the various stimuli mediate oviposition behavior.

The system used by D. antiqua to select an oviposition site is therefore fairly flexible: flies appear to discriminate between chemical stimuli in the absence of visual/structural stimuli (Vernon et al. 1978, Ikeshoji et al. 1980, Ishikawa et al. 1978), and between plant color and size in the absence of chemical stimuli (our work and Hough 1981). This flexibility could be highly adaptive, as the visual, structural and chemical stimuli emitted by A. cepa vary within fields and change drastically throughout the growing season. In Michigan, flies in the first generation encounter seedling onions and a few sprouted cull onions, whereas second generation flies generally encounter onions with tall, thick foliage and large bulbs. By the time the third generation has emerged and mated, onion fields may have been harvested. At this time, the only onions available for oviposition are those left behind by the harvester. These cull onions appear in a variety of states, some being sprouted and others submerged beneath the soil surface and therefore presenting no visual stimuli. Volatile composition also changes qualitatively and quantitatively throughout the growing season and when plants are damaged (Saghir et al. 1965, Ikeshoji et al. 1980).

Although D. antiqua oviposition preference does generally follow plant taxonomic lines, with the Allium species being preferred among plant genera and A. cepa being preferred among the Alliums, flies do occasionally ignore taxonomy and oviposit on non-Allium plants such as corn (Workman

1958) and non-A. cepa plants such as A. ampeloprasum L. (Müller 1969), A. fistulosum L. (Perron 1972) and A. sativum L. (Chapter 2). Rausher et al. (1981) have suggested that some insects do not order oviposition preferences along taxonomic lines but instead discriminate among all plants by using a few characteristics that cross plant taxonomic lines. The onion fly's preferences could therefore appear to follow taxonomic lines inasmuch as Allium species have a unique secondary plant chemistry and vary chemically within the genus (Saghir et al. 1964, 1966); however, preferences for certain visual/structural stimuli would cross plant taxonomic lines, and might cause the fly to oviposit on plants other than A. cepa.

Inasmuch as visual and structural stimuli supplement the information conveyed by chemical stimuli, the use of several sensory modalities during host finding should be adaptive. Although the onion fly may be able to locate a submerged, non-sprouted cull onion solely by the use of chemical stimuli, visual stimuli would give an accurate indication of where an onion is located. Such accuracy may be critical to the survival of the first instar larvae which move slowly (Gray 1924) and are subject to desiccation in dry soils (Workman 1958). Visual and structural stimuli might also provide information about the size of the resource available for larval development, and thereby serve as an indicator of how many eggs may safely be laid on a particular resource. In addition, it is conceivable that the spectral reflectance characteristics of the stem convey information about the physiological state of the onion (whether it is stressed by nutrient or moisture deficiencies or by disease) and therefore, give some indication of the plant's chemical and physical defenses.

The practical significance of this information is threefold. Firstly, the observation that the onion fly has a flexible system for locating oviposition sites should indicate to plant breeders that efforts to impart resistance to onions should be concentrated on toxic rather than deterrent characteristics of the plant. While some flies may leave a field when confronted with "deterrent" or less chemically stimulatory plants, others may remain in the field, and accept plants which are visually but not chemically stimulatory. Secondly, incorporating visual stimuli into the cone trap which is presently being used in Michigan for monitoring onion fly populations (Miller unpublished) might improve the ability of this trap to detect low populations. Finally, an understanding of host finding behavior may allow the development of cultural methods for control of *D. antiqua*. Such methods might utilize trap crops consisting of visually "conspicuous" sprouted cull onions which would be more preferred for oviposition than commercial onions (Gray 1924, Armstrong 1924). Trap crops might also serve to concentrate *D. antiqua* eggs in one area, and thus, make more effective releases of the egg predator *Aleochara bilineata* (Gyllo) (E. Groden, personal communication). *D. antiqua* oviposition might also be reduced if commercial onions were made less visually "conspicuous". This might be achieved by the use of cover crops which could be planted either during the growing season or after harvest of onion fields.

CHAPTER 2

Influence of Non-Propyl Radicals in Host-Range Determination of *Delia antiqua*

INTRODUCTION

Like many other herbivores, host range in the onion fly is determined by the female's propensity to oviposit on certain plants rather than limited larval feeding habits (Loosjes 1976). The stimuli responsible for the selective oviposition behavior of the female are not entirely understood. In Chapter 1, it was shown that visual and structural characteristics of host plants may play an important role. However, given the genus *Allium*, one is confronted with plant species which are remarkably similar in both color and morphological characteristics. The secondary chemistry of the *Allium* species is also quite similar, being dominated by large amounts of alkyl cysteine sulfoxides and γ-glutamyl peptides which, upon cell disruption, yield a series of alk(en)yl substituted mono-, di-, and trisulfides, as well as thiols (Whitaker 1976). Yet, in spite of the apparent similarities, *D. antiqua* concentrates oviposition on *Allium cepa*, though some less preferred species, such as *A. fistulosum*, are equally, if not better, suited for larval development (Perron and Jasmin 1963).

Allium species may be separated on the basis of more subtle differences in secondary chemistry. Proportions of major alk(en)yl radicals differ significantly among *Allium* species: *A. cepa* contains primarily propyl radicals attached to mono-, di-, and trisulfides, while *A. sativum* contains primarily 2-propenyls. According to the hypothesis of Vernon et al. (1978) and Ishikawa et al. (1978), only those alkyl sulfides containing propyl radicals should elicit oviposition by *D. antiqua*. Two questions thus arise. Does *D. antiqua* prefer certain *Allium* species simply because they contain more of the stimulatory disulfides? Or is *D. antiqua* a specialist because of limited sensitivity which allows it to perceive *A. cepa* but not other *Allium* species?

Although no studies have compared *D. antiqua* oviposition on a range of *Allium* species, field observations on larval infestation (Jones and Mann 1963, Loosjes 1976, Perron and Jasmin 1963) give evidence that, while unchecked populations of *D. antiqua* may destroy from 50 to 90% of the onions in a commercial field, serious infestations are rarely found on leek (*A. ampeloprasum* L.), Japanese bunching onion (*A. fistulosum* L.), wild onion (*A. vineale* L.) and garlic (*A. sativum* L.). It has been assumed that differences in degree of infestation seen with different *Allium* species are due to *D. antiqua*'s propensity to oviposit on attractive species, rather than larval mortality on unattractive species (Ellis et al. 1979, Perron et al. 1958). If this assumption is correct, one can rank *Alliums* from most attractive (*A. cepa*) to least attractive (*A. sativum*) based on the observations made in both field and lab (Jones and Mann 1963, Loosjes 1976, Perron and Jasmin 1963, Hough 1981) and compare the total amounts of propyl, methyl, 1-propenyl and 2-propenyl radicals found in plants which are apt to be oviposited on vs. those which are not (Table 5). However, when this comparison is made, it becomes apparent that absolute quantities of propyl

TABLE 5. Amounts and ratios of sulfur radicals in *Allium* spp. varying in attractiveness[1] to *Delia antiqua* (Meigen).

Common name	*Allium* species	RELATIVE AMOUNTS ALK(EN)YL RADICALS (cm^2/gm)[2]				
		propyl	methyl	1-propenyl	2-propenyl	ratio propyl:non-propyl radicals
onion	*A. cepa* L.	4.3	0.3	0.3	--	7.3 :1
shallot	*A. ascalonicum* hort.	1.6	0.8	0.2	--	1.6 :1
leek	*A. ampeloprasum* L.	4.9	2.3	0.7	--	1.7 :1
bunching onion	*A. fistulosum* L.	10.4	3.9	1.6	--	1.9 :1
wild onion	*A. vineale* L.	3.8	7.9	0.02	7.6	0.24:1
garlic	*A. sativum* L.	0.2	1.0	--	6.3	0.02:1

[1] *Allium* species are ranked from most attractive (*A. cepa*) to least (*A. sativum* and *A. vineale*) based on assumptions given in text.

[2] Based on data of Freeman and Whenham (1975). Data are given in peak area (cm^2) per gram on fresh weight basis.

radicals are not well correlated with different degrees of attractiveness. *A. fistulosum*, which enjoys a certain degree of resistance to *D. antiqua* due to lower egg deposition (Perron et al. 1958), produced two times more volatile propyl radicals than does onion, *A. cepa*.

Dethier (1980) has suggested that herbivore specialists show discriminatory behavior not because of "peripheral incompetence" but rather because they have evolved receptor types (including deterrent receptors) which are sensitive to a multitude of secondary plant chemicals. This suggests, that *D. antiqua* might be sensitive not only to those sulfides which are most stimulatory, but also to those which have no apparent effect on oviposition behavior when tested in isolation. *D. antiqua* might therefore respond to the summation of both stimulatory (propyl) and non-stimulatory (non-propyl) inputs. Indeed, comparisons of ratios of propyl to non-propyl (methyl, 1-propenyl, 2-propenyl) radicals show a better correlation with ovipositional attractiveness: whereas *A. cepa* has a ratio of 7:1, the ratio switches to 9:1 in *A. fistulosum*, and to 0.15:1 in *A. sativum* (Table 5). This would seem to indicate that the non-propyl disulfides play some role in oviposition behavior, be it as deterrents or simply as less stimulatory disulfides.

This chapter addresses several questions concerning the nature and diversity of chemical information used by *D. antiqua* when locating and accepting a host plant. Two *Allium* species, *A. cepa* and *A. sativum* were used to investigate these

TABLE 6. Volatile components of crushed onion (*Allium cepa*) and garlic (*Allium sativum*).[1]

Allium species	VOLATILE COMPOUNDS				
	dipropyl disulfide	methyl propyl disulfide	dimethyl disulfide	allyl methyl disulfide	diallyl disulfide
onion (*A. cepa*)	44-93%	2-34%	2-18%	0-2%	<1
garlic (*A. sativum*)	<1	<1	<1	22-33%	55-74%

[1] Percentage of total volatile components from head space (from Whitaker, 1976).

questions (Table 6). Dose-response studies using electroantennograms and the primary volatile components of A. cepa (dipropyl disulfide) and A. sativum (diallyl disulfide) were run to ascertain whether "peripheral incompetence" could explain the specialization of D. antiqua on A. cepa. The nature of chemical information conveyed by A. cepa and A. sativum was investigated with oviposition experiments wherein I tried to establish whether allyl disulfides are perceived as deterrents or as less stimulatory disulfides. Finally, larval survival studies were conducted to determine whether the observed ovipositional preferences reflected the suitability of the Allium species for larval development.

MATERIALS AND METHODS

Growth and survival of D. antiqua larvae on Allium spp.

Eggs were collected from the D. antiqua lab culture described in Chapter 1 and were placed on moist filter paper in 100 x 15 cm Parafilm^{TM}-sealed plastic Petri dishes. After approximately 3 days at 25±1° C, larvae were carefully removed with a paintbrush and placed on Allium bulbs. Onion and garlic plants were obtained, respectively, from a commercial field in Stockbridge, Michigan (variety Abundance) and from a garden (variety unknown) in Bath, Michigan in September of 1981. At the time of the experiments, plants were removed from the soil, washed and pierced with a 1 mm diameter probe halfway between stem and rootcap to allow larvae easy access to plant tissues. Infested bulbs were placed individually in glass jars (5 x 5 x 13 cm) containing 40 ml of glass beads. Bulbs were positioned so that only the uppermost tip of the bulb showed above the surface of the beads. The onion (n=30) and garlic (n=43) treatments were then placed in boxes located in an environmental chamber illuminated by cool white fluorescent bulbs on a 16:8 L:D light regime (21±1° C and 35±5% RH). Jars were checked daily for excess or insufficient moisture and additional bulbs were added if the originals had been consumed. Fourteen days into the experiment, bulbs and beads were inspected daily for pupae which when found, were removed from jars, rinsed with water, towel dried and weighed. Pupae were then put into vials containing moist sand and checked daily for adult emergence. Upon emergence, the sex of the fly and the number of days spent in the pupal stage were recorded.

Electroantennograms (EAG's)

The electroantennogram technique described by Roelofs (1976) was used with several modifications. Live flies from the culture previously described were placed dorsal side down on wax and secured at the wing bases by pins. Disposable glass pipettes containing electrodes (chlorodized silver wire) and saline solution, were covered with aluminum foil and grounded. Pipettes containing indifferent and recording electrodes were positioned so that the saline solution contacted the fly's mouthparts and antenna, respectively. A constant stream of charcoal-filtered air (linear velocity 1 m/sec) continuously flushed the antenna, and was delivered via a 1 cm diameter glass tube which terminated ca. 3 cm from the fly. Dipropyl disulfide (Eastman Kodak, Rochester, NY) was 98% pure by GLC. Both dibutyl and diallyl disulfide were obtained from Wateree Chemical Company, Lugoff, SC. However, whereas dibutyl disulfide was 98% pure by GLC, diallyl disulfide was only 60% pure and therefore was purified to 99% by preparative HPLC (packing- Li-Chroprep^{TM} RP-18; solvent- 4:1 methanol:water) (Powell and Miller, unpublished). Compounds were diluted serially in peanut oil and ca. 1 ml of each was placed in a 16 mm diameter glass culture tube. An 18 gage x 38 mm needle which accepted a 3 ml disposable syringe was fitted through a hole in the Teflon^{TM}-lined screw cap, while an L-shaped 1 mm diameter glass tube was inserted through a second hole. Ten cm of 1 mm I.D. Teflon^{TM} tubing extended from the tip of the needle to the reservoir of solution. To deliver chemicals, the L-shaped exit tube was inserted through a 5 mm aperture located 2 cm from the end of the air line, and 1 ml of air was injected into the culture tube, thereby displacing headspace into the airstream. Antennal depolarizations were amplified 100 times and displayed on a Tektronic^{TM} 564B storage oscilloscope. Each fly was exposed only once to each concentration of dipropyl and diallyl disulfides (see Figure 5 for loadings). The series began with the lowest concentrations and alternated between diallyl and dipropyl disulfide at 3 minute intervals with diallyl and dipropyl disulfide at 3 minute intervals with the dibutyl disulfide standard (0.015 molar concentration) injected before and after each at intervals of 1.5 minutes. Dibutyl disulfide was used to normalize for the diminishing antennal responses which occurred during the test period. The test was run on five females and four male flies. EAG responses were ultimately expressed as millivolt response to a treatment divided by the mean response to dibutyl disulfide standard presented before and after the treatment.

Oviposition tests

Experiments comparing oviposition on onion versus garlic plants used pairs of plants matched for stem length (6-9 cm) and bulb size (2-3 cm diameter) within a block. The sources and varieties of plants used were identical to those used in larval growth and development studies. At the time of the experiments, plants were removed from soil, washed and placed in cups which were 8 cm in diameter and 5 cm deep. Cups were filled with 200 ml of washed silica sand and watered with 50 ml of distilled water. Twenty holes, 5mm deep and 2 mm wide were made in a regular pattern in the sand of each cup.

Treatments containing onion and garlic and dipropyl and diallyl disulfide and ethyl acetate were prepared similarly to those described in Chapter 1. If the treatment contained larvae, five second instar *D. antiqua* larvae from the lab culture were rinsed in distilled water and placed on the chopped *Allium* tissues. Diallyl disulfide was identical to that used in EAG studies. The sources of dipropyl disulfide and ethyl acetate are discussed in Chapter 1. Visual and structural stimuli for treatments containing chopped Allium tissues and Allium secondary chemicals and ethyl acetate were provided by surrogate stems containing yellow papers (see Chapter 1). While experiments comparing oviposition on whole plants and fresh chopped onion and garlic were placed in cages immediately after their preparation, experiments with larvae or secondary chemicals were first "aged" in the lab for 24 hours. Design of experiments and precautions taken to avoid training to a particular stimulus were identical to those described in Chapter 1. Because of space limitations, cages (60 x 60 x 80 cm) containing several hundred flies were located in a controlled environmental chamber which was also used as a rearing facility for onion fly larvae. There was, therefore, a low but constant level of onion odor in the chamber. The environmental chamber, fly culture and collection of data were identical to those described previously. All experiments were kept in cages for 24 hours except those using synthetic chemicals. These treatments were placed in cages 9 hours into the light cycle and removed 3 hours later. When treatments remained in cages for longer periods, flies began to avoid treatments containing dipropyl and diallyl disulfides and began to lay inordinate numbers of eggs around yellow stems in the sand controls. A similar phenomenom was observed by Matsumoto and Thorsteinson (1968) when high concentrations of dipropyl disulfide were used in experiments with no visual stimuli.

Since variances for treatments were not homogeneous and normality could not be achieved by transformations, the Kruskal-Wallis one-way analysis of variance using ranks was used to determine significant differences. This test seems to be the most efficient of the non-parametric tests for K independent samples and when compared with the F test, has a power efficiency of 95.9% (Siegel 1956).

RESULTS

Growth and survival of D. antiqua larvae on Alliums

Larval development and survival was extremely poor on garlic bulbs even when bulbs were damaged and larvae were given easy access to plant tissue (Table 7). Whereas 93% of the larvae reared on onion survived to pupation, only 7% of those reared on garlic survived the larval stage and only 4.7% emerged as adults. Larvae feeding on garlic spent ca. 1.5 fold more time in the larval stage and upon pupation, weighed 57% as much as larvae reared on onion. Robinson and Zurlini (1981) have demonstrated that reductions in the pupal size of *D. antiqua* resulted in reductions of fecundity. Days spent in the pupal stage did not differ significantly for larvae reared on the two *Alliums*.

Electroantennograms (EAG's)

Dosage-response curves for diallyl and dipropyl disulfide are presented in Figure 5. Curves were not significantly different at any concentration, indicating that differences

TABLE 7. Growth and survival of *Delia antiqua* larvae reared on onion and garlic.

Larval Host Species	DEVELOPMENTAL PARAMETERS				
	Survival to Pupal Stage (%)	Days to Pupation ($\bar{X}$ ± SD)	Pupal Weight (mg)	Survival to Adult (%)	Days in Pupal Stage ($\bar{X}$ ± SD)
onion (*Allium cepa*) N = 30	83	17.8±1.0	19.3±0.8	73.3	17.5±0.8
garlic (*Allium sativum*) N = 43	7	28.3±1.5	11.3±3.5	4.7	17

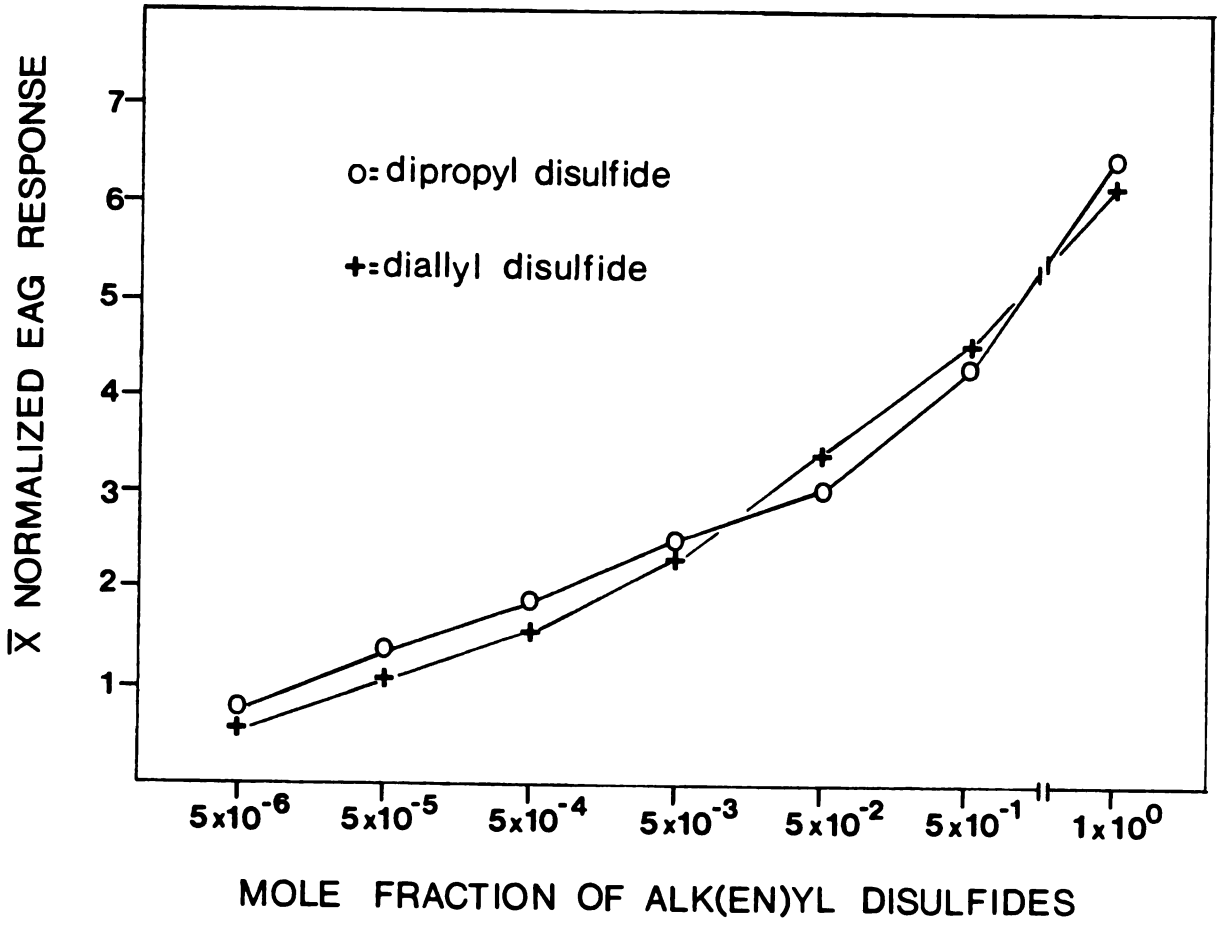

FIGURE 5. Normalized EAG responses of male and female *Delia antiqua* (Meigen) antenna *vs*. dosage of alk(en)yl disulfides loaded in peanut oil.

in behavioral responses to the two alk(en)yl disulfides were not due to filtering at the sensory periphery. Inasmuch as equal concentrations of diallyl and dipropyl disulfide in peanut oil result in very similar headspace concentrations (0.05 mole fraction diallyl and dipropyl disulfide in peanut oil showed headspace concentrations of 0.021 and 0.011 ug/ml, respectively, at ca. 25° C), EAG's gave an accurate representation of the relative responses of *D. antiqua* antenna, but did not reflect observed differences in oviposition response to the two alk(en)yl disulfides. Ikeshoji et al. (1981) also found no correlation between oviposition response and EAG response to mixtures of alcohols, esters, acids, aldehydes and dipropyl disulfide.

Oviposition tests

Flies given access to field-grown garlic and onion plants (Table 8) laid 90% of their eggs on onion. When the two *Alliums* were chopped and their stems replaced by surrogates which were identical in color, size and shape (Table 8), the same propensity to oviposit on onion was observed, indicating that the different chemical stimuli presented by the two plant species were the primary cause for the predominance of eggs on onion. When sources of the primary volatile components of onion and garlic were combined with yellow stems, ca. 80% of the eggs were laid on dipropyl disulfide whereas there was no significant difference in the number of eggs laid on diallyl disulfide treatments and yellow stem controls (Table 8).

TABLE 8. Stimulation of *Delia antiqua* (Meigen) oviposition by susceptible and non-susceptible *Alliums* and their primary volatile components[1].

Odor stimulus[2]	Exp. 1 Whole plants	Exp. 2 Chopped bulbs + surrogate stems	Exp. 3 Synthetic volatiles + surrogate stems
	Mean[3] % eggs laid/treatment[4]		
onion (Pr_2S_2)	89.7 a	84.4 a	82.9 a
garlic (Al_2S_2)	10.3 b	15.6 b	9.8 b
control (sand)	---	---	7.3 b

[1] Four replicates of each experiment.

[2] Pr_2S_2 = dipropyl disulfide, Al_2S_2 = diallyl disulfide.

[3] Means within columns followed by the same letters are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[4] Total eggs = 2725, 871 and 9672 for Experiment 1, 2 and 3 respectively.

The addition of _D. antiqua_ larvae to chopped _Alliums_ 24 hours previous to treatment presentation (Table 9) caused ca. 4 times more eggs to be laid on onion and 2.5 times more eggs on garlic; however, even in the presence of larvae, onion received 3 times more eggs that did garlic. In contrast to the result with diallyl disulfide alone, chopped garlic without larvae did receive significantly more eggs than the control. While garlic headspace does contain primarily diallyl disulfide and allyl methyl disulfide (Table 6), some (<3%) of its additional volatile components (Saghir et al. 1964) do fulfill the hypothesized requirement of propyl-thio moieties (Vernon et al. 1978, Ishikawa et al 1978), and thus may stimulate oviposition to a lesser degree than onion. Including a control containing only larvae would have aided the interpretation of these data by indicating whether the treatment, garlic plus larvae, received more eggs because the presence of larvae stimulated oviposition or because larvae were interacting with the garlic tissue, thereby making it a better oviposition stimulus.

In a final experiment comparing oviposition (Table 10) on primary volatile components of onion and garlic, both with and without ethyl acetate, ethyl acetate not only synergized dipropyl disulfide, causing 11.5 times more oviposition, but also synergized diallyl disulfide, causing 4 times as much oviposition. Oviposition on yellow stems with ethyl acetate or diallyl disulfide did not differ significantly from yellow stem

TABLE 9. Relative effectiveness of larval-infested and non-infested onions and garlic in stimulating oviposition by Delia antiqua (Meigen) when visual and structural cues were held constant[1].

	Mean[2] % eggs laid/treatment[3]			
Odor stimulus	no larvae		plus 5 larvae	
onion	14.6	b	58.4	a
garlic	7.9	c	17.8	b
control	1.3	d	---	

---

[1] Eight replicates

[2] Means followed by the same letters are not significantly different at $p \leq 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 18,632.

TABLE 10. Relative effectiveness of various combinations of microbially produced volatiles with alk(en)yl disulfides in stimulating oviposition by *Delia antiqua* (Meigen) when visual and structural cues were held constant[1].

	Mean[2] % eggs laid/treatment[3]			
	Microbial volatiles			
Allium volatiles	none		ethyl acetate	
n-dipropyl disulfide	5.6	c	69.0	a
diallyl disulfide	3.6	cd	16.4	b
control	2.6	d	2.9	d

[1] Four replicates

[2] Means followed by the same letters are not significantly different at $p \le 0.05$ (Kruskal-Wallis one way analysis of variance by ranks).

[3] Total eggs = 5112.

controls, nor did oviposition on dipropyl disulfide in this case differ significantly from diallyl disulfide.

DISCUSSION

Garlic is not a suitable host plant for D. antiqua larvae. The mechanistic explanation for garlic's lack of suitability is unknown, but could result from any or all of the following: toxins, structural impediments to feeding, lack of moisture in plant tissues, lack of nutrients and lack of microorganisms. Although many plant toxins are non-volatile and thus may be signalled by odors unrelated to the toxins (Eisner and Grant 1981), the volatile components of Allium species may be signalling their own presence and toxicity. Diallyl disulfide, for instance, has been used as an insecticide against mosquito larvae (Amonkar and Banerji 1971). Other work (Powell and Miller unpublished) indicates that the toxicity of compounds containing propyl, methyl and 2-propenyl radicals mirrors D. antiqua's propensity to oviposit on them. However, it is not known whether these toxic effects occur at biologically meaningful concentrations. A. fistulosum, which contains larger amounts of methyl radicals than leek (Freeman and Whenham 1975), is similarly resistant to oviposition by D. antiqua, but is actually a suitable larval host whereas leek is not. It might be hypothesized, therefore, that in the coevolution of D. antiqua and the genus Allium, the non-propyl radicals became associated with either non-volatile toxins, or other characteristics which rendered the plants unsuitable for larval development.

Eisner and Grant (1981) have pointed out that once "olfactory aposematism" has been established, it can be exploited by palatable species which also emit the volatile warning signals. *A. fistulosum* could thus avoid oviposition by *D. antiqua* via olfactory mimicry of less palatable *Allium* species.

Although no data are available on the nutritional characteristics of *A. cepa* versus *A. sativum*, there are obvious differences in structure and moisture content of the plant tissues. Garlic is 58% water compared to 89% for onion (Freeman and Whenham 1975) and thus may be more difficult to penetrate. Plant tissues low in moisture have been found to be more difficult to process, requiring more metabolic energy on the part of lepidoptera larvae than leaves with identical nitrogen and caloric content but increased amounts of moisture (Scriber 1977). Garlic may also affect the nutrition of the larva indirectly by inhibiting microorganisms. Garlic possesses strong antibacterial and antifungal activity (Cavallito and Bailey 1944, Tansey and Appleton 1975) and therefore might inhibit microorganisms which speed larval development (Zurlini and Robinson 1978, Friend et al. 1959, Schneider and Miller in press). Microorganisms could also conceivably play a role in detoxifying secondary plant compounds.

Having ruled out the possibility that *D. antiqua* specialization on onion results from "peripheral incompetence", there are three possible roles that non-propyl disulfides might play in oviposition behavior. First, information conveyed by non-propyl disulfides might be irrelevant to oviposition behavior, neither increasing or decreasing response to attractive stimuli. Secondly, non-propyl disulfides might elicit a bimodal response similar to that of dipropyl disulfide, being slightly attractive at low concentrations and deterrent at high concentrations. If this were the case, one would expect that oviposition response to non-propyl disulfides would be synergized by the same factors which synergize response to dipropyl disulfide. Finally, non-propyl disulfides could be deterrent at all concentrations, and reduce oviposition responses to all stimuli, whether they be chemical or visual.

Experiments indicated that information conveyed by non-propyl disulfides was relevant to oviposition behavior. Diallyl disulfide was deterrent at concentrations where dipropyl disulfide was also deterrent, but was neutral in its effect at concentrations where dipropyl disulfide was stimulatory. Furthermore, response to garlic chemicals was synergized by the same factors which synergize _D. antiqua_ oviposition response to onion chemicals. Combinations of diallyl disulfide and ethyl acetate received three times more eggs than did dipropyl disulfide alone, and in this case, there was no difference between the number of eggs laid on diallyl and dipropyl disulfide treatments. Thus, it appears that like other herbivores (Jermy and Szentesi 1978), _D. antiqua_ oviposition response to a set of stimuli is relative, rather than absolute, and therefore depends on the availability of alternative stimuli.

While non-propyl disulfides do not deter oviposition responses to chemically neutral visual stimuli, they may have very different effects when combined with chemically stimulatory dipropyl disulfides. As noted in the Introduction, the ratio of propyl to non-propyl disulfides decreases in those _Allium_ species which are less preferred by _D_. _antiqua_ for oviposition. Non-propyl disulfides may therefore deter oviposition only through some antagonistic interaction with dipropyl disulfide occurring at either the sensory periphery or the central command system (Dethier 1982).

Dethier (1980) has argued that an expanded receptor sensitivity sets the stage for discriminatory feeding behavior in insect herbivores. Such an expanded sensitivity may also set the stage for discriminatory oviposition behavior, and may allow ovipositing females to respond more flexibly to changing sets of larval food resources. In the case of _D_. _antiqua_, sensitivity to both propyl and non-propyl disulfides would allow females to discriminate between a suitable and less suitable _Allium_, without losing the ability to distinquish between plants which do and do not contain disulfides. Thus, given a situation where plants containing propyl disulfides were not available, receptor sensitivity might enable a female to find and oviposit on plants containing methyl or allyl disulfides. Though suboptimal, oviposition on these plants instead of plants containing no disulfides might be adaptive inasmuch as a small percent of the female's offspring might survive.

CONCLUSIONS

Chapter 1. SYNERGISM OF VISUAL AND CHEMICAL STIMULI IN THE OVIPOSITION BEHAVIOR OF DELIA ANTIQUA

1. Stimuli emanating from onion stems play an important role in releasing oviposition behavior.

2. Surrogate stems can be used to investigate the use of visual stimuli and compete favorably with onion stems as long as additional chemical stimuli are provided.

3. Given a constant chemical stimulus and a range of colored stems, flies laid more eggs on yellow stems. The increased stimulatory effect of yellow was expressed both in the presence and absence of chemical stimuli.

4. Tests comparing oviposition on yellow and a series of gray stems presented with a constant chemical stimulus, indicated that the increased oviposition seen on yellow stems was due to a preference for a particular hue rather than brightness characteristics.

5. There is some indication that the onion stem provides not only requisite color stimuli, but that it also provides a substrate for the performance of post-alighting, pre-oviposition behaviors.

6. Treatments combining chemical, visual and structural stimuli have a synergistic rather than additive effect on oviposition.

Chapter 2. INFLUENCE OF NON-PROPYL RADICALS IN HOST-RANGE DETERMINATION OF DELIA ANTIQUA

1. *A. sativum* is not suitable host plant for *D. antiqua* larvae. Therefore, from an evolutionary viewpoint, the threshold for acceptance of garlic as an oviposition site should be higher than the threshold for onion.

2. The virtually identical EAG responses to diallyl and dipropyl disulfide indicate that the difference seen in the attractiveness of onion and garlic is not due to "peripheral incompetence".

3. When presented with garlic and onion plants, females laid over 80% of their eggs on onion. This pattern was repeated when *Allium* stems were replaced with surrogate stems, indicating that *D. antiqua*'s preference for onion was due to differences in chemical, rather than visual characteristics.

4. Diallyl disulfide and chopped garlic were perceived as neutral or inferior oviposition stimuli rather than as deterrents inasmuch as they did not increase or decrease response to a chemically neutral visual stimulus.

5. Increased oviposition on garlic can be stimulated by the presence of *D. antiqua* larvae in spite of the fact that garlic's primary volatile components do not fulfill hypothesized specifications for stimulatory molecules. Ethyl acetate, which was known to stimulate oviposition of treatments containing dipropyl disulfide, also increased oviposition on diallyl disulfide.

6. *D. antiqua* oviposition response to a particular chemical stimulus is not absolute, i.e. the degree to which a stimulus is oviposited on depends on the availability of alternative oviposition sites.

REFERENCES CITED

REFERENCES CITED

Amonkar, S. U. and A. Banerji. 1971. Isolation and characterization of larvacidal principle of garlic. Science 174:1343-1344.

Armstrong, T. 1924. Onion maggot studies in the district of Montreal, Quebec 1923. Entomol. Soc. Ont. Annu. Rep. 54:42-45.

Borror, D. J. and D. M. DeLong. 1971. An Introduction to the Study of Insects. Holt, Rinehart and Winston, N.Y. p. 522.

Carruthers, R. 1979. Population sampling and spatial distribution of the immature life stages of the onion maggot, Hylemya antiqua (Meigen). M.S. Thesis, Department of Entomology, Michigan State University. 197 pp.

Cavallito, C. J. and J. H. Bailey. 1944. Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action. J. Amer. Chem. Soc. 66:1950-1954.

Crête, R. 1972. Chemical control of onion smut in organic soils of southwestern Quebec in 1971. Can. Dep. Agric. Pesticide Res. Rep.

Dethier, V. G. 1954. Evolution of feeding preferences in phytophagous insects. Evolution 8:33-54.

Dethier, V. G. 1971. A surfeit of stimuli: A paucity of receptors. Am. Sci. 59:706-715.

Dethier, V. G. 1980. Evolution of receptor sensitivity to secondary plant substances with special reference to deterrents. Am. Nat. 115:45-66.

Dethier, V. G. 1982. Mechanism of host-plant recognition. Ent. exp. & appl. 31:49-56.

Dindonis, L. L. and J. R. Miller. 1980a. Host-finding responses of onion and seedcorn flies to healthy and decomposing onions and several synthetic constituents of onion. Environ. Entomol. 9:467-472.

Dindonis, L. L. and J. R. Miller. 1980b. Host-finding behavior of onion flies, Hylemya antiqua Environ. Entomol. 9:769-772.

Dindonis, L. L. and J. R. Miller. 1981a. Onion fly and little house fly host-finding selectively mediated by decomposing onion and microbial volatiles. J. Chem. Ecol. 7:419-426.

Dindonis, L. L. and J. R. Miller. 1981b. Onion fly trap catch as affected by release rates of n-dipropyl disulfide from polyethylene enclosures. J. Chem. Ecol. 7: 411-418.

Eisner, T. and R. P. Grant. 1981. Toxicity, odor aversion and "olfactory aposematism". Science 213:476.

Ellis, P. R., C. J. Eckenrode and G. E. Harman. 1979. Influence of onion cultivars and their colonizers on resistance to onion maggot. J. Econ. Entomol. 72:512-515.

Freeman, G. G. and R. T. Whenham. 1975. A survey of volatile components of some Allium species in terms of S-alk(en)yl-L-cysteine sulphoxides present as flavour precursors. J. Sci. Food Agric. 26:1869-1886.

Friend, W. G., E. H. Salkeld, and I. L. Stevenson. 1959. Acceleration of development of larvae of the onion maggot, Hylemya antiqua (Meigen) by microorganisms. Can. J. Zool. 37:721-727.

Gray, H. E. 1924. The onion maggot in Alberta. Entomol. Soc. Ont. Annu. Rep. (1923) 54:36-38.

Hennig, W. 1974. Anthomyiidae, in E. Lindner (ed.) Die Fliegen der palaearktischen Region, 63a, 306:681-776.

Hough, J. A. 1981. Oviposition behavior in the onion fly, Hylemya antiqua (Meigen). Ph.D. Thesis, Cornell University. 94 pp.

Hough, J. A., G. E. Harman and C. J. Eckenrode. 1981. Microbial stimulation of onion maggot oviposition. Environ. Entomol. 10:206-210.

Howard, R. W. and G. J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27:149-72.

Ikeshoji, T., Y. Ishikawa and Y. Matsumoto. 1980. Attractants against the onion maggots and flies, Hylemya antiqua, in onions inoculated with bacteria. J. Pestic. Sci. 5: 343-350.

Ikeshoji, T., Y. Ishikawa and Y. Matsumoto. 1981. Behavioral and EAG synergism of various compounds to dipropyl disulfide in the ovipositional attraction of the onion fly, Hylemya antiqua (Meigen) (Diptera:Anthomyiidae). Appl. Entomol. Zool. 16:432-442.

Ishikawa, Y., T. Ikeshoji and Y. Matsumoto. 1978. A propyl thio moiety essential to the oviposition attractant and stimulant of the onion fly, Hylemya antiqua Meigen. Appl. Ent. Zool. 13:115-122.

Jermy, T. 1958. Untersuchungen über Auffinden und Wahl der Nahrung beim Kartoffelkäfer (Leptinotarsa decimlineata Say). Ent. exp. & appl. 1:197-208.

Jermy, T. and A. Szentesi. 1978. The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. Ent. exp. & appl. 24:458-471.

Jones, H. A. and L. K. Mann. 1963. Onions and Their Allies. Botany, Cultivation and Utilization. Leonard Hill, London. 286 pp.

Kästner, A. 1929. Untersuchungen zur Lebenweise und Bekämpfung der Zwiebelfliege (Hylemya antiqua Meigen). I. Die Bekämfung der Imago in Fruhling. Z. PflKrankh. PflSchutz. 39:49-97, 122-139.

Kennedy, J. S., C. O. Booth and W. J. S. Kershaw. 1961. Host finding by aphids in the field. III. Visual attraction. Ann. Appl. Biol. 49:1-21.

King, J. E. and J. R. Coley-Smith. 1969. Production of volatile alkyl sulphides by microbial degradation of synthetic alliin and alliin-like compounds, in relation to germination of sclerota of Sclerotium cepivorum Berk. Ann. Appl. Biol. 64:303-314.

Loosjes, M. 1976. Ecology and genetic control of onion fly, Delia antiqua. Agric. Res. Rep. 857 PUDOC, Wageningen. 179 pp.

McLeod, D. G. R. 1964. Nutrition and feeding behavior of the adult onion maggot, Hylemya antiqua. J. Econ. Entomol. 57:845-847.

Matsumoto, Y. and A. J. Thorsteinson. 1968. Effect of organic sulfur compounds on oviposition in onion maggot, Hylemya antiqua Meigen (Diptera:Anthomyiidae). Appl. Ent. Zool. 3:5-12.

Miller, J. R. and B. K. Haarer. 1981. Yeast and corn hydrolysates and other nutritious materials as attractants for onion and seed flies. J. Chem. Ecol. 7:555-562.

Moericke, V. 1969. Hostplant specific colour behavior by Hyalopterus pruni (Aphidae). Ent. exp. & appl. 12: 524-534.

Müller, J. 1969. Untersuchungen zu dem in der Ovipositionzeit von Hylemya antiqua Meig. wirksamen Orientierungsmechanismus. Wiss. Z. Ernst-Moritz-Arndt Univ. Greisfwald. 18, Math.-Nat. 5:73-78.

Perron, J. P. 1972. Effects of some ecological factors on populations of the onion maggot, *Hylemya antiqua* (Meig.) under field conditions in southwestern Quebec. Ann. Ent. Soc. Quebec *17*:29-45.

Perron, J. P. and J. J. Jasmin. 1963. Development and survival of the onion maggot under field and artificial conditions on attractive and unattractive onion varieties. Can. Entomol. *95*:334-336.

Perron, J. P., Jasmin, J. J. and J. LaFrance. 1958. Varietal resistance of seeded onions to the onion maggot, *Hylemya antiqua* (Meig.) (Diptera:Anthomyiidae). Can. Entomol. *90*:653-656.

Pierce, H. D. Jr., R. S. Vernon, J. H. Borden and A. C. Oehlschager. 1978. Host selection by *Hylemya antiqua* (Meigen). Identification of three new attractants and oviposition stimulants. J. Chem. Ecol. *4*:65-72.

Prokopy, R. J. 1968. Visual responses of apple maggot flies, *Rhagolitis pomonella* (Diptera:Tephritidae): Orchard Studies. Ent. exp. & app. *11*:403-422.

Prokopy, R. J. 1972. Response of apple maggot flies to rectangles of different colors and shades. Environ. Entomol. *1*:720-726.

Prokopy, R. J. 1977. Stimuli influencing trophic relations in Tephritidae. Colloq. Intern. CNRS *265*:305-36.

Prokopy, R. J. and E. F. Boller. 1971. Stimuli eliciting oviposition of European cherry fruit flies, *Rhagoletis cerasi* (Diptera:Tephritidae) into inanimate objects. Ent. exp. & appl. *14*:1-14.

Rahn, R. 1969. Role de la plante-hôte sur l'activité sexuelle chez *Acrolepia assectella* (Lep.:Plutel.) C.r. hebd. Séanc. Acad. Sci. Paris *266*:2004-2006.

Rausher, M. D., D. A. Mackay and M. C. Singer. 1981. Pre- and post-alighting host discrimination by *Euphydryas editha* butterflies: The behavioral mechanisms causing clumped distribution of egg cluster. Anim. Behav. *29*: 1220-1228.

Renwick, J. A. A. and J. P. Vite. 1972. Pheromones and host volatiles that govern aggregation of the six-spined engraver beetle, *Ips calligraphus*. J. Insect Physiol. *18*:1215-1219.

Riddiford, L. M. and C. M. Williams. 1967. Volatile principle from oak leaves: Role in sex life of the Polyphemus moth. Science *155*:589-590.

Robinson, A. S. and G. Zurlini. 1981. Mating success of differently sized onion flies, Delia antiqua. Ent. exp. & appl. 30:101-105.

Roelofs, W. L. 1976. The scope and limitations of the electroantennogram technique for evaluating biologically active chemicals, in N. R. McFarlane (ed.). Crop Protection Agents: Their Biological Evaluation. Academic Press, N.Y. pp. 147-165.

Saghir, A. R., L. K. Mann, R. A. Bernhard, and J. V. Jacobsen. 1964. Determinations of aliphatic mono- and disulphides in Allium by gas chromatography and their distribution in the common food species. Proc. Am. Soc. Hortic. Sci. 84:386-398.

Saghir, A. R., Louis K. Mann, and M. Yamaguchi. 1965. Composition of volatiles in Allium as related to habitat, stage of growth and plant part. Plant Physiol. 40:681-685.

Saghir, A. R., L. K. Mann, M. Ownbey and R. Y. Berg. 1966. Composition of volatiles in relation to taxonomy of American Alliums. Am. J. Bot. 53:477-484.

Scriber, J. M. 1977. Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of Hyalophora cecropia (Lepidoptera:Saturniidae). Oecologia 34:143-155.

Siegel, S. 1956. Nonparametric statistics for the Behavioral Sciences. McGraw-Hill, N.Y. 312 pp.

Sleesman, J. P. 1934. Onion maggot Hylemya antiqua (Meig.). Bull. Ohio Exp. Stn. 532:41.

Snodderly, D. M. 1979. Visual discrimination encountered in food foraging by a neotropical primate: Implications for the evolution of color vision, in E. H. Burtt (ed.). The Behavioral Significance of Color. Garland STPM Press, N.Y. pp. 237-279.

Sömme, L. and T. Rygg. 1972. The effect of physical and chemical stimuli on oviposition in Hylemya floralis (Dipt., Anthomyiidae). Norw. J. Entomol. 19:19-24.

Staedler, E. 1976. Sensory aspects of insect-plant interactions. Proc. 15th Int. Congr. Entomol. Washington, D. C. pp. 228-248.

Staedler, E. and F. E. Hanson. 1978. Food discrimination and induction of preference for artificial diets in the tobacco hornworm, Manduca sexta. Physiol. Entomol. 3:121-133.

Tansey, M. R. and J. A. Appleton. 1975. Inhibition of fungal growth by garlic extract. Mycologia *67*:407-413.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. *5*:193-218.

Ticheler, J. 1971. Rearing of the onion fly, *Hylemya antiqua* (Meigen), with a view to release of sterilized insects, in Sterility Principle for Insect Control or Eradication. (Proc. Symp., Athens, 1970). IAEA, Vienna. pp. 341-346.

Tichenor, L. H. and D. S. Seigler. 1980. Electroantennogram and oviposition responses of *Manduca sexta* to volatile components of tobacco and tomato. J. Insect Physiol. *26*:309-314.

Treherne, R. C. and M. H. Ruhmann. 1922. The imported onion maggot in British Columbia with notes on its life history and control under "dry belt" conditions. Entomol. Soc. Ont. Ann. Rep. *52*:29-33.

Vaidya, V. G. 1969. Investigations on the role of visual stimuli in the egg-laying and resting behavior of *Papilio demoleus* L. (Papilionidae:Lepidoptera). Anim. Behav. *17*:350-355.

Vernon, R. S., G. J. R. Judd, J. H. Borden, H. D. Pierce, Jr., and A. C. Oehlschlager. 1981. Attraction of *Hylemya antiqua* (Meigen) (Diptera:Anthomyiida) in the field to host-produced oviposition stimulants and their non-host analogues. Can. J. Zool. *59*:872-881.

Vernon, R. S., H. D. Pierce, Jr., J. H. Borden and A. C. Oehlschlager. 1978. Host selection by *Hylemya antiqua*: Identification of oviposition stimulants based on proposed active thioalkane moieties. Environ. Entomol. *7*:728-731.

Whitaker, J. R. 1976. Development of flavor, odor, and pungency in onion and garlic. Adv. Food Res. *22*:73-133.

Wolff, P. V. I. M. 1973. Onderzoek naar attractantia voor de uievlieg *Hylemya antiqua* (Meig.) Doctoraalverslag biologie, Rijksuniversiteit Utrecht. 35 pp.

Workman, R. B., Jr. 1958. The biology of the onion maggot *Hylemya antiqua* (Meigen) under field and greenhouse conditions. Ph.D. Thesis, Oregon State College. 81 pp.

Zohren, E. 1968. Laboruntersuchungen zu Massenanzucht, Lebensweise, Eiablage und Eiablageverhalten der Kohlfliege, *Chortophila brassicae* Bouché (Diptera, Anthomyiidae). Z. angew. Entomol. *62*:139-188.

Zurlini, C. T. and A. S. Robinson. 1978. Onion conditioning pertaining to larval preference, survival and rate of development in *Delia* (=*Hylemya*) *antiqua*. Ent. exp. & appl. *23*:279-287.

MICHIGAN STATE UNIV. LIBRARIES
31293106257037