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Host-plant Recognition in the Onion Fly,
Delia antiqua (Meigen)

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Marion Olney Harris

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James R. Miller
Major professor

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HOST-PLANT RECOGNITION IN THE ONION FLY, *DELIA ANTIQUA* (MEIGEN)

By

Marion Olney Harris

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ABSTRACT

HOST-PLANT RECOGNITION IN THE ONION FLY,
DELIA ANTIQUA (MEIGEN)

By

Marion Olney Harris

Host-plant recognition in the onion fly, Delia antiqua (Meigen) is triggered by a complex set of plant stimuli. The nature of these stimuli was investigated by presenting reproductively mature onion fly females with surrogate plants and manipulating single characteristics of surrogates against a backdrop of unchanged stimuli. When presented with dishes containing a range of "foliar" shapes, onion fly females laid the most eggs around narrow (4 mm) vertical cylinders. Response to cylinders diminished when their diameter was increased or decreased, when cylinder height was reduced to less than 2 cm, and when cylinder/substrate angle deviated from 90^{o}. Differences in egg numbers on stimulatory and non-stimulatory forms reflected primarily differences in post-alighting pre-ovipositional behaviors.

Concentration and site of release of host-plant chemicals also influenced recognition. Onion fly females laid the most eggs on ovipositional dishes having n-dipropyl disulfide release rates of 1 to 6 ng/sec from polyethylene capsules placed beneath a sand substrate. When dipropyl disulfide was released from the wax coating of surrogate foliage rather than from the substrate, ovipositing females again responded differentially to various concentrations, laying more eggs around stems containing 75 and 107 ng/stem. Factorial combinations

of several concentrations released from surrogate foliage and substrate proved that releases from surrogates were more stimulatory than those from the substrate.

Interactions among chemical and non-chemical plant stimuli are critical to the host recognition process. Removal of chemical stimulants or alterations of color or size of foliar surrogates caused similar reductions in numbers of eggs laid. Reductions were generally larger in choice bioassays than in no-choice bioassays and reflected smaller percentages of females accepting ovipositional sites. Acceptance of less optimal ovipositional sites coincided with a period of rapid egg maturation in the ovaries.

Mechanisms of resistance to egglaying were investigated in several Eastern European onion breeding lines. Mean numbers of eggs laid on the breeding lines ranged from 2 to 34 eggs per plant. Differences in ovipositional responses were mirrored by differences in size among breeding lines. Analysis of covariance revealed no significant differences in ovipositional responses to breeding lines when differences in size were taken into account.

To my parents,
with much love
and many thanks

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GENERAL INTRODUCTION

Before exploiting the "biochemical wealth" (Dethier, 1982) of green plants, insect herbivores must overcome several evolutionary "hurdles" (Southwood, 1973). Adequate nutrients must be harvested in the face of toxic and deterrent secondary plant chemicals (Slansky and Scriber, 1985). Harsh environmental conditions, and predators and parasites associated with host plants must be avoided (Price et al., 1980). However, before these more intimate insect-plant associations can be established, insects must be able to find and recognize plants as potential hosts.

Dethier (1982) has defined "recognition" as that process whereby "a particular stimulus or configuration of stimuli originating in the external world matches a model in the neural world and upon occurrence of that congruency a specific behavior usually ensues". The process of recognition therefore involves at least three steps: perception of stimuli by peripheral receptors, processing of information by the CNS, and activation of motor and/or hormonal systems.

Much progress has been made towards understanding the first of these steps, that is, how insects recognize relevant environmental stimuli. The visual systems of insects are designed to perceive movement, but are also capable of discriminating colors and complex forms (Goldsmith and Bernard, 1974). Mechanoreceptors provide information about solid environmental structures (Schwartzkopff, 1974)

and encode stimuli relevant to mating (Huber, 1978), and predator detection (Treat, 1983). Chemoreceptors allow detection of minute amounts of chemical stimuli relevant to mate and food finding (Boeckh

and encode stimuli relevant to mating (Huber, 1978), and predator detection (Treat, 1983). Chemoreceptors allow detection of minute amounts of chemical stimuli relevant to mate and food finding (Boeckh and Ernst, 1983).

Of this work, research on insect gustation and olfaction has most specifically addressed questions about host-plant recognition. Both quantitative (intensity) and qualitative information are encoded in series of action potentials. Intensity is signalled by the frequency of action potentials generated by peripheral receptors (Dethier, 1976). Some specific qualitative information may also be encoded by peripheral receptors; deterrents evoke high frequency or bursting trains of action potentials in peripheral receptors, signals which may be read by the CNS as "do not feed or lay eggs" (Dethier, 1982).

Because peripheral chemoreceptors are neither numerous nor specialized enough to specifically encode the hundreds of chemicals insects encounter during host finding and acceptance, recognition of host-plant quality is thought to occur in the central nervous system during a series of cross-comparisons of inputs from various receptors (Dethier, 1977; 1982 and references therein). These hypothetical cross-comparisons are believed to generate unique patterns of responses that are utilized in the host-recognition process. This model of neural recognition is supported by both anatomical and neurophysiological evidence. Mapping of individual neurons has

indicated that inputs from visual, chemo- and mechanoreceptors converge spatially on the mushroom bodies of the deutocerebrum (Strausfeld, 1976; Ernst et al., 1977; Suzuki et al., 1976). Because hundreds of receptor inputs may converge on single cells, cells receiving this summated input often show great sensitivity to small amounts of odors (Boeckh and Ernst, 1983). Some cells in the deutocerebrum also show specificity, responding only when certain spatial or temporal patterns of stimuli are presented (Strausfeld et al., 1984). However, though this evidence is suggestive, it is not known whether these cells actually trigger appropriate behavioral responses, or whether further filtering and integration of sensory information occurs closer to motor systems (Boeckh and Ernst, 1983).

Processes involved in host-plant recognition can also be investigated indirectly by studying behavioral responses to plant stimuli. In behavioral studies, neural aspects of recognition are regarded as a black box. Input to the black box is altered by manipulating single attributes against a backdrop of unchanged stimuli. If the altered stimulus is relevant to the process of recognition, behaviors indicating recognition will also change. Such manipulative experiments have been used with great success by ethologists for many decades (Tinbergen, 1958; Tinbergen and Perdeck, 1950; Alcock, 1979) and have yielded many insights into how animals "see" their worlds.

The world I chose to investigate by behavioral studies was that of the onion fly, Delia antiqua (Meigen). When I initiated research for my Master's degree, this anthomyiid fly was thought to "view" the plant world primarily through its chemical senses. Females could be

stimulated to oviposit by chemicals unique to the onion and its relatives (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Ishikawa et al., 1978). Because these onion compounds are toxic to both microorganisms (Cavallito and Bailey, 1944) and insects (Amonkar and Banerji, 1971), their exploitation by D. antiqua for host finding is an excellent example of one-upsmanship in the coevolutionary arms race between insects and plants (Erlich and Raven, 1965).

However, early in my studies I discovered that things were not as simple as they appeared (Harris, 1982; Harris and Miller, 1982). When added to oviposition dishes containing only moist sand or sand plus chemical stimuli, onion foliage surrogates caused D. antiqua females to lay 8 to 18 times more eggs and to localize egg deposition within 1 to 2 cm of the surrogate base. Chemical stimuli were evidently not the sole behavioral operants in the relationship between D. antiqua and its' host plants.

By fashioning surrogates and altering only color stimuli while holding all other stimuli constant (Harris and Miller, 1983), I was able to show that certain colors stimulate more oviposition than others in choice tests. Observations of individual females interacting with surrogates of different colors indicated that color influenced both alighting and post-alighting behaviors. This finding ran counter to a long-held assumption about the relative importance of plant color stimuli; plant color was believed to be important only during the early stages of host finding, and not sufficiently unique to provide useful information in the final and more critical stages of host acceptance (Thorsteinson, 1960; Beck, 1965).

This work indicated that further studies on the onion fly/onion

system might not only advance our understanding of sensory modalities functioning in host-plant recognition, but also provide a more balanced view of the relative importance of chemical vs. nonchemical stimuli. I therefore pursued these studies for my Ph.D. research. My goals were to further define the range of plant stimuli eliciting oviposition, and to use this knowledge to test the hypothesis that oviposition is stimulated by certain combinations of plant chemical, color and structural stimuli. The results are presented in the following four chapters.

In Chapter One, I address the hypothesis that plant form influences ovipositional behavior of D. antiqua. Influences on oviposition were quantified by two methods. Plant forms were first screened for effects on oviposition by counting eggs laid around foliar surrogates varying in shape, size, and angle relative to the substrate. These choice tests were followed by observations and quantification of behavioral responses of individual females to stimulatory and non-stimulatory forms.

In Chapter Two, the influence of a single host-plant chemical (n-dipropyl disulfide) was investigated within the context of non-chemical foliar cues. Dose-response curves for subterranean and foliar releases of dipropyl disulfide were generated by screening a wide range of concentrations in choice tests. The importance of site of release was also assayed in choice tests, by presenting females with factorial combinations of several concentrations of dipropyl disulfide released from surrogate foliage and the substrate.

In Chapter Three, I address the hypothesis that non-chemical foliar stimuli are primary rather than secondary indicators of "host" to D. antiqua. Effects of removing non-chemical stimuli (color and optimal size) vs. removing chemical stimuli (dipropyl disulfide) were compared in choice bioassays, in which populations of flies were given access to all treatments, and in no-choice bioassays, in which individual females were given access to a single treatment for 8-days. While choice tests were designed to reveal ranking of ovipositional preferences, no-choice tests were designed to quantify the length of time (latencies) required before females accepted ovipositional treatments lacking chemical vs. non-chemical plant stimuli. The rate at which eggs were matured in the ovaries was also quantified to determine whether ovipositional response latencies could be correlated with physiological phenomena.

In the fourth and final chapter, the role of chemical vs. non-chemical plant stimuli was investigated in a more natural setting. Eastern European onion breeding lines tested in field trials in the Netherlands (de Ponti, unpublished) were believed to be resistant to D. antiqua because of non-preference mechanisms mediated by plant chemicals. Dr. de Ponti sent me these breeding lines in hopes that chemicals conferring resistance could be identified. Breeding lines were first tested in choice bioassays to determine whether resistance to oviposition was expressed in plants grown in the greenhouse. After establishing that resistance was expressed, I investigated mechanisms of resistance using foliar surrogates that mimicked various size parameters of onion breeeding lines.

CHAPTER 1

Foliar Form Influences Ovipositional Behavior

INTRODUCTION

Although behavioral mechanisms underlying patterns of oviposition of the onion fly, Delia antiqua (Meigen) (Diptera: Anthomyiidae), are not entirely understood, chemical stimuli are generally credited as the major effectors. Oviposition on members of the genus Allium is positively correlated with the presence of stimulatory alkyl sulfides (Vernon et al., 1978; Ishikawa et al., 1978). Increased acceptance of infested and mechanically injured onions (Allium cepa) is caused by qualitative and quantitative changes in volatiles brought about by colonizing bacteria (Ikeshoji et al., 1980). However, other aspects of the onion fly's ovipositional patterns, such as high acceptability of certain developmental stages of A. cepa (Gray, 1924; Ellis and Eckenrode, 1979; Labeyrie, 1957), cannot at present be explained by differential effects of chemical stimuli.

In addition to chemical information, D. antiqua uses color and structural stimuli of onion foliage when ovipositing (Hough, 1981; Harris and Miller, 1982) and responds maximally when all three stimuli are combined (Harris and Miller, 1982). In this study we investigate further the influence of foliage form on D. antiqua ovipositional behavior. The structural characteristics that are most and least stimulatory for oviposition, and whether these characteristics exert their influence during alighting or post-alighting phases of behavior, have been determined.

MATERIALS AND METHODS

Insects and Foliar Surrogates

D. antiqua pupae were collected (Fall of 1981) from onion culls left in harvested fields in Grant, MI. Emerging adults were placed in 165 x 65 x 85 cm screened cages housed in environmental chambers illuminated by Verilux fluorescent bulbs with a LD 16:8 (21 ± 1°C and 35 ± 5% RH). Cages were provisioned with water, honey, and a dry artificial diet (Harris and Miller, 1982). Flies used in "choice" and behavioral experiments were 2-5 and 5-9 generations removed from field populations, respectively.

Foliar surrogates were made from cement, wood, or glass and, based on results of Harris and Miller (1983), were painted yellow with 5 coats of Liquitex Cadmium Yellow Light acrylic paint (value 8.7, chroma 11, Munsell 7.5 Y). Several of the experiments were repeated with surrogates painted with oil pigments (Windsor and Newton Cadmium yellow, Windsor green, Cobalt blue, Lamp blue, and Flake Everwhite) mixed to match (as detected by the human eye) the green of onion foliage. Painted objects were allowed to dry for 3 weeks before use in experiments. Inhibition of oviposition due to oil-paint was reduced by coating all oil-painted green objects with a 0.5 mm thick layer of household paraffin wax (Cullen Industries, Huntingdon Valley, PA).

Ovipositional Dishes

Ovipositional dishes were assembled 12 h before exposure to flies. Ten ml of chopped onion was placed at the bottom of each 14 x 14 x 2.5 cm plastic dish and covered with 300 ml of moist silica sand.

Surrogate foliage was added to dishes immediately before placement in cages. All non-foliar characteristics were therefore held constant in all treatments.

"Choice" Experiments - Oviposition

In "choice" experiments (Summer of 1982), arrays being tested were placed in cages (165 x 65 x 85 cm) for four 24-h periods prior to the initiation of experiments to reduce variability due to training effects (Prokopy et al., 1982) which may have occurred during previous "choice" experiments. Treatments within the replicate were spaced evenly, (c. 30 cm apart from each other and c. 40 cm from the cage walls) and were removed after 6 h. Experimental design was randomized complete block, with one replicate of each treatment per cage per sampling period. Results were recorded as percent of the total eggs laid per replicate. Experiments were replicated 10-13 times. Data generated by these experiments were heteroscedastic as explained by Harris and Miller (1983). Transformations did not result in homogeneity of variances and furthermore increased nonadditivity; therefore, results were analysed by using a Kruskal-Wallis test followed by Dunn's Multiple-Comparison test using rank sums and an experimentwise error rate of $p<0.10$ (Daniel, 1978). The experimental error rate, is an overall rate of significance and establishes the probability of making only correct decisions at 1-alpha when the null hypothesis of no difference among populations is true. The estimate of differences between treatments given by such an error rate is therefore quite conservative compared to conventional parametric tests (Daniel, 1978).

Experiment 1 - Diverse Shapes

Flies were presented with seven 3-dimensional objects molded from cement and painted yellow: a horizontal narrow cylinder (8 mm diameter and 60 mm long), a vertical narrow cylinder (8 mm diameter and 60 mm long), an inverted cone (60 mm diameter at top, 4 mm at base, 60 mm tall), a cone (same dimensions as inverted cone), a wide cylinder (60 mm diameter and height), a sphere (60 mm diameter), and a hemisphere (60 mm diameter, 30 mm height). The inverted cone and the narrow vertical cylinder were held upright in the center of the dish by a wire base and a 20 mm extension, respectively, which lay beneath the sand surface. Wide-based objects were pressed into the sand in the center of the dish to a depth of 5 mm.

Experiment 2 - Cone Width

A series of objects intermediate to and including the inverted cone and vertical narrow cylinder was cut by lathe from poplar wood and painted yellow. These consisted of 5 inverted cones (60 mm tall) with top diameters of 60, 45, 30, 15 and 8 mm and a basal diameter of 4 mm, and a vertical narrow cylinder, 60 mm tall with top and basal diameters of 8 mm (see Fig. 1b). They were held upright at the center of the dish as previously described.

Experiment 3 - Cylinder Diameter

One, 2, 4, 6, 8, 10, 12, 16, 20 mm diameter Pyrex^{R} tubing was cut into 8 cm lengths, and was heat sealed. These cylinders were painted yellow and placed upright in the center of the prepared dishes so that

6 cm of the cylinder stood above the substrate surface. Treatments were evaluated in a "choice" test as above.

Experiment 4 - Cylinder Height

Yellow surrogates were made from 4 mm diameter Pyrex^{R} (optimal diameter in Experiment 3) tubing cut into lengths ranging from 1 to 50 cm. Painted cylinders were again placed upright in the center of the dish.

Experiment 5 - Cylinder Angle Relative to Substrate

The 4 mm Pyrex^{R} glass tubing was cut into lengths of 12 cm, heat sealed at one end, and painted yellow. Three sets of the cylinders were bent at 2 cm from their open end so they formed 150^{o}, 120^{o} and 90^{o} angles, and a fourth set remained straight. Bent cylinders were placed in the corner of a square dish (3 cm in on the diagonal) so that 2 cm of the open end extended vertically beneath the sand and the cylinders stood at 90, 60, 30, and 0 degree angles with respect to the substrate surface (see Figure 4).

Experiment 6 - Alighting

A series of shapes, all having 36 cm^{2} surface areas, was placed in cages to determine whether female onion flies alighted more frequently on shapes characteristic of onion plants. The following shapes were cut from 1 mm thick formica: a horizontal rectangle (18 x 2 cm), a vertical rectangle (18 x 2 cm), 2 triangles (9 cm on a side), a square (6 x 6 cm), a circle (6.8 cm diameter) and a half circle (9.7 cm base). Extensions (2.5 x .7 cm) at the base of the shapes held

each vertically above the sand.

During periods of peak oviposition (early morning and late afternoon), treatments were placed in random positions (c. 10 cm apart) in cages containing several hundred male and female flies. The number of females alighting on each of the 7 shapes were recorded for 30 minute periods. Positions of the 7 treatments were randomized after each observation period (6 observation periods total). Data generated by these experiments met the assumptions underlying parametric tests and therefore were subjected to an F test.

Behavioral Observations

To determine why treatments with objects of particular shapes, sizes and angles received more eggs, we compared the alighting and post-alighting pre-ovipositional behaviors of D. antiqua females on "stimulatory" and "non-stimulatory" ovipositional treatments. Methods used were identical to those described in Harris and Miller (1983). During observations and 48 h prior to the initiation of observations, dishes containing the test objects were placed 6 cm apart in the middle of bioassay cages (60 x 60 x 80 cm).

Our choice of behaviors for quantification was based on observations of alighting and post-alighting pre-ovipositional behaviours on foliar surrogates. After alighting on an ovipositional dish, a female extends her proboscis to the sand and then approaches, ascends, and descends the surrogate. Upon reaching the base of the surrogate, the female again extends her proboscis and examines the surrogate and the sand around its base. The stem run is repeated several times, sometimes with the ovipositor extended and wiping from

side to side as the fly ascends and descends the surrogate. The female then runs onto and moves over the sand, repeatedly probing around the base of the surrogate with her ovipositor. Probing is occasionally interrupted by abbreviated stem runs (1 cm) or short runs away from the base of the surrogate. Eventually the female settles, generally within 2 cm of the surrogate, and lays several eggs. After ovipositing, a female often repeats the sequence of post-alighting behaviors and oviposits.

The following behaviors were recorded during a one h observation period: alighting on object and sand; stem runs; and the number of stem runs followed by probing. Stem runs were scored when a female walked from the sand onto a stem and ran at least 1.5 cm up the stem and then back down to the sand, without stopping on the stem for more than 15 s. Stem runs shorter than 1.5 cm consisted of slower circling movements around the base of the surrogate. Because it was difficult to determine where these shorter runs began and ended, we did not use them to quantify behavioral differences. Stem runs that were interrupted for more than 15 s were not recorded because they were hard to keep track of and because they were rarely completed. Stem runs followed by probing were scored when a female completed a stem run and then probed the sand repeatedly with her ovipositor upon reaching the base of the stem. When the observation period ended, treatments were removed immediately from cages and eggs were counted. Pairs of treatments being compared consisted of the vertical narrow cylinder and the cone from Experiment 1; the narrow inverted cone and the widest inverted cone from Experiment 2; the 1 mm and the 4 mm diameter stems from Experiment 3; the 4 mm and 20 mm diameter stems

from Experiment 3; the 2 cm and 15 cm tall stems from Experiment 4; and the 90^{o} and 30^{o} angles from Experiment 5. Paired observations were replicated 6 times. Alighting and oviposition were compared using a paired sign test. Ratios of behaviors were not paired because they were normalized on a per alighting, per stem run, and per probing basis and therefore were analysed by applying a Mann-Whitney Test for 2 independent samples (Daniel, 1978).

RESULTS

Experiment 1 - Diverse Shapes

Yellow curved objects (Figure 1a) having narrow bases, or straight narrow surfaces lying horizontally or rising vertically from the sand, stimulated more egg laying than objects having wide bases. The narrow vertical cylinder combined several of these characteristics and received the most eggs (39%), though not statistically more eggs than the inverted cone (31%) or the horizontal cylinder (12%). Wide-based objects did not receive significantly more eggs than dishes containing only sand and chopped onion. Experiments with identical surrogates painted green yielded a similar pattern of egg deposition.

Comparisons of behaviors on cylinders and cones indicated that shape influenced whether or not stem runs and probing occurred (Table 1) and reconfirmed the positive correlation between the frequency of these two behaviors and numbers of eggs laid (Harris and Miller, 1983). While females tended to alight more frequently on the cone (not significantly different at $p < 0.05$), very few of these females completed stem runs. Runs up cones were slow and followed the path of a parabola rather than a straight line to the top of the cone and back

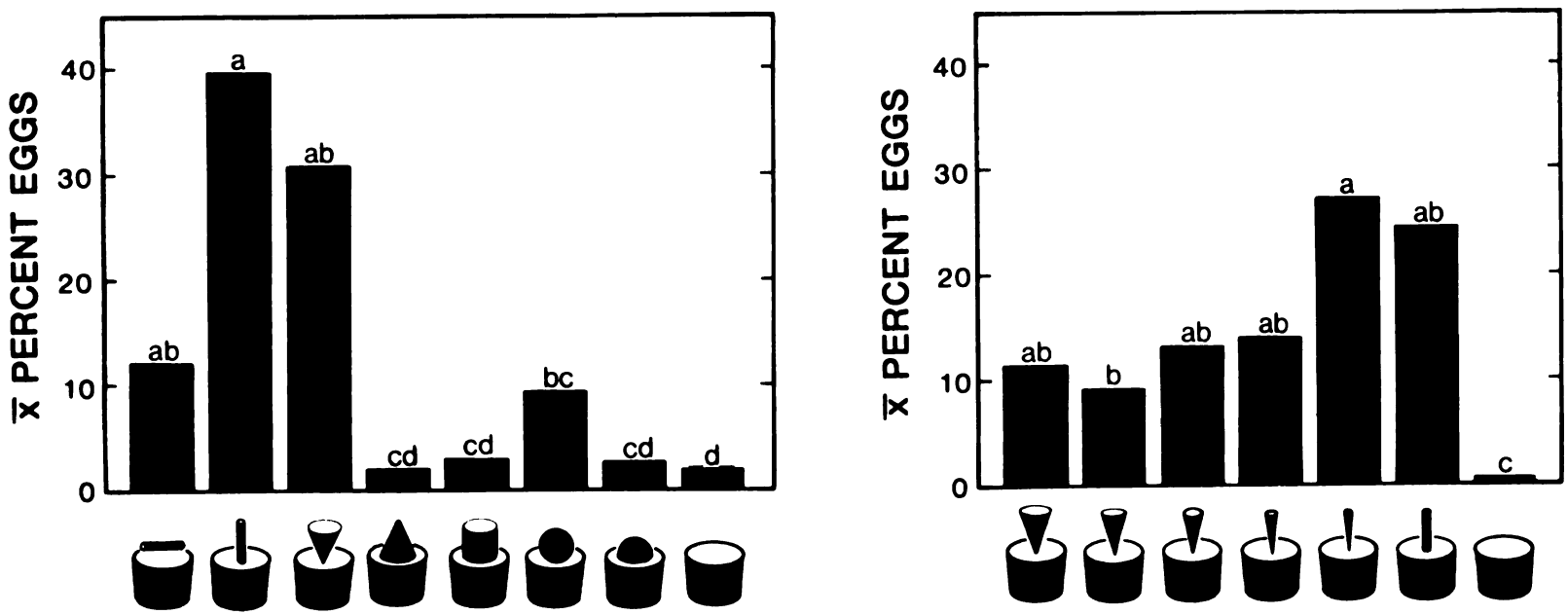

Figure 1. Influence of (a) diverse 3-dimensional shapes and (b) cone width on oviposition. Dishes used in all "choice" experiments were square but are shown here as circular for ease of drafting. Means accompained by the same letter are not significantly different, using an experimentwise error rate of a = 0.10 (Kruskal-Wallis test followed by a Dunn's Multiple-Comparison test). Total eggs for (a) = 10,958 for (b) = 3,640.

TABLE 1. Pre-ovipositional behaviours compared on stimulatory and non-stimulatory treatments from 'choice' experiments reported in Figs. 1-4.

Experiment	Surrogates[1]	Total number of visitors[2]	X number alighting on surrogates[3,4]	Stem runs per visit[3,5]	Probes per stem runs[3,5]	Eggs per probe[3,5]	Percentage of total eggs[3,4]
1. Shapes	Narrow (8 mm) vertical cylinder	935	42	0.29*	0.44*	1.10	96*
	Upright cone	1006	55	0.62*	0.14*	2.50	4*
2. Shapes	Narrow (8 mm) inverted cone	437	13*	0.62*	0.54	3.20	76*
	Wide (60 mm) inverted cone	483	31*	0.14*	0.40	0.90	24*
3. Cylinder diameter	4 mm diameter	548	13*	0.51*	0.33*	2.54	92*
	1 mm diameter	455	5*	0.11*	0.67*	2.50	8*
4. Cylinder diameter	4 mm diameter	348	7*	0.55*	0.44*	3.85	82*
	20 mm diameter	490	27*	0.06*	0.20*	0.53	18*
5. Cylinder height	15 cm height	442	22*	0.62*	0.53*	1.40	85*
	2 cm height	289	3*	0.23*	0.40*	2.20	15*
6. Cylinder angle	90°	665	28	0.44*	0.44	1.92*	92*
	30°	637	23	0.11*	0.33	0.65*	8*

[1] Surrogates used in behavioural observations were identical to those used in 'choice' experiments shown in Figs. 1-4.

[2] Total number of females alighting on surrogates and sand surrounding surrogates during six observation periods.

[3] Behaviours on surrogate pairs followed by asterisk are significantly different at $P<0.05$.

[4] Paired sign test.

[5] Mann-Whitney test.

down to the sand. After completing this parabolic path, females encountering the surrogate-sand interface often flew away without repeating the stem run or initiating probing. In contrast, 14 times more stem runs occurred per arrival on the cylinder. These stem runs were performed rapidly and without interruptions and were followed by repeated probing of the sand 3 times more often than on the cone. Eggs laid per probe were not statistically different for the cylinder and cone, perhaps because in the Delia spp., the final number of eggs laid per probe may be more affected by substrate characteristics (Zohren, 1968; Barlow, 1965; Somme and Rygg, 1972). We conclude that shape influenced oviposition primarily via effects on stem runs, and hypothesize that narrowness and slope of the running surface are sensed during the runs up and down the cylinder, while basal width is sensed during shorter stem runs which occur after repeated probing and entail sidways circling movements around the bottom 1.5 cm of the surrogates.

Experiment 2 - Cone Width

This experiment was conducted to determine why two very different shapes, the narrow vertical cylinder and the inverted cone, received similar numbers of eggs in Experiment 1. A series of 60 mm high objects (Figure 1b) was constructed which combined the narrower base of the inverted cone (4 mm diameter) with the sharper angle of curvature and more vertical surface of the cylinder. Sharpening the angle of curvature and/or making the surface of the cones more vertical while holding basal diameter constant increased oviposition (Figure 1b). The narrower inverted cone, which had a base width of 4

mm, received slightly (but not significantly) more eggs (27%) than the narrow cylinder (24%) which had a base width of 8 mm.

Greater resolution was gained when a pairwise comparison was made of pre-ovipositional and ovipositional behaviors performed on the narrowest and the widest inverted cones (Table 1). As in the previous comparison of cylinder and cone, females again alighted more frequently on the larger of the two objects, the wide inverted cone. However, females alighting on the narrow inverted cone were 4 times more likely to complete a stem run than females on wide inverted cones. After completing a stem run, females on narrow inverted cones showed a slightly (but not significantly) greater tendency to probe. More eggs were laid around the narrow cone, but, eggs per probe showed a reverse trend: wide inverted cones received more eggs per probe than narrow cones (NS at $p < 0.05$). Again, shape appeared to have its greatest impact on stem runs; sharper angle of curvature, vertical angle, and/or narrower widths stimulated females to complete more stem runs.

Experiment 3 - Cylinder Diameter

Having determined what shape characters influenced oviposition, we investigated effects of surrogate foliage size by varying the diameter and height of vertical cylindrical objects. When presented with cylinders (6 cm in height) varying in diameter from 1 to 20 mm, females laid significantly more eggs around 4 and 6 mm cylinders (23 and 24%, respectively) than around 1,16, and 20 mm cylinders (Figure 2). While diameters 2 through 20 mm received significantly more eggs than a cylinder-less control, there appeared to be a threshold below

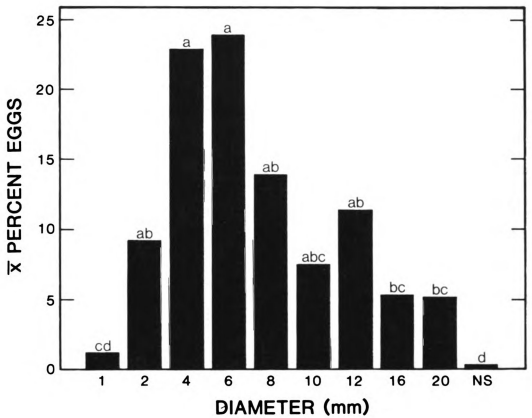

Figure 2. Influence of cylinder diameter on oviposition. Means accompanied by the same letter are not significantly different using an experimentwise error rate of a = 0.10 (Kruskal-Wallis test followed by a Dunn's Multiple-Comparison test). Total eggs = 3,198.

which D. antiqua was not stimulated to oviposit: the 1 mm treatment (1%) did not receive significantly more eggs than control (1%), while the 2 mm treatment received 9% of the eggs. When this series of diameters was run with taller cylinders (16 cm) or green cylinders, the same pattern of oviposition emerged (Harris, unpublished data).

Two pairs of treatments from the diameter choice experiment were compared in behavioral observations to ascertain why narrower and wider cylinders received fewer eggs than did 4 mm diameter cylinders. When 1 and 4 mm cylinders were placed in cages, females landed more frequently on the object with the larger surface area (Table 1). After alighting, females were c. 4 times more likely to perform stem runs on the 4 mm cylinder and were c. 4 times more likely to probe after completing a stem run. Once probing occurred, there was an equal probability that females would lay eggs. When compared to a cylinder of wider diameter, the 4 mm cylinder elicited one-fourth the alightings elicited by the 20 mm cylinder, but stimulated 6 times as many stem runs per arrival. After completing a stem run, females on the 4 mm cylinder were c. 2 times more likely to probe (significant at $p < 0.05$), but significantly more eggs in total.

Experiment 4 - Cylinder Height

While a cylinder 6 cm tall elicited significantly more oviposition than did a 1 cm tall cylinder and cylinder-less control (Figure 3), there were few significant differences between cylinders 2 to 50 cm tall.

Greater resolution was gained when a pairwise comparison was made of behaviors occurring on the 2 and 15 cm cylinders, two treatments

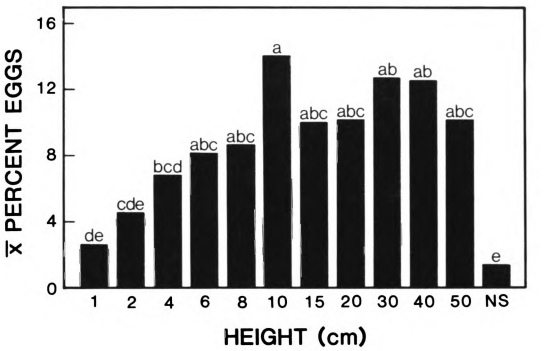

Figure 3. Influence of cylinder height on oviposition. Means accompanied by the same letter are not significantly different using an experimentwise rate of a = 0.10 (Kruskal-Wallis test followed by a Dunn's Multiple-Comparison test). Total eggs = 13,884.

that did not receive significantly different numbers of eggs in the choice experiment. The 15 cm cylinder, which had c. 7 times the surface area of the 2 cm tall cylinder, elicited 8 times more landings than the smaller cylinder (Table 1). Females arriving on the taller stems were c. 3 times more likely to complete stem runs, and after completing stem runs were c. 3 times more likely to probe. Females probing around short and tall cylinders laid similar numbers of eggs per probe.

Experiment 5 - Cylinder Angle Relative to Substrate

Females laid more eggs around the vertical cylinders and laid progressively fewer eggs as cylinder angle deviated from the perpendicular (Figure 4).

Quantification of behaviors on the vertical cylinder (90^{o}) and 30^{o} cylinder indicated that post-alighting behaviors were most affected by cylinder angle (Table 1). As would be expected with two objects of identical surface area, there was no significant difference in the number of females alighting on the two types of cylinders. After alighting, females on vertical cylinders were 4 times more likely to complete stem runs; however, after completing a stem run, females on the two treatments were equally likely to probe. This same pattern was observed when the narrow inverted cone and the wide inverted cone were compared, suggesting that deviations from the vertical hinder the initiation or completion of stem runs. If stem runs are successfully completed; however, females on either surrogate type are equally likely to probe. Stem angles of 30^{o} also reduce numbers of eggs laid per probe, an effect not seen with other shapes.

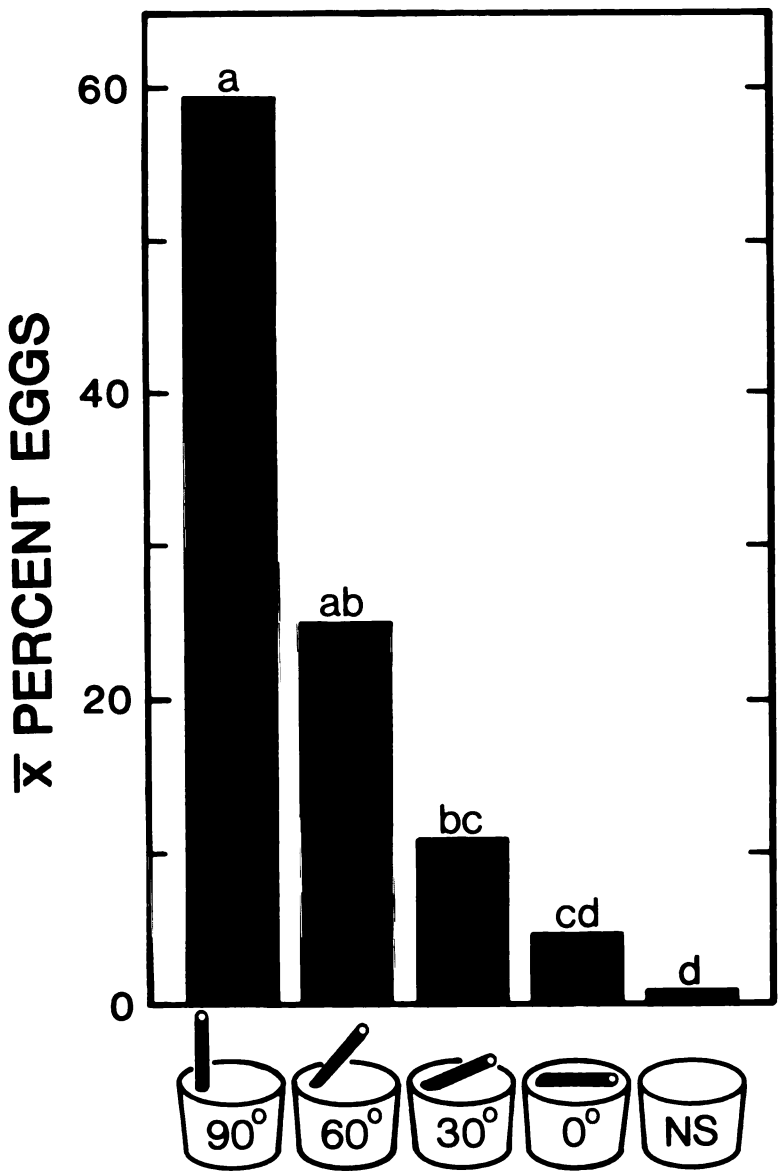

Figure 4. Influence of cylinder angle on oviposition. Means accompanied by the letter are not significantly different using an experimentwise error rate of a = 0.10 (Kruskal-Wallis test followed by a Dunn's Multiple-Comparison test). Total eggs = 7,699.

Angled cylinders may not provide a vertical surface against which females can firmly position themselves while ovipositing into the crevice which is formed at the sand/surrogate interface.

Experiment 6 - Alighting

While more females alighted on vertical cylinders (Figure 5), there were no significant differences between numbers alighting on different shapes. Because we were not able to distinguish between females searching for oviposition sites and those alighting for other reasons, the possibility remains that pattern discrimination in ovipositing females was masked by indiscriminate alighting by other females.

Discussion

In spite of the recognized versatility of insects and the diversity of sensory modalities they use to monitor their environments (see Griffin, 1981), many studies on insect-plant relationships have focused on only the chemical sense. The preponderance of these studies has allowed permeation of the literature by a "subtle causality" (Dethier, 1982) which links particular plant secondary compounds to patterns of herbivory, often ignoring the possibility that "compounds may in fact be directly correlated with other unknown factors which are the real behavioral operants" (Dethier, 1982). Summary attention has also been paid to the possibility that insects sense plants via multiple sensory modalities and that interactions among stimuli provide far more information than any one stimulus taken

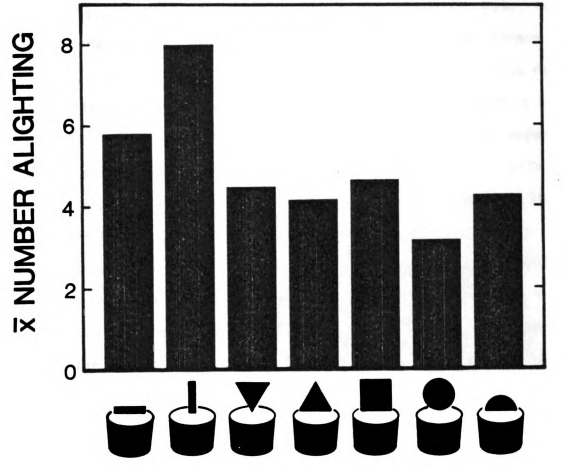

Figure 5. Influence of shapes having identical surface areas on alighting behavior. Means were not significantly different at $P < 0.05$ (F test). Total alighting = 208.

alone (Kennedy, 1965).

Given the literature on D. antiqua, it is understandable that such a causality has come into existence. Studies have shown that the characteristic volatile sulfides and thiols of onions stimulate alighting, probing (Matsumoto and Thorsteinson, 1968), and ovipositional behavior (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Ishikawa et al., 1978), and that microorganisms play a role in the release of these and other stimulatory compounds (Hough et al., 1981; Ikeshoji et al., 1980). Correlations established between chemical stimuli and ovipositional behavior correspond with patterns of herbivory seen within the genus Allium (Ellis and Eckenrode, 1979) and within A. cepa (Ikeshoji et al., 1980; Ikeshoji et al., 1982).

Chemical stimuli are not, however, the sole behavioral operants in the relationship between D. antiqua and its host plants. When added to ovipostion dishes containing only sand (Hough, 1981; Harris and Miller, 1982) or sand plus chemical stimuli (Harris & Miller, 1982), onion foliage surrogates caused D. antiqua females to lay 8 to 18 times more eggs and to localize egg deposition within 1 to 2 cm of the surrogate base. Such localization does not occur when only chemical stimuli are provided.

In the present experiments, we have shown that, in the presence of color and chemical stimuli, size, shape, and angle of foliar surrogates also influence oviposition. Of these characteristics, size alone influences both alighting and post-alighting phases, albeit with opposite effects; there was little or no relationship between sizes stimulating alighting and sizes accepted for oviposition. Color also influences both alighting and post-alighting behaviors; however,

colors that stimulate more alighting also stimulated more stem runs, probing and oviposition (Harris and Miller, 1983). All of the three foliar characteristics tested influenced post-alighting pre-ovipositional behaviors. The frequency of these behaviors is highly correlated with numbers of eggs laid (Harris and Miller, 1983). After alighting, and in the presence of onion chemicals, females were stimulated to approach colored surfaces arising from the substrate. Females rapidly ascended narrow (4-8 mm) vertical cylinders to a height of 3 to 6 cm, then descended. After several stem runs, females reaching the stem/sand interface probed the sand repeatedly with the ovipositor. Unless interrupted, females completing these pre-ovipositional behaviors oviposited within 1 cm of the base of the surrogate. Females alighting on other shapes, or larger, smaller, or non-vertical cylinders either: did not initiate or complete stem runs, or did not go on to probe after completing a stem run. Such females rarely oviposited.

How then did D. antiqua females perceive these characteristics? The poor correlation between alighting and ovipositional preferences, which was also observed with D. radicum (Zohren, 1968), indicates that females do not determine the suitability of foliar shape and size characteristics simply by looking at them from a distance. Instead, form is sensed after alighting, during runs over the plant or surrogate surface. Such exploratory runs over plant surfaces and around stem bases are common among dipteran herbivores (Bohlen, 1967; Städler, 1977; Ibbotson, 1960; Zohren, 1968; Wiesmann, 1937; Prokopy and Boller, 1971). Herbivores engaging in such runs respond to structural and chemical characteristics of foliage and fruits of host

plants (Städler, 1977; Städler and Buser, 1982; Prokopy and Boller, 1971; Ibbotson, 1960).

It is not known whether runs over the plant surface allow the central nervous system to construct a point-by-point image of the entire foliage or fruit form, or whether such point-by-point images stimulate subsequent pre-ovipositional behaviors. A simpler explanation is that females perceive only small parts of the form at any one point during the run; if each part stimulates the female to continue and complete the run, the completed run (rather than the point-by-point image) stimulates females to perform subsequent pre-ovipositional behaviors. At present, we cannot differentiate between these hypotheses.

Foliage shape, size, and angle may provide D. antiqua and other herbivores with information critical to the survival of their offspring. D. antiqua larvae are subject to desiccation in dry soils (Workman, 1958). Therefore, survival is better if eggs are laid in moist soil near food resources. Foliar form stimulates such a localization of eggs. Information about nutritional suitability may also be provided. Rausher et al.(1981) suggested that some insects may not order ovipositional preferences along taxonomic lines, but instead may discriminate among host plants using a few characteristics unrelated to taxonomic affinities. While some chemical stimuli are common to numerous plant species (Visser et al., 1979), plant form, size, and color can cross plant taxonomic lines and might provide generalised information about resource size and nutritional characteristics such as leaf toughness and water content.

However, perception of plant form might also provide more

specific information. While not unique to one type of fruit, structural characteristics most acceptable to the cherry fruit fly do correspond to the size and developmental stages of cherries that are optimal for larval development (Prokopy and Boller, 1971). In the onion fly, foliar characteristics stimulating oviposition are relatively uncommon outside the genus Allium, and seem to be correlated positively to those developmental stages that may be optimal for larval growth (Perron, 1972). Because chemical and color stimuli might be expected to covary with plant shape and size as host plants age or become stressed by diseases or insects, herbivores could receive and integrate information about host plant suitability from multiple sensory modalities.

Although there are indications in the literature that D. antiqua's host acceptance behaviors are correlated with host plant suitability (Perron, 1972; Harris, 1982; Hough, 1981), alternatives to the adaptationist's perspective should be considered (Gould and Lewontin, 1979). Instead of being strictly adaptative, behaviors observed in the laboratory may be evolutionary relics, indicating more about the suitability of previous rather than present host plants. Studies comparing ovipositional preferences with host suitability would help to resolve this issue.

CHAPTER 2

Behavioral Responses to n-Dipropyl Disulfide: Effects of Concentration and Site of Release

INTRODUCTION

Like many other herbivore specialists, the onion fly, Delia antiqua (Meigen) has a narrow host range delimited by preferences of egglaying females. Newly eclosed larvae have limited mobility and are subject to desiccation in open areas and dry soils; eggs must therefore be placed close to or on proper hosts (Workman, 1958).

The question of what stimuli signal "host" to ovipositing D. antiqua has been investigated in some detail. Females oviposit only on a small number of species in the genus Allium. Initial studies focused on sulfur compounds unique to this plant genus. Secondary chemicals are formed in alliums when cells are disrupted, and the normally compartmentalized cysteine sulfoxides contact the enzyme alliinase (Whitaker, 1976). The labile thiosulfinates produced by this reaction then react further at room temperatures to form thiosulfonates and sulfides such as dipropyl disulfide (Pr_2S_2) and propanethiol. Secondary chemicals are also formed from intact onions when propenyl cysteine sulfoxides are exuded by roots into the soil and then converted into sulfides by soil bacteria (Coley-Smith and King, 1969; Hough et al., 1981; Ikeshoji, 1984).

Host finding and acceptance in the onion fly are stimulated by the end-products of Allium defensive reactions, the stabile mono-, di- and trisulfides. Increased numbers of flies are caught in traps baited with Pr_2S_2, propanethiol (Matsumoto, 1970), methyl propyl and

propenyl propyl disulfide and several other mono- and disulfides (Vernon et al., 1981). Trap catches in response to Pr_2S_2 are significant at release rates of 60 ug per hour (Dindonis and Miller, 1981) and may be influenced by reproductive state, with gravid virgin females being the most responsive (G. Judd, personal communication). Final acceptance of oviposition sites is also influenced by onion chemicals. Propanethiol and Pr_2S_2 stimulate females to probe with their ovipositors and lay eggs (Matsumoto and Thorsteinson, 1968), as do numerous other compounds containing a sulfur atom having two unshared pairs of electrons and bonded to a saturated hydrocarbon chain 3 to 5 carbons long (Vernon et al., 1978; Ishikawa et al., 1978).

Non-chemical plant stimuli also play an important role in host recognition. Addition of surrogate foliage to oviposition dishes synergizes responses to onion slices buried beneath moist sand (Harris and Miller, 1982). Color (Harris and Miller, 1983),shape, and size (Harris and Miller, 1984) of surrogates have major effects on acceptance of ovipositional sites. These foliar stimuli are sensed by females during extended runs over the surface of surrogates.

While observing females engaged in these pre-ovipositional examining behaviors, we wondered whether runs over the surface of onion foliage might expose flies to plant chemicals in addition to color, size and shape stimuli. The importance of chemicals released from foliage had been dismissed by Vernon et al. (1977) who reported that extracts from onion bulbs were more stimulatory than extracts of foliage; however, recent work on the carrot fly, which performs similar runs over host plant foliage, has shown that a complex mixture

of chemicals in surface waxes of foliage is critical to host recognition (Städler and Buser, 1984).

Our approach to the question of whether D. antiqua females obtain chemical information from onion foliage was to quantify effects of releasing a range of doses of the ovipositional stimulant, Pr_2S_2, from the substrate as well as from foliar surrogates. Locations of various pre-ovipositional behaviors were also recorded to clarify whether females examine certain areas of the foliage and substrate more thoroughly than others before committing their eggs to a host.

MATERIALS AND METHODS

Insect Rearing

Delia antiqua pupae were collected (autumn of 1981 and 1983) from onions left in harvested fields in Grant, Michigan. Adults emerging from these pupae were placed in 60 x 60 x 80 cm screened cages housed in environmental chambers (21 + 1° C and 35 + 5% R.H.) illuminated by Verilux fluorescent bulbs with a LD 16:8 cycle. Because this environmental chamber was also used for rearing D. antiqua larvae in onion bulbs, flies were exposed to onion odors during their pre-reproductive and reproductive periods. Cages were provisioned with water, honey, a dry artificial diet (Schneider et al., 1983) and the ovipositional dishes of Harris and Miller (1982).

Foliar Surrogates

Foliar surrogates were of two types. Surrogates used in the wind-tunnel experiment were identical to those described in Harris and Miller (1983) and consisted of Pyrex tubes (8mm diam x 9 cm long)

into which were inserted strips of yellow silk-screened paper colored on both sides. Tubes were sealed by heat at the tip and by corks at the base. In all other experiments, surrogates consisted of 4 mm diam Pyrex tubing cut into 12 cm lengths and heat sealed at the tip. Color stimuli were provided by oil pigments (see Harris and Miller, 1984) mixed to match (as detected by the human eye) the green of onion foliage. Painted surrogates were allowed to dry for 3 wks before use in experiments and were coated with a thin layer of wax to avoid any inhibition of oviposition due to oil paints. In all oviposition dishes, surrogates were placed vertically in the centers of 4 cm diam x 4 cm deep plastic dishes filled with moist white silica sand so that all but 3 cm of the "foliage" stood above the substrate surface. The small diameter of the cups does not interfere with normal ovipositional behavior as females confine their movements to the substrate within 2 cm of the base of the stem and lay ca. 87% of their eggs within 1.0 cm of the stem base even when given 15 cm diam oviposition dishes (Harris, unpublished data).

"Choice"Tests

Flies used in experiments were 2-5 generations removed from the original field-collected populations and were 7-14 days old. The exception was the experiment conducted in the wind-tunnel, where flies were of variable age and were exposed to ovipositional dishes in the culture room before being used in choice experiments. Flies used in all other experiments were transferred when 7 days-old to food and male-provisioned screened cages (165 x 65 x 85 cm) situated in an environmental chamber identical to the previously described chamber

but without onion odor. These flies were "naive" in the sense that they had not been exposed to ovipositional sites before choice experiments. After being exposed to the ovipositional dishes in choice experiments for 1 day, "naive" groups were considered to be "experienced" even though probably not all females had in fact oviposited.

Because Price (1971) and Goth et al. (1983) have observed an inverse relationship between increasing female age and reproductive success of offspring in Delia species, we chose to use a young and well-defined cohort of D. antiqua at their reproductive peak. Choice tests and behavioral observations were therefore initiated at age 7 days and terminated at 14 days. Females reared under the conditions outlined normally begin ovipositing at age 7-10 days and rarely oviposit before 7 days.

Arrays of treatments within replicates were spaced evenly (ca. 10 cm apart) and were surrounded by wire-screen barriers (16 cm high x 9 cm diameter) in all experiments except that in the wind-tunnel. Experimental design was randomized complete block. Eggs were separated from sand by flotation and were recorded as numbers of eggs laid per ovipositional dish per cage per sampling period. Resultant data required log or square root transformation before being subjected to analysis of variance (ANOVA). Means were separated by the lsd test at $p<0.01$. Experiments comparing oviposition on treatment pairs were analyzed by two-way ANOVA.

Dose-response Curves for Pr_2S_2 Released from the Substrate

Six variant Pr_2S_2 release rates from the substrate of oviposition cups were generated by inserting size 3 BEEM polyethylene embedding capsules (Pelco Electron Microscopy Supplies, Ted Pella Co., Tustin, CA, see Dindonis and Miller, 1981 for discussion of capsule properties) containing 100 ul of a mixture (range 0.05-0.9 M) of Pr_2S_2 (Eastman Kodak, Rochester, New York, 98% pure by GLC) and peanut oil (PVO International, Inc.). These capsules, which allowed outward diffusion of the Pr_2S_2 but not of the peanut oil, were set, lid-side up, in ovipositional dishes with 2 cm of moist silica sand. Capsules were then covered with 2 cm of additional sand. Color and structural stimuli were provided by the aforementioned 8 mm diam yellow surrogate. Twelve hr after assembly, the 6 different mole fractions of Pr_2S_2 in peanut oil, plus treatments of one and ten capsules of neat Pr_2S_2, as well as controls of peanut oil alone and a dish with an empty capsule, were randomly placed in a line of 12 holes bissecting the long axis of a 74 x 60 cm styrofoam board 2.5 cm thick. The sand surface of each dish was flush with the surface of the styrofoam board. This board was snugly fitted into the bottom of a cage (60 x 74 x 58 cm) having plastic walls on the 2 sides perpendicular to the treatment line, and screening on the other two sides and top. Moisture in the dishes was maintained by wicks running from each cup to individual reservoirs located below the styrofoam board. Styrofoam boards were replaced after each replicate of the experiment to avoid possible contamination problems.

This cage was situated at the distal end of a wind tunnel (Carde and Hagaman, 1979) constructed of 0.3 cm thick clear plastic and

having interior measurements of 1.4 x 0.8 x 2.8 m. The velocity of the relatively laminar air flow within the tunnel was 10 cm/sec. The cage was oriented so that the wind line ran perpendicular to the treatment line. Titanium dioxide smoke tests indicated that chemical plumes from ovipositional dishes did not overlap before exiting the cage. After exiting the cage, all Pr_2S_2 plumes were evacuated from the building.

Because preliminary experiments showed that Pr_2S_2 is not adsorbed by sand, we measured release rates from individual ovipositional cups by assembling 2 sets of each treatment (6 replicates) and analyzing the amount of Pr_2S_2 in capsules before (12 hr after being assembled) and after (24 hr after being assembled) the normal experimental period (12 hr). Both initial and final concentrations of capsules with neat chemical were obtained gravimetrically. All other capsules were opened and placed in 8 ml vials containing 1 ml of 10:1 methanol: water. Extracts were placed in the freezer. Concentrations in peanut oil were analyzed by injecting 1 ul of the methanol layer of the extract onto a 10% carbowax 20M GLC column programmed for an initial 2 min hold at 70^{o} C, followed by temperature increases of 10^{o} C/minute and a final temperature of 160^{o} C. Diallyl disulfide was used as an internal standard because of its similar solubility in methanol and similar flame ionization detector sensitivity. Differences in Pr_2S_2 content between the two sets of treatments were expressed as ng released/second, with the approximation that release rate over the 12 hour experimental period was linear.

Because flies suffered elevated mortality rates in cages held in

the wind-tunnel, subsequent experiments were conducted in cages (165 x 65 x 85 cm) housed in environmental chambers having a rapid turnover of air. To determine whether this change in experimental procedure would significantly alter dose-response curves, we again exposed females to the range of release rates shown in Figure 6a. Four other changes were made in experimental procedure; two treatments were deleted from the experiment (empty-capsule control and the 10-capsule neat Pr_2S_2 treatment), densities of flies in cages were lowered from ca. 800 flies to 100, green surrogates were used instead of yellow surrogates, and treatments were separated by wire-screen barriers. Fly densities were lowered because of suspected social facilitation; females are stimulated to lay more eggs in areas where oviposition is occurring or has occurred (G. Judd, personal communication). We have found that this social facilitation leads to greater variability in ovipositional responses and heteroscedastic data that cannot be transformed to conform to the requirments for parametric statistical tests such as ANOVA. Green stems were used in this second experiment and all other experiments because they constituted a more natural color stimulus than yellow stems. Wire-screen barriers were used because Städler (1977) found that barriers around oviposition treatments reduced variability of ovipositional responses in the carrot fly. He hypothesized that barriers hinder females from rapidly visiting and ovipositing on what are normally less stimulatory treatments after being aroused on neighboring stimulatory treatments.

Dose-response Curves for Pr_2S_2 in Wax Layer on Surrogate

Various concentrations of Pr_2S_2 were formulated on the surface of surrogate foliage by adding different amounts (0.5, 1, 5, 10, 50, 100, 500, 1000 and 2000 ul) of Pr_2S_2 to 100 ml of melted paraffin wax (Cullen Industries, Huntingdon Valley, PA). Green surrogate foliage was dipped in melted Pr_2S_2-containing wax so that a 0.5 mm thick layer was retained on the surrogate surface. Loading rates per stem were estimated to be 0.002, 0.004, 0.022, 0.044, 0.22, 0.44, 2.21, 4.41 and 8.82 mg Pr_2S_2 per stem. Much care was taken to insure that the temperature of the melted wax when adding Pr_2S_2 (65^{o} C) and when coating stems (68^{o} C) was identical for all replicates. Stems were allowed to equilibrate for 24 hr and then were placed in plastic dishes filled with moist silica sand. Treatments were placed in cages for 24 hr in this and all remaining experiments. For better resolution, no Pr_2S_2, the optimal dose (50 ul Pr_2S_2/ 100 ml wax) and the highest dose (2000 ul Pr_2S_2/ 100 ml wax) were compared using the same experimental design and procedures used in the larger dose-response experiment.

Concentrations of Pr_2S_2 in wax coverings of surrogate stems at the initiation of choice experiments were estimated by coating glass (unpainted) surrogates (4 mm diam x 12 cm length) with different concentrations (10 replicates of each dosage) of Pr_2S_2-containing wax, allowing them to age for 24 hr and then scraping the Pr_2S_2-containing wax from the surrogates directly into vials containing 2 ml of distilled pentane. One ul of Pr_2S_2-containing-wax in pentane (replicated 3 times) was then injected onto a DB-5 GLC capillary column (column temp. 130^{o} C). Concentrations in wax were calculated by

comparing peak areas with those of Pr_2S_2 (in pentane) standards containing comparable amounts of unadulterated wax.

Interaction Between Pr_2S_2 Released from "Foliage" and Substrate

Five substrate dosages (peanut oil control, 0.15, 0.5, 0.7, and 1.0 M) and five surrogate foliage dosages (wax control, 5, 10, 50, and 2000 ul Pr_2S_2/ 100 ml wax) were combined factorially to investigate possible interactions between Pr_2S_2 released from the two sites. Formulations were identical to those described previously. A smaller experiment was also run to test again for possible interactions between Pr_2S_2 released from the substrate and from surrogate foliage; stems containing optimal doses of Pr_2S_2-treated wax (50 ul/100 ml wax) were placed in dishes with capsules containing various concentrations of Pr_2S_2 in peanut oil (05, 0.15, 0.5, 0.7, 0.8, 0.9 and 1.0 M).

Effectiveness of Pr_2S_2-treated Stems Over Time

Onion foliar surrogates coated with an optimal dosage of Pr_2S_2 (50 ul/100 ml wax) and prepared 15, 10, 5, 3, 2, and 1 day(s) before treatments were bioassayed. Surrogates coated with wax containing no Pr_2S_2 served as controls and were also prepared 15 days before use. A final set of Pr_2S_2-stems was prepared immediately (0 hr) before treatments were placed in cages.

Pr_2S_2 Placement on Surrogate Stems

Effects of placing Pr_2S_2 at different locations on surrogate foliage were examined by presenting flies with surrogates coated with Pr_2S_2-treated wax (50 ul/100 ml wax) in 7 different locations: on the top 1 cm, on the top 4.5 cm, on the top 9 cm, on the bottom 3 cm, on the bottom 4 cm, on the bottom 7.5 cm and on the entire 12 cm length. Areas not coated with Pr_2S_2-treated wax were coated with unadulterated wax. A surrogate covered with unadulterated wax served as a control. Twenty-four hr after their preparation and immediately before placement in cages, surrogates were placed in plastic cups filled with moist silica sand so that 9 cm of the 12 cm surrogate stood above the sand surface.

Location of Pre-ovipositional Examining Behaviors

Groups of 5-15 newly emerged female flies were placed along with equal numbers of males in clear plastic cages (15 cm diam x 30 cm height) provisioned with water and food. For 2 hr every day at various times between 3 and 8 pm, flies were exposed, starting on day 7, to an optimal surrogate stem standing upright in the middle of a pot (15 cm diam x 20 cm deep) containing organic "muck" soil. This period (3-8 pm) was chosen for behavioral observations because ca. 78% of egg production occurs during this interval (Havukkala and Miller, submitted).

During each observational period, the location and duration of the following stances and behaviors of individual females were noted: no movement (still), grooming, running, running with the mouthparts repeatedly brought in contact with the plant or soil surface

(mouthpart examining), running with the abdomen bent and the tip of the abdomen (and sometimes the mouthparts) touching the plant or soil surface (ovipositor examining) and stationary probing where the ovipositor was fully extended and wedged into soil crevices (probing). The foliar surrogate and substrate were divided into five zones: foliage 0-1 cm from the surrogate-substrate interface, foliage 1-3 cm, foliage 3-9 cm, substrate 0-1 cm from the stem base, and substrate 1-4 cm from the stem base. Observations began when individuals entered the 1 cm diameter zone around the base of the surrogate or flew to the surrogate. Observations of individuals were terminated after females left the surrogate area either by flying away or by walking more than 3 cm from the surrogate stem base. Behavioral observations of individuals (identified by acrylic-paint color codes on the thorax) were recorded on a Radioshack TRS 80, Model 100 using a program developed by T. Bierbaum, Dept. Zoology, Michigan State University. The experiment consisted of 24 different encounters with the surrogate, 12 of which resulted in oviposition.

Surrogate Onions vs Real Onions

The best surrogate onion was compared with real onion plants to evaluate whether the major determinants of D. antiqua oviposition were effectively simulated. The best surrogate (50 ul Pr_2S_2/100 ml wax 4 mm diam green cylinder) was compared to three types of onions in pair-wise choice tests: (i) onion plants (grown from sets) having 4 leaves, a height of 14 cm and a basal diameter of 4-5 mm, (ii) onions grown from seed (Downing Yellow Globe-6 weeks old) having 3 leaves, a height of 180-240 mm and a basal diameter of 3.0-3.5 mm, and (iii) a

single onion leaf ca. 120 mm long cut from the foliage of onion sets (4-5 mm diam) and positioned upright in moist sand.

Comparisons of oviposition on surrogates and onion seedlings were also made using a no-choice bioassay. Individual 7-day-old females (n=16) were placed in cages (10 cm diam x 30 cm height) with food, water, two males, and the surrogate or onions grown from seed. Oviposition dishes were replaced and eggs counted every 24 hr on each of the next 7 days.

RESULTS

Pr_2S_2 Dose-response Effects

In the wind-tunnel, females laid the most eggs in response to treatments having a mean release rate of 1 and 5 ng Pr_2S_2/ sec from polyethylene capsules placed beneath the sand substrate (Figure 6a). Release rates on either side of this optimum stimulated less oviposition. When this range of concentrations was tested in cages having no rapid turnover of air, females responded in much the same way (Figure 6b).

When Pr_2S_2 was released from the wax coating of surrogate foliage rather than from the substrate (Figure 7), ovipositing females again responded differentially to various concentrations of Pr_2S_2. Maximal responses occurred around surrogates loaded with 0.22 and 0.44 mg/stem and dropped off sharply with the next lowest loading rate of 0.044 mg/stem. The highest loading rate of 8.82 mg/stem appeared to depress oviposition responses relative to the plain wax control. A smaller experiment consisting of a subset of the original treatments (including the wax control, the optimal loading rate (0.22 mg/stem)

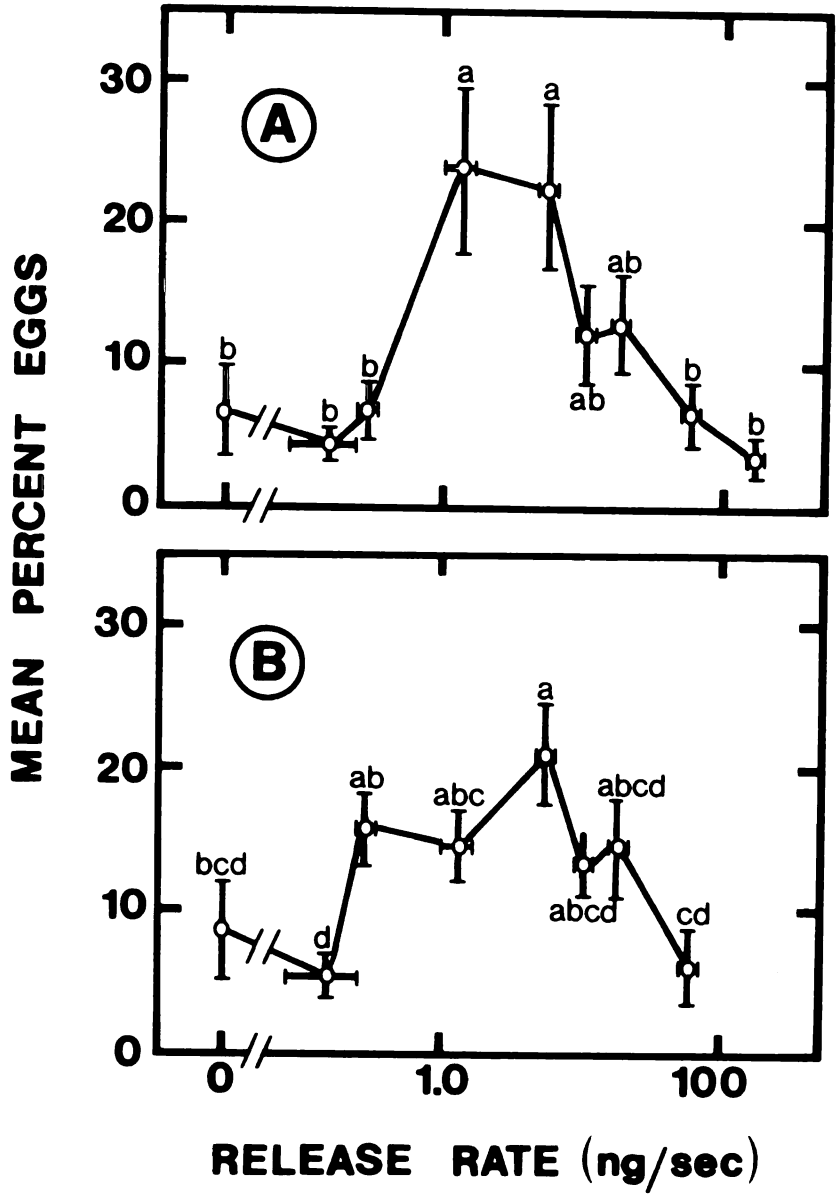

Figure 6. Semi-logarithmic plot of Delia antiqua ovipositional responses (means and standard error bars) to n-dipropyl disulfide released from the substrate using cages of flies placed in a wind-tunnel (a) or in normal environmental chambers (b). Treatments accompanied by the same letter in each figure are not significantly different at $p<0.01$ (ANOVA followed by lsd test for separation of means). Total number of eggs for 12 replicates for (a)=22,428, for 15 replicates for (b)=1,517 eggs.

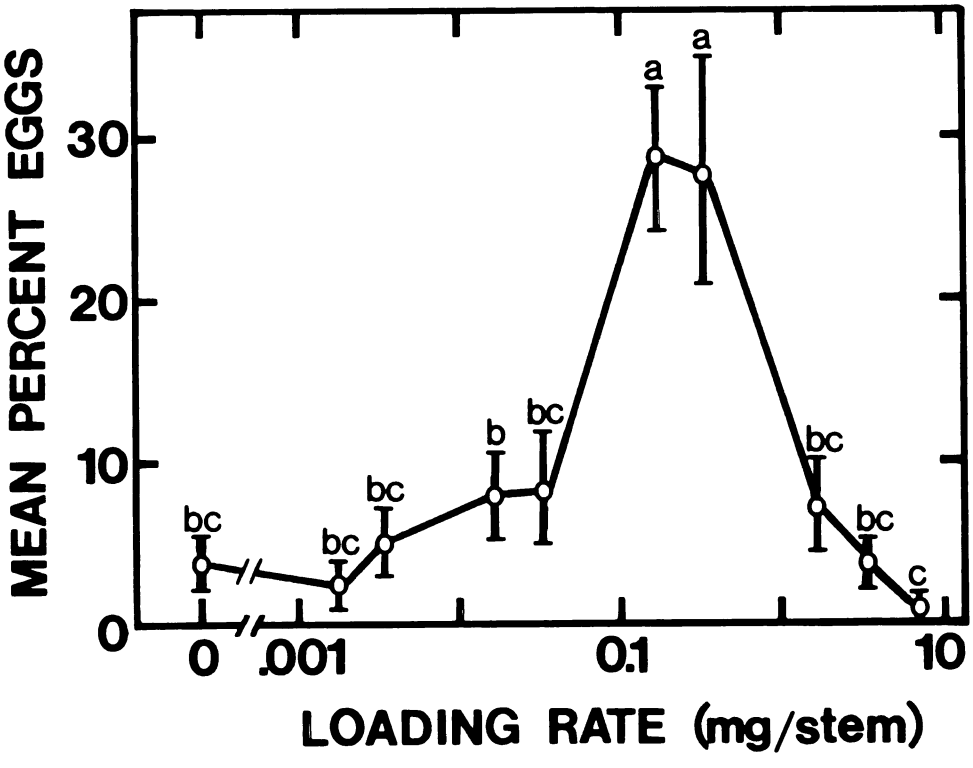

Figure 7. Semi-logarithmic plot of Delia antiqua ovipositional responses to n-dipropyl disulfide released from surrogate foliage. Treatments accompanied by the same letter are not significantly different at $p<0.01$ (ANOVA followed by lsd test for separation of means). Total number of eggs for 7 replicates=676.

and the highest rate) proved that inhibition did occur at the highest loading rate: the high rate received a mean of 3 eggs vs 16 and 55 eggs laid on the wax stem control and the optimal loading rate, respectively (8 replicates, ANOVA F=24.06, significant at $p<0.001$, all three treatments significantly different from each other at $p<0.01$, lsd test).

GLC analysis of actual amounts of Pr_2S_2 remaining in wax at the beginning of the experimental period revealed no detectable levels of the chemical at the lower loading rates of .002, .004, and .022 mg/stem. Stems with optimal loading rates of .22 and .44 mg/stem contained 0.075 and 1.07 mg/stem. Stems inhibiting oviposition (loading rate=8.8 mg/stem) contained 3.85 mg/stem. The loss of Pr_2S_2 from wax from the time of loading to the initiation of experiments was probably due primarily to volatilization during formulation using heated wax.

Interactions Between Pr_2S_2 Released from "Foliage" and Substrate

Females laid few eggs (Figure 8) around treatments with Pr_2S_2 released only from the substrate. The previously determined optimal substrate dose (Figure 6b) received only 4% eggs, hardly twice as many eggs as were received by the wax stem-peanut oil control. Flies were far more responsive to changes in concentration of Pr_2S_2 in the wax coating of surrogates. Loading rates of 0.044 and 0.22 mg/stem caused two and nine-fold increases in oviposition, respectively; these increases were similar to those seen in Figure 7.

Dose-response effects were significant both for Pr_2S_2 released from the substrate (ANOVA F=18.2, significant at $p<0.001$) and from

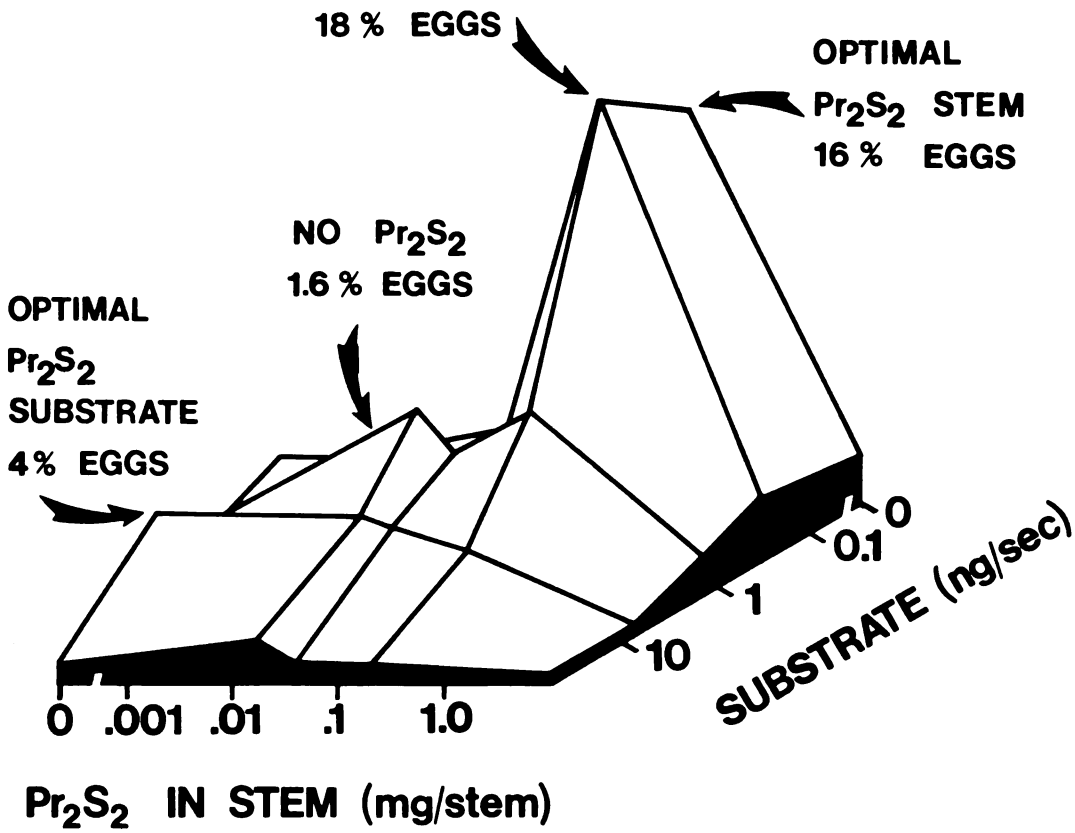

Figure 8. Response-surface generated by presenting gravid onion flies with doses of n-dipropyl disulfide released from substrate and surrogate foliage combined factorially (discontinuous log-log plot). Mean number of eggs laid on each of the 25 treatments is indicated by height on the vertical axis. Total number of eggs for seven replicates= 4,157.

the stem (ANOVA F=14.3, significant at $p<0.001$). There was also a significant interaction between Pr_2S_2 released from the two areas (ANOVA F=3.07, significant at $p<0.001$). Whether this interaction consisted of both synergy and interference could not be determined; however, a subsequent experiment in which a range of Pr_2S_2 substrate concentrations was presented with optimal Pr_2S_2-treated wax stems (14 replicates) indicated that small concentrations of Pr_2S_2 released from the substrate did not synergize optimal concentrations of Pr_2S_2 on stems. On the other hand, large release rates from the substrate did interfere with the optimal stem concentration; release rates of 5.83 ng/second from the substrate significantly inhibited egglaying relative to a Pr_2S_2-treated wax stem control.

Effectiveness of Pr_2S_2-treated Stems Over Time

Optimal Pr_2S_2-treated stems (loading rate 0.22 mg/stem) retained their stimulatory effects on oviposition for up to 5 days after being formulated (Figure 9). Stems formulated more than 10 days before exposure to females received significantly fewer eggs.

Pr_2S_2 Placement on Surrogate Stems

Stems covered both above and below the substrate with optimal doses of Pr_2S_2-treated wax, and stems covered on the area above the substrate or on all but the top 4.5 cm of the stem elicited the most oviposition (Figure 10). Stems having Pr_2S_2-treated wax placed on the section beneath the substrate and on the bottom 1.0 cm of the stem received fewer eggs, but more eggs than the wax control and treatments having Pr_2S_2-treated wax only beneath the substrate or on

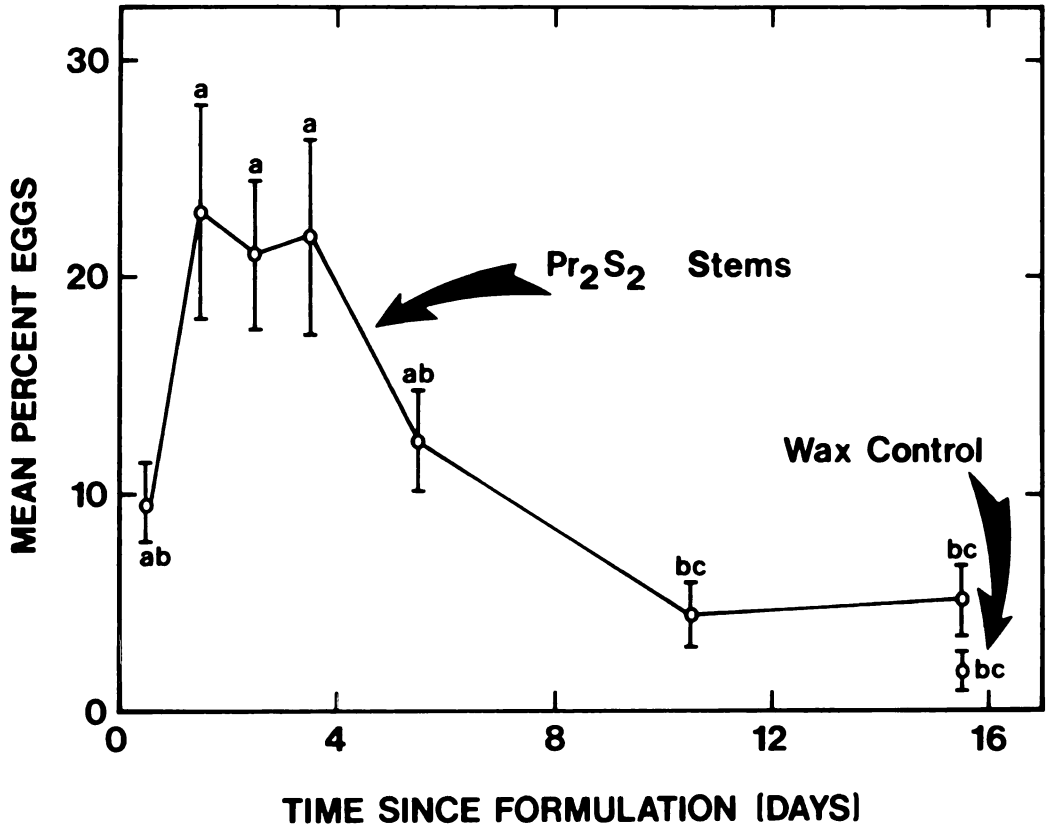

Figure 9. Ovipositional responses to n-dipropyl disulfide-treated surrogate foliage formulated 0 to 15 days before being used in ovipositional bioassays. Treatments accompanied by the same letter are not significantly different at $p<0.01$ (ANOVA followed by lsd test for separation of means). Total number of eggs for 10 replicates=2,518.

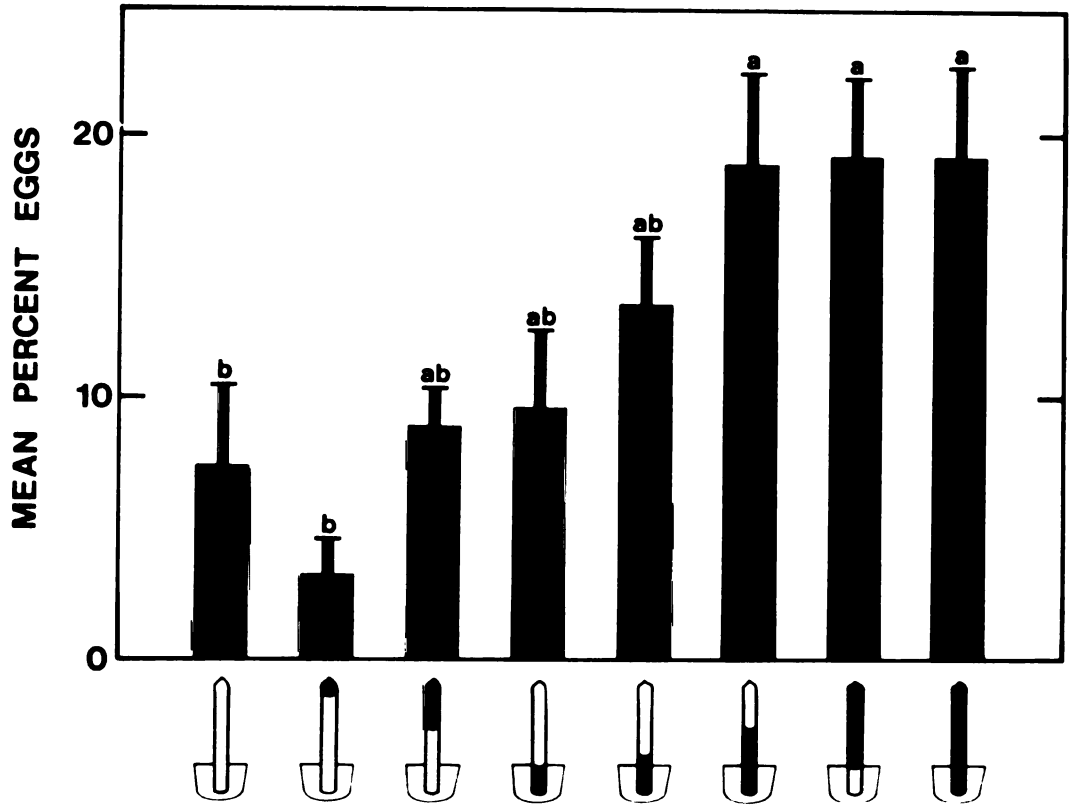

Figure 10. Delia antiqua ovipositional responses to surrogate foliage with n-dipropyl disulfide-treated wax placed in different locations. Dark portions of surrogate foliage indicate treated areas, white areas were covered with plain wax. Treatments accompanied by the same letter are not significantly different at $p<0.01$ (ANOVA followed by lsd test for separation of means). Total number of eggs for 10 replicates= 4,312.

the upper half of the stem. A smaller experiment including the top ranking 4 treatments confirmed no significant differences in ovipositional responses (6 replicates, $p<0.01$).

Location of Pre-ovipositional Examining Behaviors

Encounters with Pr_2S_2-treated wax surrogates began when individual females (n=24) either flew to the surrogate (n=12) or walked up to the surrogate via the substrate (n=12). Females not ovipositing during these encounters (n=12) stayed on the surrogate or in the immediate area of the surrogate for only 167 seconds on the average and spent 67% of their time grooming or sitting immobile on the surrogate. The remainder of their time was spent running over the surrogate surface and examining the surrogate with mouthparts while running. These non-ovipositing visitors did not proceed beyond mouthpart examining; ovipositor examining and probing were never observed.

Females that eventually oviposited during encounters (n=12) were on the average 8.1 days old and laid 13.6 eggs (SE=2.4). Encounters lasted ca. 17 min and 41 sec. Ovipositor probing accounted for the largest proportion of this time (mean $\pm$ SE=65 $\pm$ 2.1%), with the remainder spent sitting still and grooming (3%) or examining the surrogate and substrate (32%). Examining behaviors included running, running with the mouthparts repeatedly brought in contact with the plant or soil surface (mouthpart examining) and running with the abdomen bent and the tip of the ovipositor touching the surrogate or soil surface (ovipositor examining).

Examining behaviors were primarily confined to areas of

soil and surrogate within one centimeter of the soil/surrogate stem interface (mean ± SE tenure time= 253 ± 50.8 and 98 ± 16.8 sec, respectively, for substrate and surrogate-see Figure 11). Females spent significantly less time (mean ± SE tenure time=6.2 ± 1.3 sec) examining the upper portion (foliage> 3 cm) of the surrogate. When flies did examine this area (Figure 12) it was by running over the surface or by mouthpart examining; examining with the ovipositor at this height on the surrogate occurred infrequently (mean ± SE= 0.8 ± 0.05 sec). Below 3 cm and above 1 cm (mean ± SE tenure time=41 ± 8.7 sec), females continued to use their mouthparts to examine the surrogate but were more likely to also engage the tip of the ovipositor to examine the surface. This latter form of examining was even more common at the base of the surrogate (foliage 0-1 cm).

Behaviors on the substrate differed somewhat from those seen on the surrogate itself. In the area immediately surrounding the base of the surrogate (foliage 0-1 cm) females spent most of their time examining and probing the soil with their ovipositors. This latter behavior differed from ovipositor examining in that it was done in a stationary position, involved a full extension of the ovipositor, and placed the abdomen deeply within crevices in the soil surface. Ovipositor probing was highly correlated with egglaying. The time spent in this behavior corresponded closely with the numbers of eggs laid by individual females (r^2=.90, F for regression=91.84, $p<0.001$). As females moved farther away from the stem base (substrate 1-4 cm) they were less apt to probe (oviposit) and more apt to examine the soil with their ovipositors, sit still or groom.

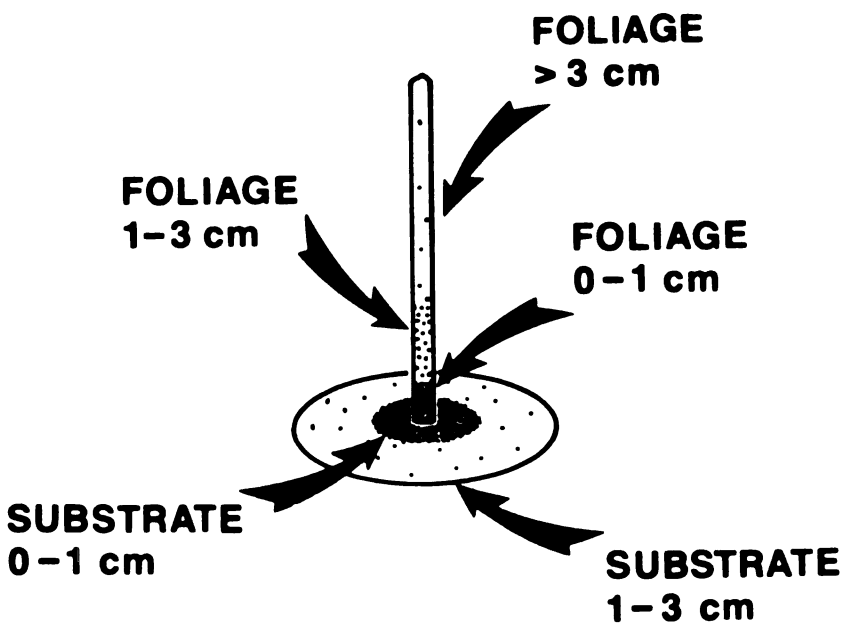

Figure 11. Location and relative duration of Delia antiqua preovipositional examining behaviors on n-dipropyl disulfide-treated surrogate foliage. Density of shading indicates how much time is spent examining each zone.

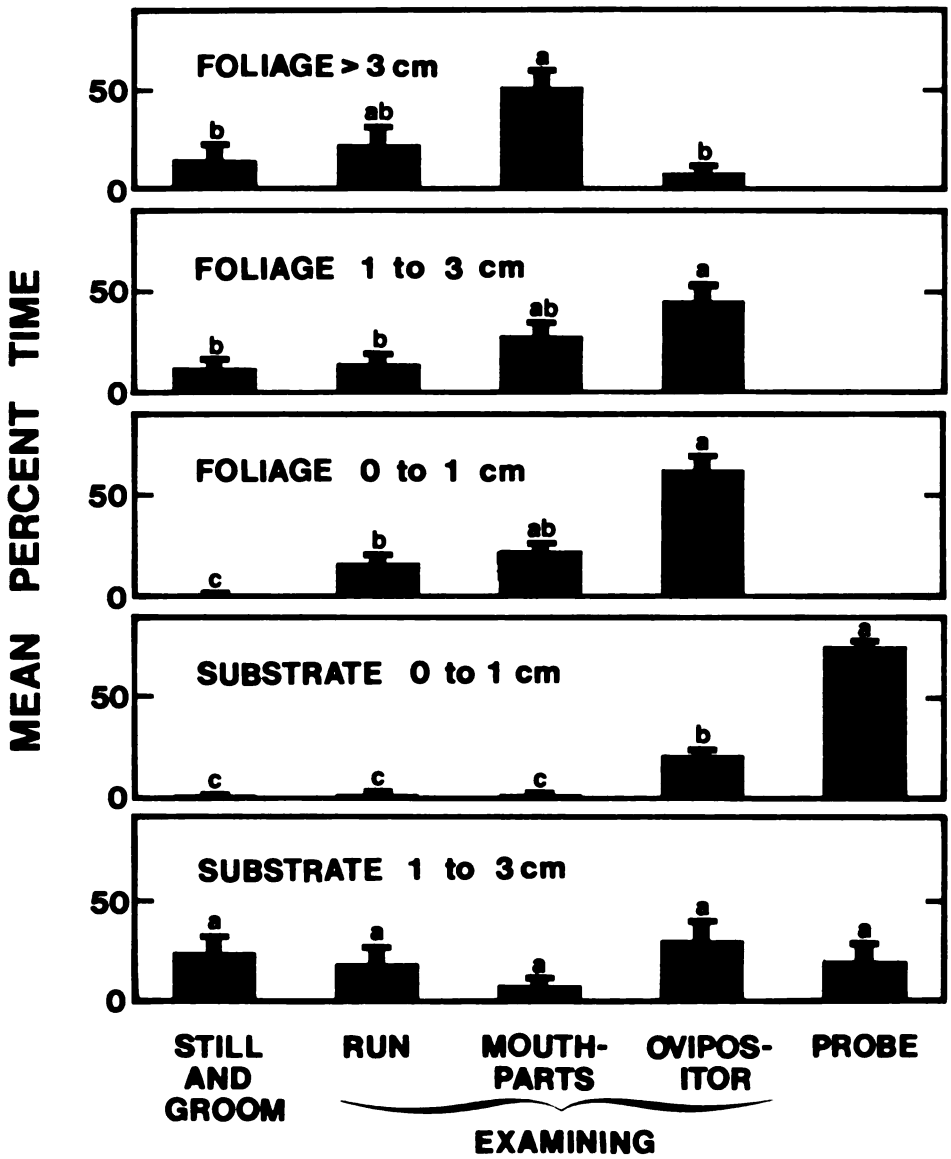

Figure 12. Time allocated by ovipositing Delia antiqua females to 5 different behaviors in 5 different zones comprising oviposition sites. Values for percent time spent in behaviors within a single zone accompanied by the same letter are not significantly different at $p<0.01$ (ANOVA followed by lsd test for mean separation).

Surrogate Onions vs Real Onions

When presented with the optimal surrogate (4 mm basal diam x 12 cm height) and a 6 wk-old onion seedling (3 mm diam x 20 cm height) in the choice bioassay, females laid as many eggs around the surrogate (mean=43 eggs) as the onion plants (mean= 30 eggs, difference not significant at $p< 0.05$, 2-way ANOVA). Significantly more eggs were laid around surrogates in no-choice bioassays (mean total eggs per female per day=6.7 vs 3.2 for onion seedling, significantly different at $p<0.01$, 2-way ANOVA) in which females were given access to either surrogates or onion seedlings for the first 8 days of their reproductive lives. The larger number of eggs laid around surrogates was in part due to the fact that more females accepted the surrogate as an oviposition site during the 8-day test period (44 vs 22% ovipositing per day, significantly different at $p<0.01$).

However, when surrogates were placed in cages with 2- wk-old onions grown from sets (4 mm diam x 18 cm height) they received significantly fewer eggs (mean=56) than did the real onions (mean=194, $p<0.05$, 2-way ANOVA). Foliage removed from these same sets (4 mm diam x 9 cm height) and placed upright in moist sand received fewer eggs (mean=12) than did surrogates (mean=47, not significantly different at $p<0.05$, 2-way ANOVA).

DISCUSSION

Because onion bulbs produce more of the stimulatory thiopropyl chemicals (Vernon et al., 1977, Ishikawa et al., 1978), it has been assumed that, when ovipositing into soil adjacent to onion plants, female onion flies are stimulated by volatile compounds that originate

in the bulb. These assumptions have lead to the design of bioassays that test effects of chemicals administered in a similar fashion; volatiles are presented either in conjunction with small holes and moistened filter paper (Vernon et al., 1977, 1978; Pierce et al., 1978) or are adsorbed onto charcoal granules and covered with moistened glass beads (Matsumoto and Thorsteinson, 1968; Ishikawa et al., 1978; Ikeshoji et al., 1980). Chemicals presented in this way never elicited more than fifty percent of the oviposition stimulated by onion slices.

Onion fly females however do not perceive onion plants solely through the chemical sense. Even when chemical stimuli are separated spatially from surrogate foliage by placing onion slices beneath the substrate, the addition of foliar surrogates to onion slices synergizes ovipositional responses to odors, causing females to lay 18 times more eggs (Harris and Miller, 1982). Females respond strongly to changes in surrogate color, shape and size and lay more eggs around yellow or green cylinders 4 to 8 mm in diameter and greater than 2 cm in height (Harris and Miller 1983, 1984). These stimuli are sensed at least in part during runs up and down surrogate foliage.

Evidence presented in this paper indicates that acceptance of ovipositional sites may occur more readily if females perceive host plant chemical cues in addition to foliar structural cues during runs over the plant surface. Optimal doses of Pr_2S_2 released from surrogate foliage stimulated 4 times as much oviposition as optimal doses released from the substrate. While this differential response to Pr_2S_2 may have occurred because of differences in formulation (polyethylene capsules vs wax), wax formulations of Pr_2S_2 applied to the substrate

(perforated with holes to allow oviposition) did not stimulate oviposition (Harris, unpublished results).

Placement of dipropyl disulfide on the surrogate itself also influenced oviposition. Females tended to lay more eggs around surrogates having the disulfide at the base rather than on the upper half of "foliage". Observations of individual females performing pre-ovipositional examining behaviors on Pr_2S_2-treated surrogates suggested a reason for this trend; females tended to land on the upper portions of the foliage but after landing, spent very little time in this area. The lower portions of the surrogate and areas of substrate immediately adjacent to the surrogate base were extensively examined (Figure 11) during runs with or without the mouthparts and/or the tip of the ovipositor repeatedly contacting the wax surface.

During these examining behaviors, Pr_2S_2 formulated in wax could be detected by contact or olfactory receptors on the tarsi, antennae, mouthparts or ovipositor. Indeed, running over the surface of artificial or natural onion foliage may be akin to drumming in Lepidoptera (Ilse, 1937) and palpation in grasshoppers (Blaney and Chapman, 1970); abrasion of foliage by spines or hairs located on the tarsi, mouthparts and ovipositor may disrupt plant cells thereby increasing concentrations of chemical stimuli the female is exposed to. Antennae of D. antiqua are covered with trichoid, basiconic, grooved and clavate sensilla (Honda et al., 1983) and give EAG responses to Pr_2S_2 and numerous other compounds which have stimulatory or neutral effects on oviposition (Ikeshoji et al., 1981; Guerin and Stadler, 1982). Preliminary experiments on tarsal hairs (done in the lab of Dr. Frank Hanson) indicate that contact chemoreceptors also

respond to Pr_2S_2. Other herbivorous flies, whose examining behaviors closely resemble those of D. antiqua, perceive host chemicals via chemoreceptors located on the antennae, tarsi and maxillary palps (Städler, 1977; Städler, 1978; Guerin and Städler, 1982).

Since we do not know how accurately our present optimal surrogate approximates the stimuli presented by onion foliage, in depth analyses of leaf surface chemistry and bioassays must be done to ascertain what compounds are present and whether compounds function as stimulants or deterrents for ovipositing onion flies. For example, stimulatory chemicals such as Pr_2S_2 may be accompanied by other onion secondary chemicals such as diallyl disulfide. While this chemical does not appear to inhibit or stimulate oviposition when presented singly (Harris, 1986), it does inhibit responses when added to dipropyl disulfide (Harris and Miller, in preparation). Dimethyl disulfide may function in the same way. Both of these non-propyl-containing disulfides are more toxic than dipropyl disulfide to onion fly adults and larvae (Powell and Miller, in preparation) and are present at higher concentrations in Allium species (Freeman and Whenham, 1975) that are less preferred by ovipositing females and less suitable for larval development (Loosjes, 1976). On the other hand, disulfides may not be the primary chemicals used in host recognition. The leek moth, Acrolepiopsis assectella, which also specializes on Allium species, responds positively to thiosulfinates in olfactometers and to thiosulfonates when ovipositing (Thibout et al., 1982). Methanol extracts of leek leaves are also highly stimulatory to ovipositing leek moths but do not contain sulfur compounds (Auger and Thibout, 1983).

Foliar surrogates and wax formulations of plant chemicals provide excellent tools for both applied and basic researchers studying insect/plant interactions. Chemicals present in resistant breeding lines can be formulated in wax coatings of surrogates and tested in lab bioassays before carrying out expensive and time-consuming field trials. The production of identical surrogates will provide realistic ovipositional resources that could be used to standardize responses to breeding lines tested at different times or in different countries, and furthermore could be used to study variation in host-acceptance behaviors due to deprivation and learning. And finally, manipulation of surrogates, involving alterations of one or more plant character(s), will provide needed information on whether host acceptance is triggered by a few "key" stimuli or by interactions among a larger set of stimuli sensed by multiple sensory modalities.

CHAPTER 3

Influence of Chemical and Nonchemical Stimuli on Host Acceptance Under Choice and No-choice Situations

INTRODUCTION

Surrogate host plants have provided many insights into the perceptual world of onion flies. Thought for many years to be stimulated primarily by "key" chemical stimuli originating in the onion bulb (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Ishikawa et al., 1978), ovipositing onion flies are, in fact, also very sensitive to stimuli of onion foliage. These stimuli can be mimicked by foliar surrogates and manipulated so that single foliar characters are altered while holding all other characters constant. Such manipulations have proven that ovipositing onion flies are sensitive to color (hue and saturation), shape, and size stimuli of onion foliage (Harris and Miller, 1982; 1983; 1984). A more realistic and effective presentation of chemical stimuli has also been made possible by the development of foliar surrogates (see Chapter 2); wax formulations of n-dipropyl disulfide (Pr_2S_2) added to the surface of foliar surrogates stimulate more oviposition than optimal concentrations of Pr_2S_2 released from the substrate.

Though structural, color, and chemical stimuli are all involved in host acceptance, questions remain about the relative importance of chemical vs. non-chemical stimuli. Earlier experiments with less refined surrogates indicated that foliar color and structural cues synergize responses to chemical cues (Harris and Miller, 1982); however, color and chemical stimuli used in these experiments (yellow

papers and chopped onion) may have been supernormal (Prokopy, 1972) and therefore not realistic representations of stimuli onion flies might encounter in a natural setting. Furthermore, because sources of chemical stimuli were placed beneath the sand substrate, structural and color stimuli representing onion foliage were spatially separated from host chemicals.

We have reinvestigated the hypothesis that non-chemical foliar stimuli are primary rather than secondary indicators of "host" to D. antiqua using a newer and more realistic surrogate. Effects of removing non-chemical stimuli (color and optimal size) vs. removing chemical stimuli (dipropyl disulfide) were compared in choice bioassays, in which populations of flies were given access to all treatments, and in no-choice bioassays, in which individual females were given access to a single treatment for 8 days. While choice tests were designed to reveal ranking of ovipositional preferences, no-choice tests were designed to quantify the length of time (latencies) required before females accepted ovipositional treatments lacking chemical vs. non-chemical plant stimuli. The rate at which eggs were matured in the ovaries was also quantified to determine whether ovipositional response latencies could be correlated with physiological phenomena.

MATERIALS AND METHODS

Insect Rearing

Pupae were collected from onion bulbs left in harvested fields in Michigan and Ontario. Adults emerging from these pupae were placed in screened cages housed in environmental chambers (21+ 1^{o} C and 35+

5% RH) illuminated by Verilux fluorescent bulbs with a LD 16:8 cycle. Cages were provisioned with water, honey, a dry artificial diet (Schneider et al., 1983) and ovipositional dishes containing chopped onion and surrogate foliage (Harris and Miller, 1982).

Foliar Surrogates

Foliar surrogates were of four types. (1) The unaltered surrogate (see Harris and Miller, 1984) consisted of a 4 mm diameter glass cylinder cut into 12 cm lengths and heat sealed at the tip. Color stimuli were provided by oil pigments mixed to match (as detected by the human eye) the green of onion foliage. After being painted, surrogates were oven-dried for 3 wk. Twenty-four hr before use in experiments, surrogates were dipped in warm paraffin wax containing 0.01 or 0.05% Pr_2S_2 (Eastman Kodak, Rochester, NY, 98% pure by GLC) by volume so that a 0.5 mm thick layer of wax was retained on the surrogate surface (see Chapter 2 for details). (2) Surrogates with altered color stimuli were identical to the above optimal surrogate but were not painted and therefore were translucent white rather than green. (3) Surrogates lacking chemical stimuli differed from the optimal surrogates in that they were covered with plain wax rather than Pr_2S_2-treated wax. (4) Surrogates with altered size stimuli consisted of a 15 mm cylinder of the same height and color as the optimal surrogate; these surrogates were also covered with a 0.5 mm thick layer of Pr_2S_2-treated wax. All surrogates were placed vertically in the centers of plastic dishes filled with moist sand so that all but 3 cm of the surrogate foliage stood above the substrate surface. Dishes containing only moist sand were used as controls.

Choice Bioassay- Unaltered and Altered Surrogates

Because flies were reared from eclosion to day 6 in an environmental chamber used for rearing larvae on onion bulbs, all females were exposed to onion odors during their pre-reproductive period. For choice experiments, groups of ca. 50 6-day-old females were transferred to 3 large screened cages (165 x 65 x 85 cm) containing food, water and 50 reproductively mature males. Flies used in this experiment and all other choice and no-choice bioassays were from a culture 2-6 generations removed from the original field collected populations. These cages were located in an identical environmental chamber (Chamber II) free of plant odors. The following morning, one of each of the five treatments (unaltered surrogates, altered color, altered chemical, altered size, and sand control) were placed ca. 20 cm apart in each of the three cages. Each treatment was surrounded by a 16 cm high x 9 cm diam wire-screen barrier (see Chapter 2 for rationale). Treatments were removed 24 hours later. Eggs laid in each dish were separated from sand by flotation and counted. This procedure was repeated each day with the same groups of flies for the next 6 to 7 days (20 replicates total). Data generated were heteroscedastic and thus were analyzed using the nonparametric Friedman two-way analysis by ranks followed by a multiple-comparison procedure designed for use with the Friedman test (Daniel, 1978).

No-choice Bioassay- Unaltered and Altered Surrogates

In the no-choice experiment, females emerging from pupae over a 24-hour period (average age=0.5 day) were transferred to separate cages containing food, water and several reproductively mature male

flies. When 5 days old, individual females were transferred out of these cages to small cages (10 cm diam x 30 cm height) housed in Chamber II. The following morning, one of the 5 treatments used in the choice experiment was randomly assigned to and placed with 16 of the individually-caged females (average age=6.5 days). Ovipositional cups were replaced and eggs were counted every day for the next seven days. On the final day of the experiment (average age of flies=14.5 days) all females were removed from cages and sacrificed so that the number of stage 10 eggs (Theunissen, 1973) remaining in ovaries could be counted. In stage 10, the oocyte fills the entire egg chamber and trophocytes are barely visible. Eggs at this stage can be oviposited. Data were transformed using log or square-root transformations and analyzed via a one-way or two-way ANOVA. When significant differences between treatments means were established by ANOVA, means were separated by the LSD test at a comparison-wise error rate of $p<0.01$ and an experiment-wise error rate of $p<0.10$. The experiment-wise error rate for data on ovipositional rate was higher ($p<0.24$) because of the larger number of treatments (8 days total). Distributions of percent of total eggs laid per day were analyzed the Wilcoxon two-sample test (Daniel, 1978).

Choice Bioassay-Fat Stems with Varying Concentrations of Pr_2S_2

A second choice experiment that presented 15 mm diameter surrogates with varying concentrations of Pr_2S_2 determined whether the larger diameter surrogate was inhibitory because it contained higher <u>total</u> amounts of Pr_2S_2. Since the larger surface area of the 15 mm surrogate required 5 times as much wax to achieve a 0.5 mm

covering, 15 mm surrogates were formulated with the normal concentration of Pr_2S_2 (0.05% in wax), one fifth the concentration (0.01% in wax) and with plain wax (control). These three treatments were tested using methods identical to those described previously.

Mature Eggs in Ovaries of Deprived and Non-deprived Females

Female flies emerging from a laboratory strain of D. antiqua over a 24-hour period (designated 0.5 day flies) were placed in screened cages (15 cm diam x 32 cm height) provided with food, water and 20 reproductively mature males. The laboratory strain (25 generations removed from original field-collected populations) was used for dissections of ovaries because newer cultures (2-6 generations removed from field) were not available in large enough numbers. When 7 days old, females were divided into 2 groups and placed in separate cages. One of these two cages contained a Pr_2S_2-treated surrogate in a standard ovipositional dish (nondeprived flies) in addition to the food, water and 10-15 males. Females in the other cage were given access to food, water and males but were deprived of ovipositional dishes. Deprived (4.5-15.5 day-old) and non-deprived flies (7.5-15.5 day-old) of known ages were removed from cages on a daily basis, chilled and dissected. Eggs fitting the description given by Theunissen (1973) for mature (stage 10) eggs were counted and the age of the female was noted. Data were analyzed with one- and two-way ANOVAs.

RESULTS

Choice Bioassay- Unaltered and Altered Surrogates

When populations of onion fly females were presented with an array of ovipositional dishes containing various combinations of structural (4 mm vs 15 mm diameter), color (green vs white), and chemical stimuli (Pr_2S_2-treated wax *vs.* unadulterated wax), females laid the most eggs on green surrogates with a diameter of 4 mm and a layer of Pr_2S_2-treated wax (Figure 13a). Removal of chemical stimuli, and alteration of color and size stimuli caused 84, 70 and 89 percent reductions in oviposition, respectively. Cups containing moist sand elicited significantly less oviposition than all other treatments (Friedmans test, experiment-wise error rate a=0.10).

No-choice Bioassay- Unaltered and Altered Surrogates

In the no-choice bioassay, reductions in eggs laid per female per day around altered surrogates (Figure 13b) were consistent with, but generally smaller than, those observed in the choice experiment. Individually-caged females given access to single treatments laid 49% and 54% fewer eggs on surrogates with altered size and chemical characteristics, respectively ($p<0.01$, lsd test). Reductions for altered color were not significant at $p<0.01$. All three altered surrogates received significantly more eggs than the sand control ($p<0.01$, lsd test).

Reductions in oviposition were due primarily to reduced acceptance of altered surrogates and sand controls (Figure 13b). A significantly higher percent of females accepted (44%) the unaltered surrogate each day during the eight-day experimental period. Of the

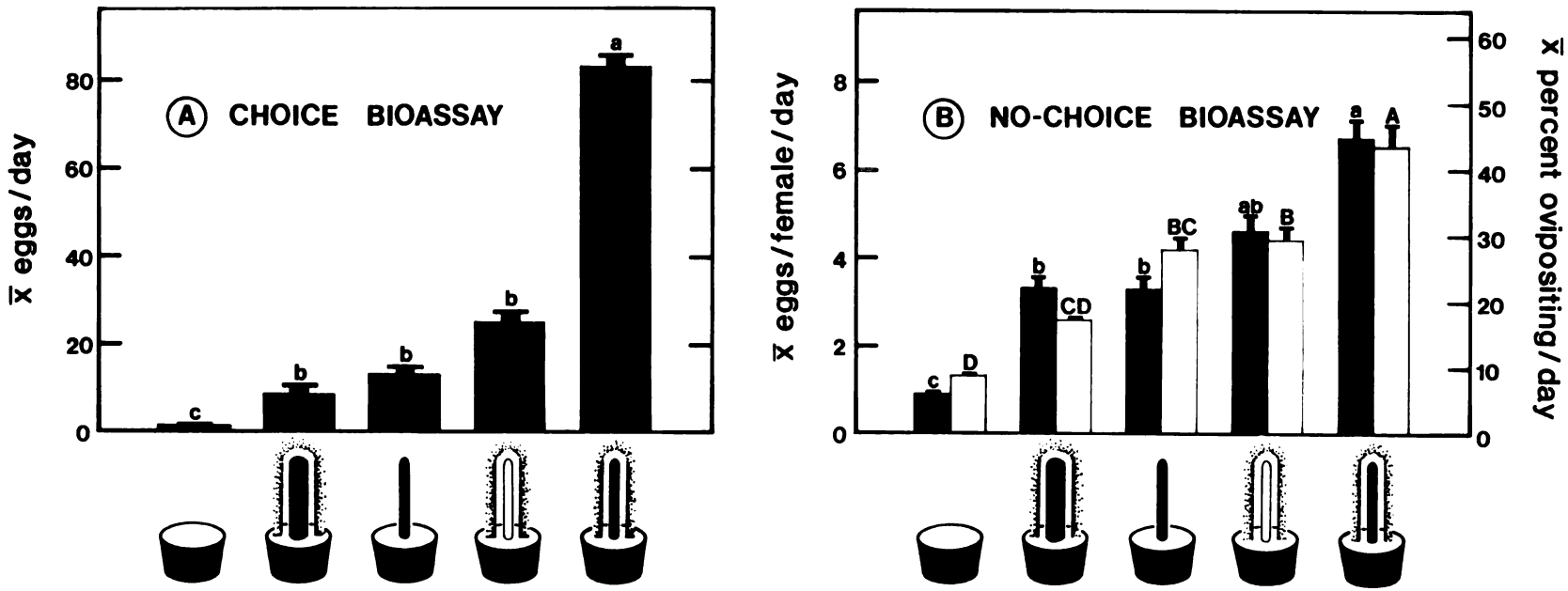

Figure 13. Delia antiqua ovipositional responses (means and standard error bars) to unaltered foliar surrogates (far right) and surrogates altered by removing color (white stem), chemical (no halo) and size stimuli (fat stem). The empty dish represents the sand control. Responses shown in (a) and (b) were measured in choice and no-choice bioassays, respectively. Black bars in (b) represent mean eggs/female/day (left vertical axis). White bars in (b) represent mean females ovipositing/day. Treatments accompanied by the same size letter within each figure are not significantly different at $p<0.01$ (lsd test). Total number of eggs for (a)= 2,621 (20 replicates), for (b)=2,409 (80 individual females).

altered surrogates, the 15 mm diameter cylinder stimulated the fewest females to oviposit (17.7%). There were no significant differences in numbers of eggs laid on the four surrogates when data were normalized to a per ovipositing female basis (Table 2). Females accepting surrogates without Pr_2S_2 did not lay significantly more eggs than females accepting the sand control ($p<0.01$, lsd test). Therefore, the total number of eggs laid on any one treatment depends on how many females lay eggs on (accept) the treatment and how many eggs are laid by the female once the treatment has been accepted.

Due probably to small sample sizes, numbers of eggs laid per treatment were not significantly different until day 8.5 (Figure 14a), when the unaltered surrogate received significantly more eggs than the sand control ($F=2.56$, $p<0.05$, ANOVA). Eggs laid by females accepting unaltered surrogates were more evenly distributed over the eight day period than eggs laid around altered surrogates and on sand controls (Table 3). Females given access to the altered surrogates and the sand control laid a significantly higher percent of their eggs on day 9.5 ($p\leq0.06$, Wilcoxon two-sample test). Also shown is the cumulative percent of females accepting the five treatments during the experimental period (Figure 14b). All four surrogates were accepted by at least one 6.5 day-old female (out of 16 total). The sand control was not accepted until day 8.5. However, ages of females accepting the five treatments were not significantly different ($F=1.54$, ANOVA-see Table 2).

Females given access to the unaltered surrogate contained significantly fewer mature eggs upon dissection than females given only sand (Table 2). Intermediate numbers of mature eggs remained in

Table 2. Ovipositional responses of individual ovipositing Delia antiqua females to unaltered and altered foliar surrogates and sand controls.

	Response parameters (x)			
	Eggs per ovipositing female per day	Days until first oviposition	Mature eggs remaining in ovaries	Total eggs matured
Sand control	6.8 b	12.2 a	63 a	73 a
Altered size	17.6 a	10.3 a	50 ab	78 a
Altered chemical	12.8 ab	10.5 a	55 ab	81 a
Altered color	15.9 a	9.8 a	41 ab	85 a
Unaltered surrogate	16.1 a	10.1 a	34 b	88 a

[1] Means within columns accompanied by the same letter are not significantly different at $p<0.01$ (lsd test).

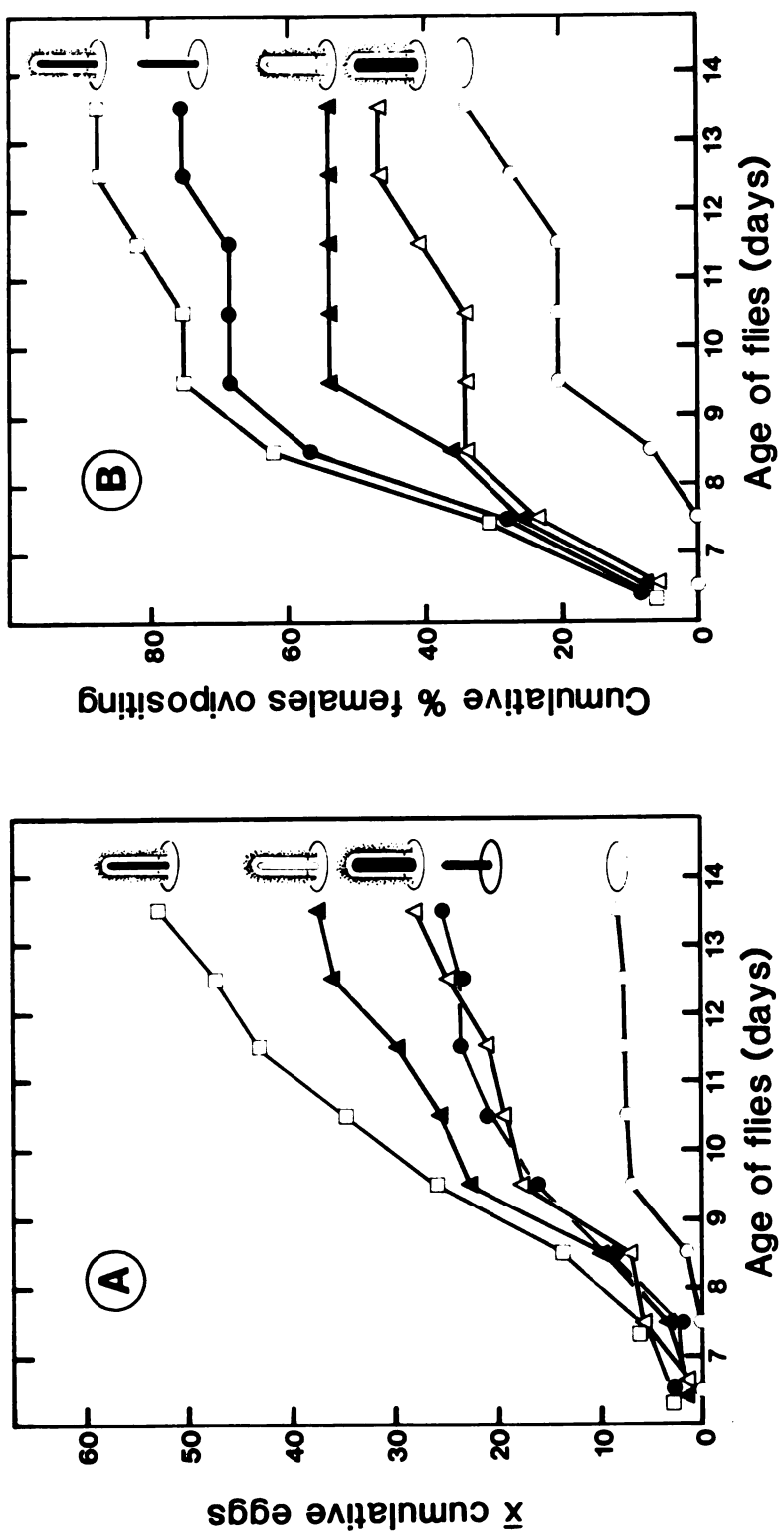

Figure 14. Cumulative numbers of eggs laid (a) and cumulative percent of Delia antiqua females accepting (b) altered and unaltered foliar surrogates and sand controls in no-choice bioassays. Symbols for treatments are as given in Figure 13.

Table 3. Mean percent of total eggs laid per ovipositing Delia antiqua female on unaltered surrogates vs all other treatments on each dayof the experiment.

Mean age of ovipositing females	Unaltered surrogates (n=13)	Altered surrogates and sand controls (n=27)
6.5	1.8 c	7.0 c
7.5	4.4 bc	5.3 bc
8.5	18.2 ab	14.4 b
9.5	24.6 a, ***	40.1 a, ***
10.5	12.4 abc	5.0 bc
11.5	16.0 ab	7.3 bc
12.5	8.5 abc	13.9 bc
13.5	14.0 abc	6.8 bc
Total eggs	837	1,572

[1]The number of females ovipositing on treatments is shown as n.

[2]Means within rows followed by *** are different at $p<0.06$ (Wilcoxon two-sample test). Means within columns accompanied by the same letter are not significantly different at $p<0.01$ (lsd test, on log-transformed data).

females given access to altered surrogates. A strong negative correlation existed between mean total eggs per treatment and mean numbers of mature eggs remaining in the ovaries of females exposed to those treatments (r^2=.97, F=102.4, $p<0.005$). Females given access to the more acceptable treatments tended to mature more eggs (total eggs laid plus eggs in ovaries) during the eight-day experimental period (Table 2); however none of these differences were significant ($p<0.05$, ANOVA).

Choice Bioassay- Fat Surrogates with Varying Concentrations of Pr_2S_2

Because 15 mm diameter surrogates were coated with 5 times as much Pr_2S_2-treated wax as 4 mm diameter surrogates (wax layer on both was 0.5 mm thick) reductions in oviposition seen in Experiment 1 for 15 mm diam surrogates could have resulted from elevated <u>total</u> amounts of Pr_2S_2 rather than alterations in size stimuli. However, when the concentration of Pr_2S_2 in wax coatings was reduced so that the total amount of chemical per 15 mm surrogate was equal to the total amount found in 4 mm diameter surrogates, ovipositional responses were reduced ($p<0.01$). Fifteen mm diameter surrogates coated with reduced amounts of Pr_2S_2 did not stimulate more oviposition than surrogates coated with unadulterated wax (ns at $p<0.05$, lsd test) and received 88% fewer eggs than the 15 mm surrogate used in Experiment 1. Oviposition on all treatments in this experiment was minimal (12 replicates, total eggs=22) even though flies were given no alternate ovipositional dishes for the six-day experimental period.

Mature Eggs in Ovaries of Deprived and Non-deprived Females

Females given access to unaltered surrogates (Figure 15) when 7.0 days-old (nondeprived) retained 8.9 mature eggs in their ovaries (range 0-51 eggs) in contrast to the 28.7 eggs (range 0 to 104) retained in the ovaries of females given no oviposition dishes (significant at $p<0.01$, $F= 49.5$, two-way ANOVA). Differences in eggs retained by the two groups were not significant until flies were 8.5 days-old ($p<0.01$, $F=15.63$, one-way ANOVA). Differences in numbers of eggs retained by 7.5- to 15.5-day-old non-deprived females were not significant from numbers of eggs found in the ovaries of 5.5- to 6.5-day-old deprived females ($p<0.05$, $F=1.71$, one-way ANOVA). However, deprived females of ages 7.5-15.5 days retained significantly more eggs than 5.5-6.5 day-old deprived females ($p<0.001$, $F=8.84$, one-way ANOVA). Differences in eggs retained by deprived flies 7.5-15.5 days-old were not significant ($p<0.05$, $F=1.56$, ANOVA). The ovaries of 15.5-day-old deprived females showed signs of oosorption; eggs appeared shrunken and more highly pigmented than eggs found in the ovaries of younger flies. Females showing signs of oosorption also possessed substantial amounts of fat body.

DISCUSSION

Removal of a chemical stimulant or alterations of color or size of foliar surrogates caused similar reductions in D. antiqua oviposition. In choice experiments in which populations of females were exposed to all treatments, these reduction were expressed in numbers of eggs laid per treatment during the 24-hour experimental period; reductions ranged from 70% for the color-altered surrogate to

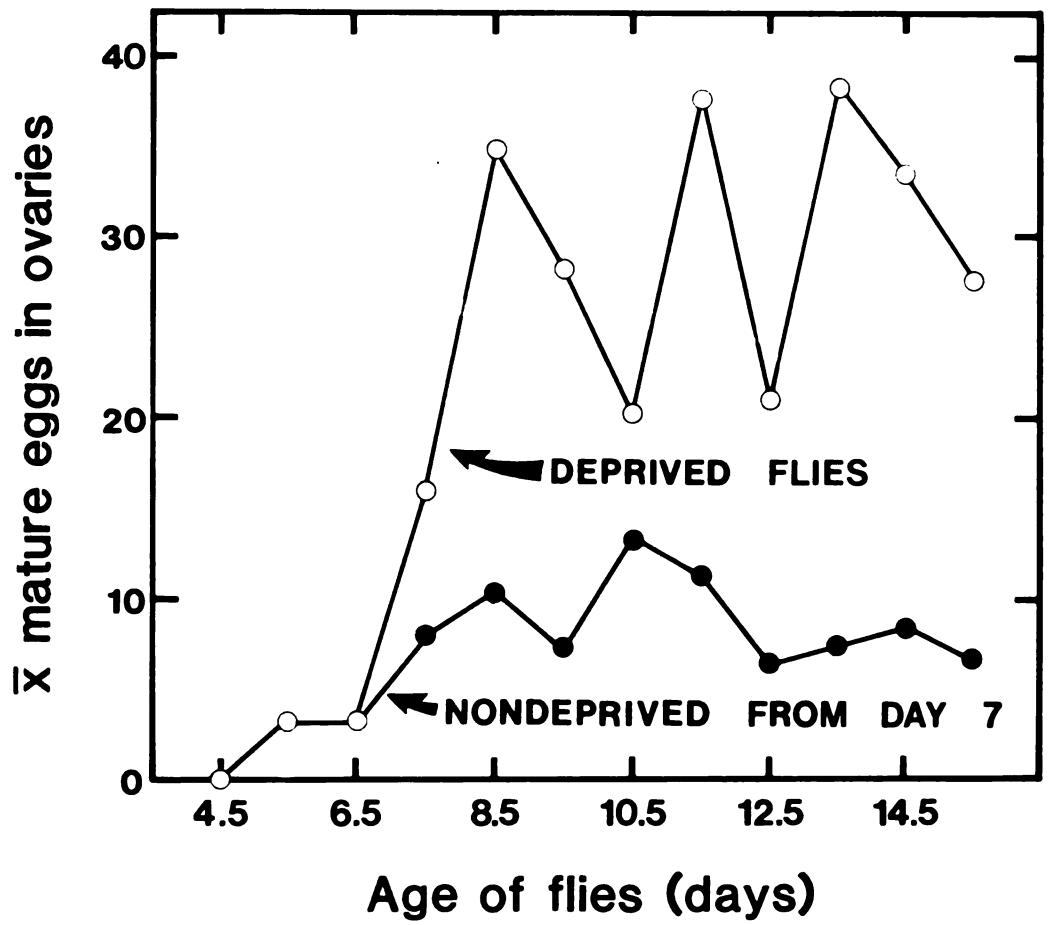

Figure 15. Mature eggs remaining in the ovaries of Delia antiqua females given no oviposition dishes (deprived) or oviposition dishes containing unaltered surrogates (nondeprived). Oviposition dishes were introduced into cages of nondeprived flies on day 7. Each data point represents mean numbers of mature eggs for 18-31 females. Total eggs= 6,997.

84% for the chemical-altered surrogate to 89% for the size-altered surrogate. Behavioral mechanisms underlying these reductions were not evident from these results; reduced egg counts could have occurred because smaller numbers of females accepted altered surrogates or because females accepting altered surrogates laid fewer eggs. No-choice experiments indicated that the former was the major factor. Surrogates with altered color, size or chemical cues were less likely to be accepted by females during the 8-day experimental period; however, once accepted, altered and unaltered surrogates received similar numbers of eggs.

Choice and no-choice bioassays yield different insights into ovipositional responses and thresholds. Choice tests using populations of females reveal which treatments are most acceptable to the largest percent of the population. Though the majority of the flies in this population would presumably have similar thresholds of acceptance, small numbers of eggs laid on less acceptable treatments could reflect the presence of females with lowered thresholds. Since some females do mature substantial numbers of eggs before the age at which we initiated experiments (6.5 days), groups of females carrying larger egg loads could be less discriminating and make "wrong" choices even when given a choice of ovipositional sites, including the optimal surrogate. Thresholds of individual females could also be lowered after visiting, examining or accepting a stimulatory ovipositional site. Sites visited immediately thereafter might therefore be more acceptable even though only a subset of the stimuli sensed in the original site are present. Preliminary observations of marked individuals interacting with the five treatments used in the original

choice test (Harris, unpublished) revealed that females do in fact visit and examine numerous treatments before accepting any one treatment and will accept altered surrogates after visiting unaltered surrogates. Visits consisted of flight onto the surrogate or sand surrounding surrogates followed by no movement or runs up and down vertical surfaces of surrogates. Because dishes containing no foliar surrogates were rarely visited, it appears that surrogates rather than dishes stimulated alighting.

No-choice experiments revealed more about the "rank order" of treatments and the latencies of ovipositional responses (specificity of Singer, 1982). Though Singer measured these parameters by offering different plants to individual females and measuring time to acceptance, they can also be estimated from graphs representing responses of populations of individually-tested females. For example, in Figure 14b we can see that on day 9.5, approximately 75% of the females tested had accepted the unaltered surrogate while only 68%, 55%, and 35% had accepted surrogates lacking chemical, color and size stimuli, respectively. Thus, in terms of rank order or the relative rates at which the five treatments become acceptable, the unaltered surrogate ranks highest followed by surrogates lacking chemical, color and size stimuli. The specificity of onion flies, or the relative rates at which females become less discriminating (Singer, 1982) is indicated by the slopes of the lines and the vertical distance between curves. For example, on day 6.5, all females rejected the sand control and most females rejected the unaltered and altered surrogates. Between days 7.5 and 8.5, all females still rejected the sand control but ca. 22-30% of the females accepted altered and unaltered

surrogates. This period would correspond to the discriminating phase of Singer (1982).

Though 30% of the females exposed to the altered and unaltered surrogates did not appear to go through a measurable discriminatory phase, the other 70% apparently did. This is shown by the increasing differences in slopes and vertical distances between curves after day 8 and by the level attained by the curve on the final day of the experiment. Whether these differences in length of discriminatory phases for these populations are due to different egg maturation rates or more permanent differences in thresholds is not known. Discriminatory phases could be more accurately measured by using intervals of less than 24 hours; Rhagoletis pomonella showed significant shifts in host acceptance thresholds 5-10 minutes after being deprived of hosts (Roitberg and Prokopy, 1983).

Both Singer's (1982) and our no-choice experiments measure thresholds of acceptance that have probably been modified by experience. In Singer's case this experience consists of encounters with other host plants. Previous host encounters do alter acceptance/rejection thresholds in Rhagoletis pomonella (Prokopy et al., 1982) and numerous other insect herbivores (see Papaj and Rausher, 1983, for review). In our bioassay, females experience a single oviposition site for the entire experimental period. If stimuli comprising this site both prime responses, for example by causing eggs to be matured (Weston and Miller, in press) and release responses, we may be measuring thresholds modified by egg load. Whether either type of experience significantly influences host acceptance thresholds of D. antiqua is not known.

Thresholds for acceptance appear to be influenced by egg load. The period during which behavioral thresholds for sand controls and altered surrogates are lowered (day 9 to 10) is parallelled by a buildup of mature eggs in the ovaries (Figure 15). We doubt whether this relationship is coincidental; the presence of large numbers of mature eggs in ovaries is probably communicated to the CNS (Mesnier, 1984) or to the peripheral nervous system by humoral pathways and may alter responses of cells in the CNS or sensitivity of peripheral receptors to external stimuli (Davis, 1984). Either change could effectively reduce acceptance thresholds. A behavioral analog of this has been observed in sphingid moths (Knoll, 1922 cited in Hinton, 1981). Orientation, alighting and acceptance are normally triggered by plant color and chemical stimuli; however, deprivation of ovipositional sites causes females to respond to reduced sets of stimuli and to lay eggs on anything with appropriate color stimuli.

What do these experiments tell us about the relative importance of color, size and chemical stimuli? Non-chemical stimuli are generally assumed to be of secondary importance in host finding and acceptance (for precedents see Thorsteinson, 1960; Beck, 1965). However, in the onion fly (and probably many other insect herbivores) this assumption does not hold. Alterations of chemical and non-chemical stimuli all reduced oviposition albeit in somewhat different manners. For example, increasing the diameter of surrogates greatly reduced numbers of females accepting surrogates but had no apparent effects on numbers of eggs laid once the surrogate had been accepted. Surrogates lacking chemical stimuli had the opposite effect; though a larger percent of females eventually oviposited on the surrogate,

these females also tended to lay fewer eggs.

However, we cannot in all fairness conclude from this experiment that color, size and chemical stimuli are equally important. It is not possible to alter stimuli perceived by different sensory modalities in an equivalent manner (say by 50%), nor do we not know whether alterations have rendered stimuli neutral or inhibitory. For example, size stimuli were altered in our experiments from a diameter that is known to stimulate oviposition to a diameter that does not elicit oviposition. The absence of a behavioral response could have occurred because the larger diameters were not stimulatory or because they were actually inhibitory. If the size alteration was inhibitory rather than neutral, an equivalent alteration in a chemical stimulus should have not only removed the stimulatory chemical (Pr_2S_2) but also added an inhibitory chemical. It is also difficult to determine the relative importance of various stimuli if they are important at different points in the behavioral sequence leading up to oviposition. For example, if color is important early in the sequence and is removed, the additional removal of stimuli important later in the sequence, such as size, may have no apparent effect simply because the behavioral sequence has been disrupted at an earlier stage. Thus we need to know more about mechanisms of host-plant recognition before we can fully address the question of the relative importance of chemical vs. non-chemical stimuli.

We can, however, conclude unequivocally that interactions among stimuli are critical to the host recognition process. Host finding and acceptance require combinations and sequences of specific stimuli (Kennedy, 1965); behavioral responses to host plant stimuli cause

insects to move, thereby altering the position of peripheral sensory receptors so that new stimuli can be perceived. This process is well-illustrated by data on host acceptance behaviors of onion flies (Harris and Miller, 1983; 1984). Flies are stimulated to alight by large areas of color and chemical cues. After alighting, shape and size cues are perceived during runs over the surface of surrogates. Specific size and shape cues stimulate females to continue these runs, extend their mouthparts, curl the abdomen downwards and extrude the tip of the ovipositor so that it is brought in contact with the waxy surface of the surrogate or plant. Stimuli eliciting runs therefore change the behavior of females so that chemoreceptors on the tarsi, antennae, mouthparts and ovipositor are exposed to chemical stimulants embedded in surface waxes. Examination of foliar surfaces is followed by examination of the substrate by mechanoreceptors (and possibly chemoreceptors) located on the tip of the ovipositor.

Combinations of stimuli must be perceived by D. antiqua not only to trigger single behaviors (size and shape trigger runs) but also to allow sequences of behaviors to progress towards acceptance of ovipositional sites (runs to probing). This behavioral requirement for simultaneous or sequential perception of stimuli of different modalities may reflect physiological requirements of higher order neurons in the central nervous system. Central body neurons in bees and crickets respond only when certain combinations or temporal patterns of stimuli are presented (Strausfeld et al., 1984). Onion flies may possess similar neurons which fire only when combinations of host stimuli are perceived.

These cells or higher integrative centers might also be influenced by hormones signalling the presence of mature eggs in the ovaries and could conceivably fire in response to subsets of complete host stimulus patterns (e.g. only chemicals or structural stimuli) when large numbers of eggs are ready to be oviposited. The firing of these cells could in turn trigger the release of oviposition-stimulating hormones by neurohaemal organs. In Galleria mellonella (Lepidoptera), oviposition-stimulating hormones (OSH) are stored in, and released into the hemolymph from, segmental nerves and neurosecretory cells associated with the CNS (Mesnier, 1984). These hormones act on the visceral muscles of the oviduct, triggering contractions which allow movement of mature eggs through the ducts. Final deposition of eggs, however, is controlled by the last abdominal ganglion, which integrates information gathered by sensory hairs during probing movements by the ovipositor.

If such a regulatory system also functions in D. antiqua, acceptance of ovipositional sites could pass through two phases, acceptance of the plant itself which triggers the release of OSH by the CNS, and acceptance of the substrate which is mediated by the terminal ganglion and triggers final expulsion of eggs. Such a system also suggests a mechanism for differences in numbers of eggs laid by ovipositing females on different surrogates; highly stimulatory patterns of neural input could trigger release of larger amounts of OSH, thereby causing contractions of the oviducts and egglaying to persist for longer periods of time. Less stimulatory patterns would presumably trigger the release of smaller amounts of the hormone. Such hypotheses are presently being investigated in our lab.

CHAPTER 4

Mechanisms of Resistance to Onion Fly Egg-laying

INTRODUCTION

For several decades, host colonization by herbivorous insects has been viewed as a process stimulated mainly by plant chemicals and deterred by other plant chemicals (Thorsteinson, 1960; Beck, 1965; Renwick, 1983) as well as by certain textural characters such as trichomes (Pillemer and Tingey, 1976; Norris and Kogan, 1980). Physical characters such as plant color and shape have also been shown to influence colonization (Prokopy et al., 1983; Prokopy and Owens, 1983), but are thought to be less important because they are presumed to influence early (pre-alighting) rather than later (post-alighting) stages of colonization. Given this conceptual framework, it is not surprising that in seeking to explain resistance discovered in certain plant species or breeding lines, investigators tend to invoke chemical and textural characters while ignoring others.

Chemically-mediated forms of resistance have been postulated for onion cultivars exhibiting resistance to onion fly. Ikeshoji (1984) found a significant correlation between bacterial counts of bulbing onion roots and susceptibility of cultivars in the field. Soil bacteria are believed to metabolize S-propenylcysteine sulfoxides exuded by onion roots into dimethyl disulfide (Coley Smith and King, 1969; Ikeshoji, 1984). Dimethyl disulfide stimulates host finding in D. antiqua larvae but does not stimulate egg-laying in the adult (Matsumoto and Thorsteinson, 1968). Ikeshoji (1984) also hypothesized that soil bacteria increase acceptability of cultivars to ovipositing

females by perforating root epidermal cells. Such a disruption of root cells could trigger the formation of propylthio compounds through enzymatic reactions. It is the propylthio compounds which stimulate oviposition (Ishikawa et al., 1978; Vernon et al., 1978).

However, recent studies (Harris and Miller, 1982; 1983; 1984) on the host-colonization behavior of the onion fly have shown that plant chemicals are not the sole behavioral operants in this insect-plant relationship. In addition to chemicals typical of onion, females respond to color and structural characters of onion plants when ovipositing. Maximal responses occur only when there is a confluence of certain levels of chemical,visual, and physical stimuli.

In light of these findings, we examined the resistance of several selected onion (Allium cepa) breeding lines to D. antiqua. These breeding lines were found to stimulate less oviposition in choice and no-choice field trials in the Netherlands (de Ponti, unpublished results) and therefore were believed to be resistant because of nonpreference mechanisms (antixenosis of Kogan and Ortman, 1978).

MATERIALS AND METHODS

Insect Rearing

The laboratory culture used in these experiments originated from pupae collected from onions left in harvested fieldsnear Grant, Michigan. Flies emerging from these pupae were placed in screened cages housed in environmental chambers illuminated by Verilux fluorescent bulbs with LD 16:8 cycle (21 + 1^{o} C and 35 + 5% R.H.). Cages were provisioned with water, honey, a dry artificial diet

(Schneider et al., 1983) and oviposition dishes consisting of moist sand, chopped onion, and surrogate foliage (Harris and Miller, 1983).

Plant Material

Breeding lines were developed by Dr. de Ponti: line 1 = #81559 (F_2 of I_2 Rawska x K1979); line 2 = #81555 (F_2 of I_2 Zittauer x K1979); line 3 = #81548 (F_2 of I_2 Wolska x K1979); line 4 = #81565 (F_2 of I_2 Rawska x K1979); line 5 = #81573 (F_2 of I_2 Rawska x K1979); line 6 = #81575 (F_2 of I_2 Rawska x K1979); line 7 = #81571 (F_2 of I_2 Rawska x K1979); line 8 = #81544 (F_2 of I_2 DeNys Extra x K1979); line 9 = #81578 (F_2 of I_2 Rawska x K1979); and line 10 = #79165, "Jumbo" susceptible. This means, using line 1 as an example, that Rawska was selected and inbred two times and then polycrossed with a crossing population (K1979) consisting of selected I_2 lines of different origin. Seed was harvested from the I_2 Rawska. This half-sib seed was tested in field trials in 1980 and selected bulbs were grown for seed in 1981.

Twelve seeds were planted in 20 cm diam x 20 cm deep plastic pots containing organic soil. Pots were placed in a glasshouse and watered and fertilized (Peters 20-20-20 Soluble Plant Food, Fogelsville, PA) for six weeks. After 4 weeks of growth, plants in each pot were thinned so that 3 plants remained. The remaining three plants stood ca. 7 cm apart from each other. Before pots were placed in fly cages for choice tests, a 1.5 cm layer of soil was removed from the surface and replaced with moist silica sand.

Surrogate onions

Lengths (145, 180, 195, 200 and 245 mm) were cut from Pyrex tubing (1, 2, 3 and 4 mm diameters) and one end was heat sealed. These surrogate stems were painted with oil paints mixed to match the green of 6-week-old onion foliage (Harris and Miller, 1984). Painted cylinders dried for 3 weeks and then were coated with a thin layer of household paraffin wax.

Ovipositional dishes containing surrogate foliage were assembled 12 hr before presentation to flies. Ten ml of chopped onion were placed at the bottom of each 14 x 14 x 2.5 cm plastic ovipositional dish and covered with 100 ml of moist silica sand. Foliar surrogates were added to dishes immediately before placement in cages; they stood at the center of dishes, with all but 20 mm of their full length extending vertically above the sand.

Choice Tests

Five different choice experiments were run using breeding lines and surrogate onions. In the choice test with breeding lines, one pot (3 plants) of each breeding line was placed in each of four cages (1.7 x 0.7 x 0.9 m). Pots were spaced evenly (ca. 20 cm apart) and arranged randomly within the cage. Data were recorded as numbers of eggs laid around the base of each plant; also recorded were basal diameter at the interface with the soil and distance from the soil to the tip of each leaf. Because data for 1 pot (3 plants) of line 9 were lost, data were analyzed using a completely randomized design.

The four other choice test experiments were run using a randomized complete block design, with single linear block spaced

evenly within a single cage. The experiments consisted of the following treatments: Experiment 2= four foliar surrogates of varying heights (125, 175, 225, 275 mm) and of constant diam (3.0 mm), Experiment 3= four foliar surrogates of varying diam (1, 2, 3, and 4 mm) and of constant height (200 mm), Experiment 4= four foliar surrogates of varying height and diameter (1 mm diam x 125 mm height, 2 mm diam x 175 height, 3 mm diam x 225 mm height, and 4 mm diam x 275 mm height), Experiment 5= seven plants (breeding line 10) of various sizes (2, 3, 4, 5, 6, 7, and 8 mm diam), and Experiment 6= seven foliar surrogates of varying diameters (2, 3, 4, 5, 6, 7, and 8 mm) and of constant height (200 mm). Treatments were placed in 1.7 x 0.7 x 0.9 m screened cages housed in the aforementioned environmental chambers. Cages contained several hundred reproductively mature onion flies (8 to 20 days old) 2 to 4 generations removed from field populations. After a 24-hr exposure to flies, treatments were removed from cages. Eggs were separated from sand by flotation and counted.

Breeding line data were analyzed by two-way analysis of variance (ANOVA) and analyis of covariance (ANCOVA). Eggs laid on breeding lines and foliar surrogates were regressed on basal diameter and height. Slopes of these lines were compared for equality by the methods of Sokal and Rohlf (1981). The coefficient of determination (square of product-moment correlation coefficient) was computed for interdependent variables (height and diameter).

RESULTS

Breeding lines exhibited a wide range of acceptability to ovipositing D. antiqua ($F=8.61$, $p<0.01$, ANOVA) (Figure 16). The

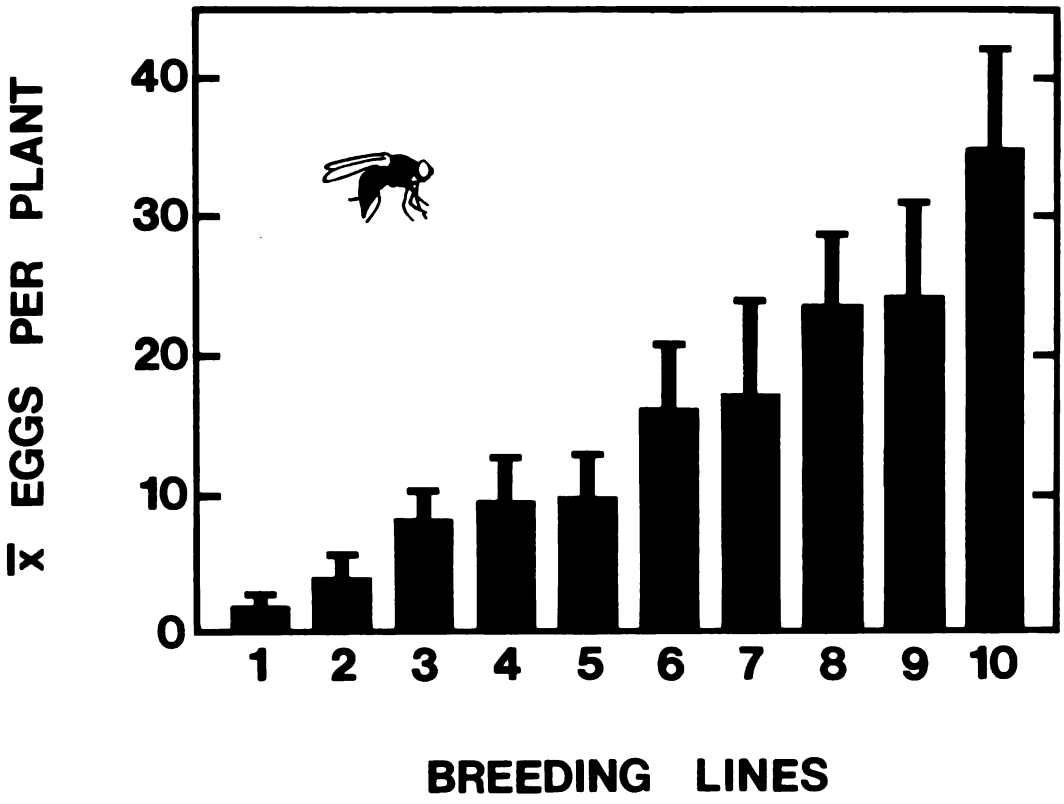

Figure 16. Ovipositional responses ($\bar{x} \pm SE$) to selected onion breeding lines in laboratory choice tests. See text for description of lines. Total eggs for 117 plants = 1,715.

susceptible cultivar "Jumbo" (line 10) received 16 times more eggs than line 1. However, breeding lines also differed significantly in size (F= 14.24 for basal diameter, F=11.15 for height, $p<0.01$, ANOVA). "Jumbo" had a mean height and diameter 1.9 and 2.4 times greater than line 1.

Because differences in oviposition appeared to mirror differences in size, we reanalyzed the data using analysis of covariance, with egg numbers as the dependent variable, breeding lines as the nonmetric independent variable, and size as the independent covariate. Both basal diameter and height of foliage served as measures of size and were highly correlated with one another ($r^2=0.70$). Analyses using either height or diameter as the independent covariate revealed linear relationships (Figure 17 a,b) between size and numbers of eggs (F=39.01 and 17.16 for diameter and height, respectively, $p<0.0001$, ANCOVA) but no significant differences in ovipositional responses to the 10 breeding lines when differences in size were accounted for (F=0.94 and F=0.39 for breeding lines using height or diameter as the covariate, respectively, ANCOVA).

When the range of heights shown by the 10 breeding lines was mimicked by foliar surrogates differing only in height (Figure 17 a), there was no evidence of a linear relationship between surrogate height and eggs (F=1.02, ANOVA). Two-way ANOVA also revealed no differences in ovipositional responses to the 4 different heights (F= 1.12). However, when onion basal diameters were mimicked by foliar surrogates differing only in diameter (Figure 17 b), there was a strong linear relationship between eggs and diameter (F=29.03, $p<0.01$, ANOVA). The slope of this line was not significantly different from

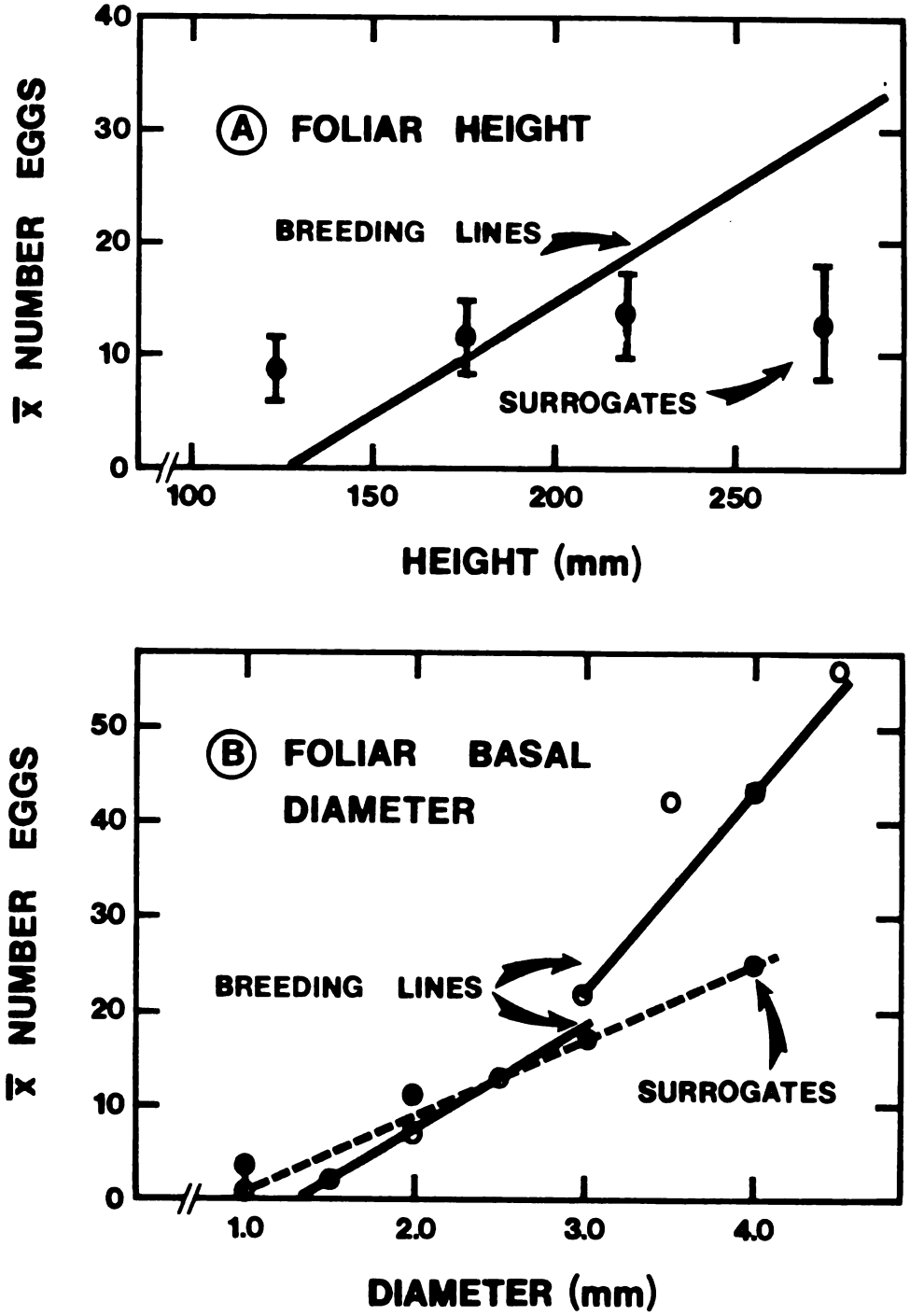

Figure 17. Ovipositional responses to height (a) and basal diameter (b) of onion breeding lines and foliar surrogates. Solid circles and open circles represent mean eggs laid for surrogates and breeding lines, respectively. Standard error bars are given for responses to surrogate heights in (a). Total eggs for 18 replicates of (a) = 874, for 18 replicates of surrogates (b) = 1,030.

the slope of the line generated for eggs vs onion basal diameters of 1 to 3 mm ($F=0.57$, equality of slopes test). Responses to onions and surrogates tended to diverge beyond diameters of 3 mm; the slope of the line for onions was steeper (20.4) and significantly different from the slope of the line (8.4) for surrogates at $p<0.10$ ($F=3.89$, equality of slopes test) but not at $p<0.05$. Ovipositional responses to surrogates that mimicked both the height and diameter differences of onion plants (1 mm diameter x 150 mm height, 2 mm diameter x 200 mm height, etc.) did not differ significantly from responses to surrogates that mimicked only diameter differences ($F=0.0016$, equality of slopes test).

When presented with a broader range of plant sizes (Figure18), females again laid fewer eggs on smaller authentic plants. In the range of 2-4 mm, this relationship was linear ($F=8.98$, $p<0.05$, ANOVA); however, beyond 4 mm, there was no evidence of a linear relationship ($F=1.98$, ANOVA). Surrogates also showed linearity in the diameter range of 2 to 4 mm ($F=4.89$, $p<0.05$, ANOVA) and once again did not differ significantly from onion plants ($F=0.25$, equality of slopes test). Beyond surrogate diameters of 4 mm, the linear relationship between eggs and diameter ($F=9.63$, $p< 0.01$, ANOVA) had a negative slope of 26.7.

DISCUSSION

Though herbivorous insects are often categorized as specialists on particular plant species, many are far more specialized than their common names denote. Oviposition and feeding may not only be limited

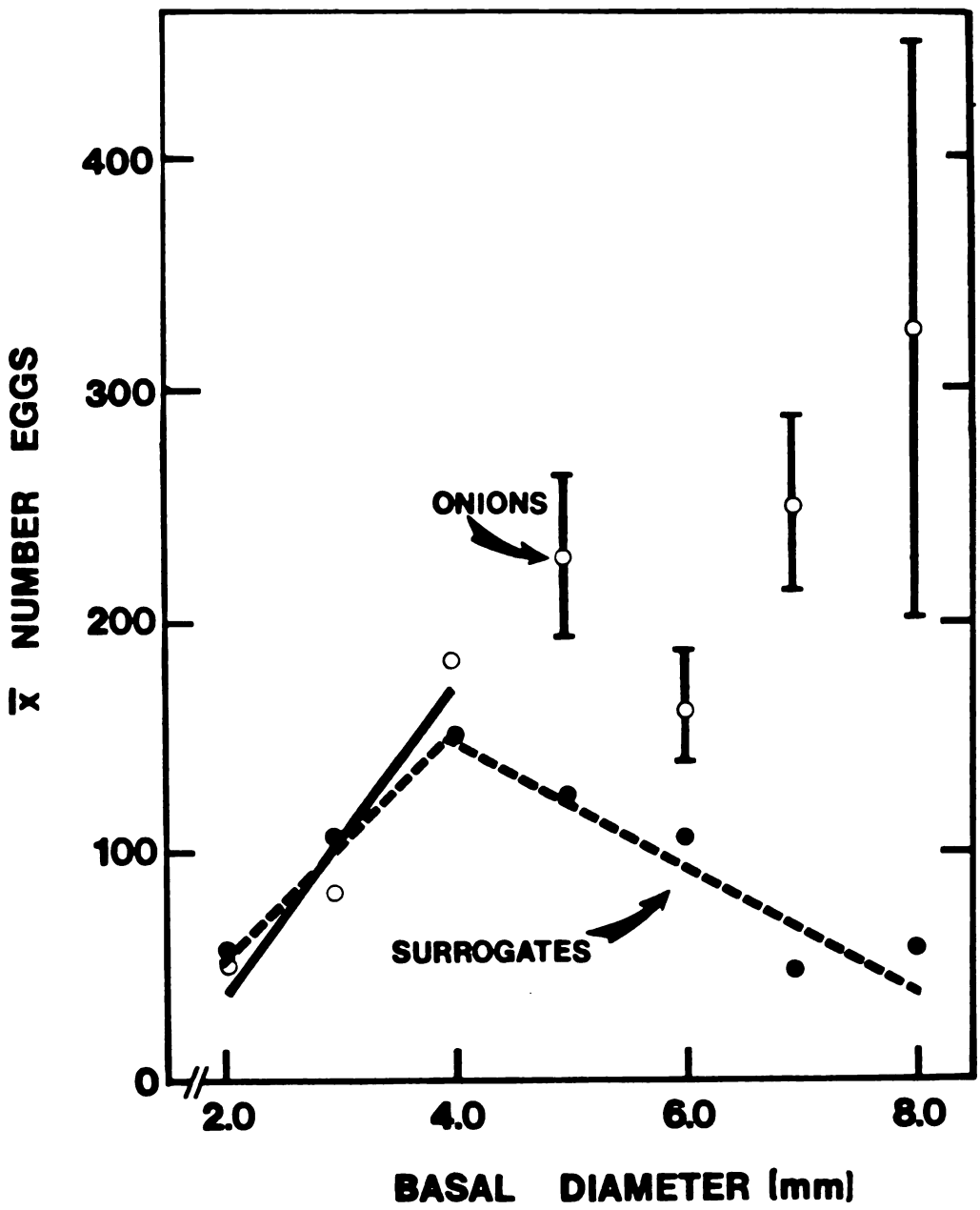

Figure 18. Ovipositional responses to a single breeding line and surrogates ranging in diameters from 2 to 8 mm. Solid circles and open circles represent mean eggs laid for surrogates and breeding lines, respectively. Standard error bars are given for responses to authentic onion plants 4-8 mm in diameter. Total eggs for authentic onions= 6,416, for surrogates = 5,764.

to single plant species and specific plant parts, but also to particular growth stages. If these growth stages are not synchronized within a population of plants, herbivores will be confronted with plants of varying acceptability and often will concentrate egg-laying or feeding on the most acceptable plants (Ives, 1978; Rausher and Papaj, 1983).

Plants therefore can gain a certain degree of immunity if non-preferred growth stages coincide with periods of insect feeding and oviposition. This immunity would presumably be transient if non-preferred stages grew into preferred stages while insects were still present. Because of its transience, immunity of this type is considered to be a form of pseudoresistance (Painter, 1951) and has been termed "host evasion". Knowledge of the existence of this form of resistance has been used to alter sowing dates of several different grain crops so that plants pass through susceptible stages when herbivore densities are minimal (Painter, 1951; Jonasson, 1980; Delobel, 1982).

Eastern European onion breeding lines grown under glasshouse conditions reached susceptible stages at different times. Breeding lines that grew slowly, attaining basal diameters of 1-2 mm and heights of 90-200 mm after six weeks of growth, did not stimulate ovipositing D. antiqua females and received very few eggs relative to those lines attaining diameters of 3-4 mm and heights of 200-300 mm. Analysis of covariance revealed that there were no significant differences in ovipositional responses to breeding lines when differences in size were taken into account. However, beyond the size range of 1-4 mm basal diameters and 90-300 mm heights, egg-laying was

not linearly related to plant size. Therefore, if breeding lines had been tested at a later growth stage, differences in ovipositional response would presumably not have occurred, unless some other mechanism of resistance came into play.

Field studies support our observation that seedling onions are not particularly stimulatory to ovipositing onion flies. In the spring, when most of the damage to the onion crop occurs, early sown onions and vigorously growing onions receive more eggs than smaller seedlings (Ellis and Eckenrode, 1978). Sprouted onion bulbs planted deeply in the soil receive so many more eggs than seedlings that they were advocated as trap crops before the advent of pesticides (Gray, 1924).

After passing through a period of susceptibility, onions once again become less acceptable to ovipositing onion flies. Cultivars which stimulate oviposition and support larval growth in the early part of the growing season become unacceptable to egg-laying females during formation of the onion bulb (Perron, 1972). If less mature cultivars are available when other cultivars are bulbing, female D. antiqua preferentially oviposits on smaller plants. Onion flies will also shift from laying eggs on onions to laying eggs on slower growing Allium species, such as shallots and leek, late in the season (Labeyrie, 1957).

How does D. antiqua distinquish between different growth stages of onion? Though we do not know why older plants become less acceptable, responses to onion seedlings can be attributed almost entirely to differences in stem basal diameter; egg-laying around onion surrogates varying in basal diameter mimicked responses to

onion plants of similar sizes. Changes in surrogate height in the range of 90-300 mm had no effect on ovipositional responses. Females apparently measure basal and foliar diameter during examining runs over vertical surfaces and circling movements around the plant base (Harris and Miller, 1984). If the cylinder is too narrow, runs are discontinued and females leave the plant or surrogate plant before ovipositing. If the cylinder is of an optimal size (4 mm), females move from the foliage to the soil, and subsequently lay eggs in crevices near the base of the plant.

Precise measurement of host plant size has been noted in several other insect herbivores. The ragwort seed fly, D. seneciella, lays eggs on unopened and newly opened buds (Frick, 1970). Tests with surrogate buds proved that responses to developmental stages could be attributed to size alone. Coincidentally, spheres preferred by D. seneciella for oviposition had diameters of 4 mm. Flies in the genus Rhagoletis are also noted for their abilities to distinquish sizes and shapes of host fruits (Prokopy and Boller, 1971; Prokopy and Bush, 1973). Size also influences host acceptance in the bruchid, Callosobruchus chinensis (Avidov et al., 1965 a,b). Indeed, all seeds sharing a particular set of size, shape, and textural characteristics were accepted by ovipositing females regardless of their suitability as larval hosts.

We can only speculate why ovipositional responses of D. antiqua to authentic onions and surrogate onions were similar for basal diameters of 1-4 mm but diverged beyond basal diameters of 4 mm. It is possible that small onions do not generate significant amounts of chemical stimulants in foliar surface waxes and therefore are

perceived to be identical to foliar surrogates mimicking their size characteristics. Levels of chemicals produced by larger plants may excede perceptual thresholds; responses of ovipositing females to plants of this size would therefore reflect effects of chemical stimuli superimposed on responses to size stimuli. Responses to surrogates of this size range could therefore indicate that chemical stimuli present in larger plants in effect neutralize inhibitory effects of larger size stimuli. Why D. antiqua would have evolved preferences for such a narrow range of basal diameters is also not known. Such preferences may reflect the superior suitability of certain developmental stages of onions, but could also conceivably be evolutionary relics, indicating more about the suitability of previous rather than present host plants.

Although our experiments proved that stem diameter was the most important determinant of differential oviposition by D. antiqua on onion breeding lines tested in the laboratory, we emphasize that oviposition resulted from the interaction of multiple stimuli across sensory modalities (Harris and Miller, 1982; Miller and Harris, 1985). In addition to structural characters, seedlings provided chemical, color and tactile stimuli; however, these stimuli were apparently less variable or influential across these breeding lines than was stem diameter.

SUMMARY

SUMMARY

CHAPTER 1: Foliar Form Influences Ovipositional Behavior

When presented with dishes containing a range of "foliar" shapes, onion fly females laid the most eggs around narrow (4 mm) vertical cylinders. Response to cylinders diminished when their diameter was increased or decreased, when cylinder height was reduced to less than 2 cm, and when cylinder/substrate angle deviated from 90^{o}. Differences in egg numbers on stimulatory and non-stimulatory forms reflected primarily differences in post-alighting pre-ovipositional behaviors. Females alighting on narrow vertical cylinders initiated and completed stem runs rapidly and without interuptions, and frequently went on to probe and oviposit. Females alighting on other shapes, or larger, smaller, or non-vertical cylinders, either did not initiate or did not complete stem runs, or did not go on to probe after completing a stem run. Such females rarely oviposited. Possible causes of such examining behaviors are discussed.

CHAPTER 2: Behavioral Responses to n-Dipropyl Disulfide: Effects of Concentration and Site of Release

Onion fly females laid the most eggs on ovipositional dishes having n-dipropyl disulfide (Pr_2S_2) release rates of 1 to 6 ng/sec from polyethylene capsules placed beneath a sand substate. When dipropyl disulfide was released from the wax coating of surrogate foliage rather than from the substrate, ovipositing females again responded differentially to various concentrations, laying more eggs

around stems containing 75 and 107 ng/stem. Factorial combinations of several concentrations released from surrogate foliage and substrate proved that releases from surrogates were more stimulatory than those from the substrate. Females tended to lay more eggs around surrogate stems having Pr_2S_2 at the base rather than on the upper half of foliage. Observations of individual females performing pre-ovipositional examining behaviors on Pr_2S_2-treated surrogate stems indicated that females tended to land on the upper portions of the foliage, but after landing, spent most of their time examining areas of soil and surrogate within one centimeter of the soil/surrogate interface. Surrogate stems provide a realistic context for investigating effects of plant chemicals on host-acceptance behaviors.

CHAPTER 3: Influence of Chemical and Non-chemical Stimuli on Host Acceptance under Choice and No-choice Situations

When presented with ovipositional dishes containing various combinations of foliar shapes, colors, and chemical stimuli in a choice bioassay, onion fly females laid the most eggs around 4 mm diameter green cylinders coated with a thin layer of paraffin wax containing n-dipropyl disulfide (Pr_2S_2). Fly responses to foliar surrogates diminished 70 to 89 percent when cylinder diameter was increased to 15 mm, when the color green was removed, and when Pr_2S_2 was removed from wax coatings. In a no-choice bioassay in which females were given access to a single surrogate type for 8 days, reductions in ovipositional responses to altered surrogates were consistent with, but generally smaller than, those observed in the choice bioassay. Reductions in numbers of eggs laid in no-choice

experiments occurred because surrogates with altered color, size or chemical cues were less likely to be accepted by females during the 8-day experiment; however, once accepted, altered and unaltered surrogates received similar numbers of eggs. Acceptance of altered surrogates and sand controls coincided with a period of rapid egg maturation in the ovaries. The importance of sensory interactions and the difficulties associated with assessing the relative importance of chemical and nonchemical plant stimuli are discussed.

CHAPTER 4: Mechanisms of Resistance to Onion Fly Egglaying

When gravid onion fly females were presented with 6-week-old Eastern European onion breeding lines and a susceptible control in laboratory choice tests, mean numbers of eggs laid ranged from 34.8 to 1.6 eggs per plant. Differences in ovipostional responses were mirrored by differences in size among breeding lines. Analysis of covariance revealed no significant differences in ovipositional responses to breeding lines when differences in size were taken into account. Tests using foliar surrogates that allowed single size parameters to be varied while holding all other plant stimuli constant revealed that among plants with basal diameters of 1 to 3 mm and heights of 100 to 250 mm, diameter alone influenced responses of ovipositing females. Ovipositional responses to plants beyond this size range could not be explained by differences in basal diameter.

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APPENDIX

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1986-2

Title of thesis or dissertation (or other research projects):

Host-plant Recognition in the Onion Fly, Delia antiqua (Meigen)

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Marion Olney Harris

Date 3-5-86

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page ___ of ___ Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Delia antiqua Meigen	MI: Grant Newaygo Co. M. Harris 1986		28		10	10	10		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Marion Olney Harris

Date 3-5-86

Voucher No. 1986-2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 5 March 1986

Date