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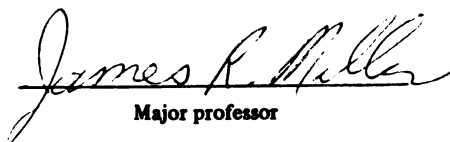
HOST-FINDING BEHAVIOR OF THE
ONION FLY, HYLEMYA ANTIQUA (MEIGEN)

presented by

Liene Liga Dindonis

has been accepted towards fulfillment
of the requirements for

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Major professor

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HOST-FINDING BEHAVIOR
OF THE
ONION FLY, HYLEMYA ANTIQUA (MEIGEN)

by
Liene Līga Dindonis

A THESIS

Submitted to
Michigan State University
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ABSTRACT

HOST-FINDING BEHAVIOR

OF THE

ONION FLY, HYLEMYA ANTIQUA (MEIGEN)

by

Liene Līga Dindonis

Host-finding behavior of the onion fly, Hylemya antiqua (Meigen) is due, in part, to chemical stimuli originating from the host plant, the onion. In field tests, both female and male behavior varied with host plant condition. Decomposing onion seedlings and bulbing plants elicited a greater host-finding response by female (but not male) onion flies, than did healthy plants. Clear acetate cone traps baited with the onion chemical, n-dipropyl disulfide, caught more female and male onion flies than traps baited with plants or propanethiol. Of variously damaged onions, only mechanically injured plants released a significant male response, whereas female onion flies were caught by traps baited with maggot infested, Fusarium inoculated, and mechanically injured hosts. These results suggest that distinctive blends of host plant volatiles, differing quantitatively or qualitatively, release varying degrees of female and male onion fly host-finding behavior.

To assess the effect of quantitative differences of a host-finding stimulus, H. antiqua response was tested to five

loadings of n-dipropyl disulfide (.1, 1, 10, 100, and 10 x 100 μ l) dispensed in polyethylene enclosures. Loadings of at least 1 μ l for females and 10 μ l for males were required to effect significant trap catches. Catches increased with loadings up to 100 μ l per capsule but then plateaued. As determined gravimetrically, the release rate from enclosures containing a reservoir of chemical remained constant under isothermal conditions but increased exponentially as a function of temperature.

Responses of onion flies to various onion and microbial volatiles, as well as volatiles from microbial cultures and decomposing onions were tested to characterize the most effective host-finding stimulus. Of nine onion and microbial volatiles tested individually, only the known attractant n-dipropyl disulfide caught significant numbers of female onion flies. However, a blend of these volatiles attracted more flies than any single chemical including n-dipropyl disulfide. In another experiment, agar plates inoculated with microorganisms from decomposing onions did not attract onion flies. However, cut onions, inoculated with microorganisms and conditioned four days, caught more female and male onion flies than freshly cut onions and n-dipropyl disulfide. These results suggest that a blend of chemicals, rather than a single key chemical, is the most effective host-finding stimulus, and that microbial activity enhances the attractancy of a blend of onion volatiles.

In addition, large numbers of Fannia canicularis (L.), the little house fly, responded to the microbial cultures, demonstrating a potential long range host-finding stimulant for this muscid.

The host-finding behavioral mechanism of H. antiqua was directly observed in the field in response to sliced onions, barnyard grass (Echinochloa sp.), and an unbaited control. More flies landed within 0.5 m of the onion bait than within 0.5 m of any of the other treatments. Flies which landed downwind of the onion bait flew directly or in a series of short flights toward the volatile source. These behaviors were not observed in the vicinity of the other treatments. Traps designed to assess flight direction with respect to the wind substantiated the observations of female and male upwind flight in response to distant host-plant odors. These results show that onion flies locate host plants as a result of chemically stimulated positive anemotaxis.

To my grandfather and his son,

Peteris and Aleksandris Dindonis

ACKNOWLEDGEMENTS

To Dr. James R. Miller, my major professor, I am indebted for his unyielding support, encouragement, and enthusiasm which guided me throughout this study. He has been the major force in developing my awareness of and respect for scientific research. The time and ideas he so generously shared with me will never be forgotten.

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INTRODUCTION

Host finding and host selection are the initial and possibly most significant events in the array of interactions between insects and their host plants. All ensuing host-related behaviors, such as feeding and oviposition, depend on the successful completion of this process. Although host finding is governed by a complex set of factors, (including environmental effects and the insect's physiological condition) the chemical stimuli produced by the host plant appear to play the predominant role in releasing the overt host-finding behavior (Shorey 1977). Host selection of phytophagous insects has been investigated since the early 1900's (Dethier 1978). However, most of these studies have dealt with contact chemoreception via gustation or taction. Olfactory responses, especially those occurring at some distance from the plant, have been frequently neglected or discounted as having a significant effect on host finding (Kennedy 1977). Recently, long-range host finding mediated by host plant volatiles has been demonstrated for a few insects (Kellogg et al. 1962, Haskell et al. 1962, Kennedy and Moorhouse 1969, Hawkes et al. 1978). Current laboratory

bioassay techniques allow the analysis of chemical stimuli to define effective host-finding stimuli. Identification of such behavior-modifying chemicals and an increased understanding of the corresponding behaviors may provide agricultural entomology with new approaches to possible pest control methods either through selective breeding or manipulation of pest populations. Furthermore, analysis of host-finding mechanisms may elucidate the evolutionary history of the plant-insect relationship and its interaction within the ecosystem.

The onion fly, Hylemya antiqua (Meigen) (Figure 1) was utilized in this investigation to study the chemical stimuli and behaviors involved in locating or selecting its host plant. This insect is well-suited for the study of host-finding behavior because it is a specialist whose survival is dependent on its ability to locate plants within the Allium genus (Loosjes 1976). Furthermore, some onion volatiles identified as oviposition stimulants have been successfully used as trap bait (Matsumoto 1970, Loosjes 1976).

A major pest of onions in North America, Europe, and Asia, the onion fly has developed resistance to organo-chlorine insecticides and more recently to many organophosphates (Ellis and Eckenrode 1979). Thus, future control of this onion pest may be dependent on the use of naturally occurring chemicals to modify the flies' behavioral patterns.

This investigation examines the onion fly's host-finding behavior by experiments designed to 1) demonstrate the occurrence of long-range host finding and describe the preferred host plant condition, 2) assess the effect of quantitative volatile differences on host finding and characterize the dispensing system used for controlled release of onion volatiles, 3) examine the effect of qualitative differences of the chemical stimulus as produced by mixtures of onion and microbial volatiles, and 4) describe the behavioral mechanism by which host location occurs.



Figure 1. Scanning electron micrograph of the head
of a female onion fly. 500X.

CHAPTER 1

Host-Finding Responses of Onion and Seedcorn Flies to Healthy and Decomposing Onions and Several Synthetic Constituents of Onion

INTRODUCTION

Survival and progenitive success of phytophagous insects are dependent on a well-orchestrated sequence of host-related behaviors including among others: 1) host finding, 2) host accepting, 3) feeding, and 4) reproducing. Various component behaviors may be mediated by visual, mechanical, and chemical stimuli originating from the plant. Although certain insect-plant interactions have been investigated extensively, long-range host finding as defined by Kennedy (1977), especially chemically mediated host finding, has been seriously neglected (Schoonhoven 1968, Dethier 1970, Kennedy 1977).

The degree to which insects rely on chemicals for locating host plants remains largely a matter of speculation. Thornsteinson (1960) suggested that the effect of host plant odors is generally restricted to arrestment after insects arrive at the plant. However, there is substantial support for the hypothesis that various insect specialists locate "unapparent" (Feeny 1976) host plants via directed orientation mediated by distinctive wind-borne host volatiles (Beeson 1930, Cripps 1947, McMullen and Atkins 1962, Kellogg et al. 1962, de Wilde et al. 1969, Kennedy 1977). Hawkes and Coaker (1976) and Hawkes et al. (1978) have recently demonstrated that the cabbage root fly, Hylemya (=Delia)

brassicae (Bouché), does, in fact, locate its host plant as a result of host volatile-stimulated upwind orientation and flight.

We have chosen to investigate the host-finding behavior of the onion fly H. antiqua, as affected by onion, Allium cepa L., volatiles. This plant-insect relationship is particularly suitable for such study because: 1) H. antiqua is a specialist whose survival has become limited evolutionarily to a few Allium species (Loosjes 1976) and hence this insect has probably evolved effective host-finding strategies; 2) Allium, an "unapparent" plant (Feeny 1976) in a natural situation, may be susceptible to discovery by H. antiqua because of its distinctive secondary chemistry; 3) an onion volatile, n-dipropyl disulfide, has already been identified as an oviposition stimulant for H. antiqua (Matsumoto and Thornsteinson 1968) and traps baited with this chemical have also caught significant numbers of H. antiqua in several studies (Matsumoto 1970, Loosjes 1976); and finally, 4) the chemistry of onion volatiles has been thoroughly investigated (Carson and Wong 1961, Bernhard 1970, Boelens et al. 1971).

An underlying hypothesis in this work is that different blends of plant volatiles produce graded host-finding responses by phytophagous insect specialists. Furthermore, the optimal olfactory stimuli for such host finding may be distinctive combinations of host and host-related volatiles. The latter components need to be considered in light of the findings of Eckenrode et al. (1975) that microorganisms

associated with germinating seeds produce the stimulants for oviposition by the seed corn fly, H. platura (Meigen). Likewise, several reports (Workman 1958, Loosjes 1976) suggest that maggot-infested and decomposing onions are selectively colonized by H. antiqua.

This initial report presents results of a study designed to establish methodology for quantifying H. antiqua host-finding behavior as well as to define the naturally occurring host condition eliciting greatest host finding.

METHODS AND MATERIALS

Trap design

Observations of H. antiqua responses to decomposing onion plants in a 2 x 1 x 0.3 m PlexiglasTM wind tunnel suggested that host finding in response to a wind-borne stimulus was largely a result of chemically stimulated positive anemotaxis. In light of these observations, it was judged that traps designed to selectively quantify this type of behavior in the field should: 1) have stimulus plumes emanating and remaining at ground level, 2) have entrance ports restricted to exiting plume dimensions, 3) require entrance into the traps for ensnarement, and 4) be able to contain and sustain live onion plants as baits.

Large cone traps (Figure 2), similar to the design of Matsumoto (1970) were constructed from .76mm clear acetate stock. A 30 cm-diam x 32 cm-high cone with a 2 cm

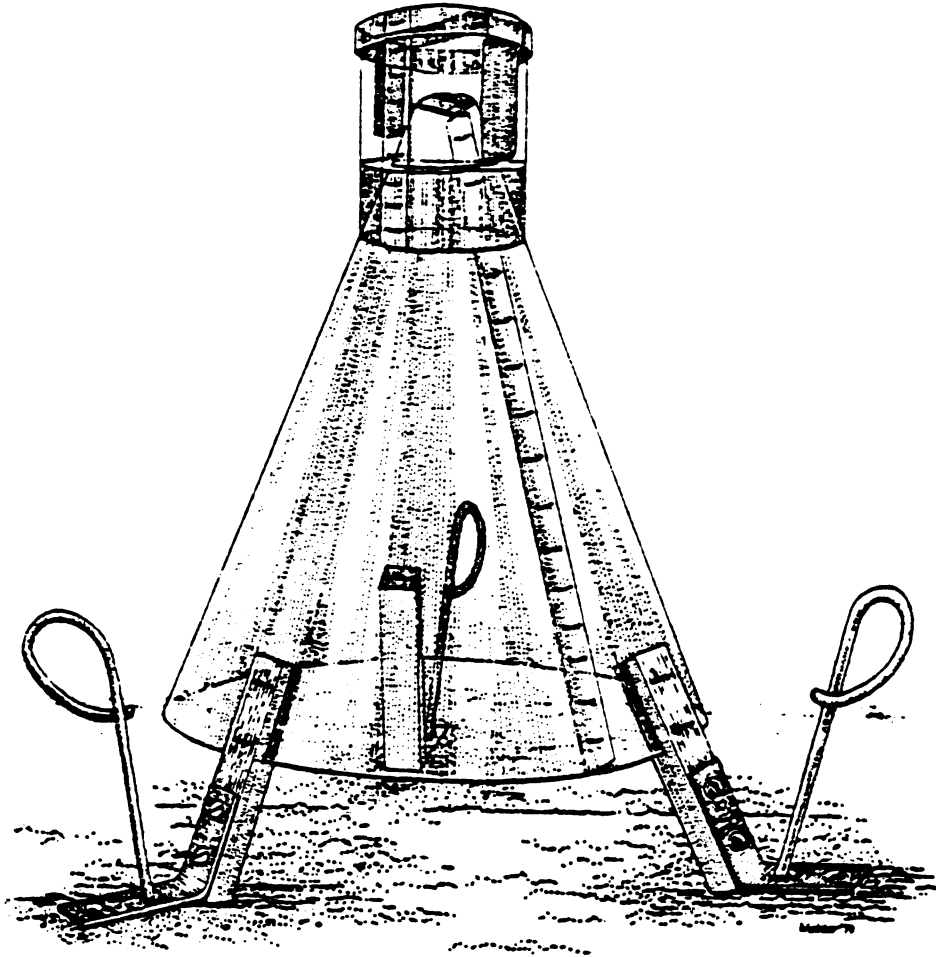


Figure 2. Acetate cone trap designed to quantify onion fly host-finding behavior.

opening was topped with a 5 cm-diam x 10 cm-high cylinder which was capped with a removable Petri dish cover (Figure 2). Wooden legs with angle brackets held the cone 5 cm above ground, while wire stakes secured the trap to the ground. Air could circulate beneath the trap and carry volatiles downwind and along the soil. Only walking insects or those flying close to the ground were able to enter the trap directly. Ethylene glycol (30% aqueous) was placed in the silicone-sealed groove between the cylinder and cone. This solution ensnared the flies, usually within one minute after they entered the cylinder, preserved them between collections, and allowed removal by pouring during fly collections. Since flies were undisturbed upon entering the traps, they could investigate and oviposit on the source. When leaving, they usually flew upward into the cone and were eventually caught. Thus, we could assess treatment effectiveness, not only as a stimulus for host finding, but also for oviposition.

Experiment I - Host finding responses to healthy and maggot-infested onion plants

The eight treatments tested were: both healthy and decomposing seedling and bulbing Stuttgarter Risen onion plants, two synthetic constituents of onion and two controls. The 8 cm-high onion seedlings were grown in nonsterilized muck soil, 30-50 per 15 x 20 cm plastic pot. The 20 cm-tall bulbing onion plants were grown from sets, seven per pot.

To generate the infested and decomposing condition, seedlings and larger plants were punctured with forceps and inoculated with 20 second and third instar H. antiqua larvae from a lab culture. The onion volatiles, n-dipropyl disulfide and propanethiol (Eastman Kodak Company), were released from size 3 (ca. 300 μ l) BeemTM polyethylene embedding capsules (Pelco Electron Microscopy Supplies, Ted Pella Company, Tustin, Ca). Loaded capsules were positioned with pointed end downward and were 3/4 embedded in pots of nonsterilized muck soil. As determined gravimetrically, the capsules released chemicals at ca. 900 μ g.hr⁻¹ (Dindonis and Miller, unpublished). A 20 cm-high barnyard grass, Echinochloa sp., planted in muck soil was the control used to determine if a green vertical object affected host-finding behavior. Traps over a pot of muck soil containing no plants were considered unbaited. In the field, the treatments were placed level with the ground in pot-lined cavities which facilitated rerandomization.

A randomized complete block design with treatments typically spaced ca. 10 m apart is commonly used in pheromone studies because, presumably, this spatial orientation decreases the probability of treatment interactions while allowing a degree of treatment competition for available insects (Roelofs and Cardé 1977). We compared a linear trap design having treatments spaced 3 m apart within blocks to a clustered trap design with 60 cm between adjacent treatments. The closely spaced trap design permitted mixing of treatment volatiles and allowed for the possibility of increased

treatment differences due to direct competition. Trap catches in a cluster design may provide information on fly responses to mixtures of varying stimuli as they would exist within an onion field.

A randomized complete block design with six replicates of blocks was used for each arrangement. Three replicates of both the linear and cluster design were located at each of two different onion farms, one in Stockbridge and the other in Eaton Rapids, Michigan. All treatments were placed on weed-infested field borders, ca. 3 m from the cultivated onions which were then 10-20 cm-high seedlings.

Trapped flies were collected and all treatments were watered and rerandomized every two days from May 30 to June 17, 1978. Chemicals were replenished when no reservoir was visible inside the capsules. Although other flies were found in the traps, only H. antiqua and H. platura were identified and sexed using the keys of Hockett (1923, 1965) and Brooks (1951). At the end of the experiment, pots were inspected for eggs and new maggot infestation.

Experiment II - H. antiqua host finding of variously damaged onions

The 20-30 cm-tall bulbing plants were inflicted with four types of damage: 1) punctured three times with a knife, 2) infested with first-third instar H. antiqua larvae, 3) mechanically injured plus maggot infested, and 4) inoculated with Fusarium oxysporum var. cepa, a common onion vascular wilt

disease causing lesions at the bulb base. Traps baited with the treated onions, four per muck-filled plastic pot, were deployed on the borders of an onion field in Hudsonville, Michigan, during August 7-28, 1978. At this time, the field sustained a damaging population of H. antiqua but very few H. platura. A linear randomized complete block design with a 3 m treatment spacing was used. Treatments within the five replicates were rerandomized and watered five times and flies were collected twice over the three weeks.

RESULTS AND DISCUSSION

Experimental designs

The linear and cluster designs proved equivalent in allowing differentiation among treatment means. To provide optimal resolution between possible treatment effects, the better design would consistently produce large treatment mean differences relative to corresponding variances. Comparison of the ratios of these two parameters for the linear and cluster designs, using coefficients of variation ($s/\bar{x}$) followed by Fisher's combined evidence test (Gill 1978), resulted in no significant differences between designs. The statistical advantage gained from arithmetically greater treatment mean differences in the linear design was negated by a correspondingly greater treatment standard deviation (Figure 3).

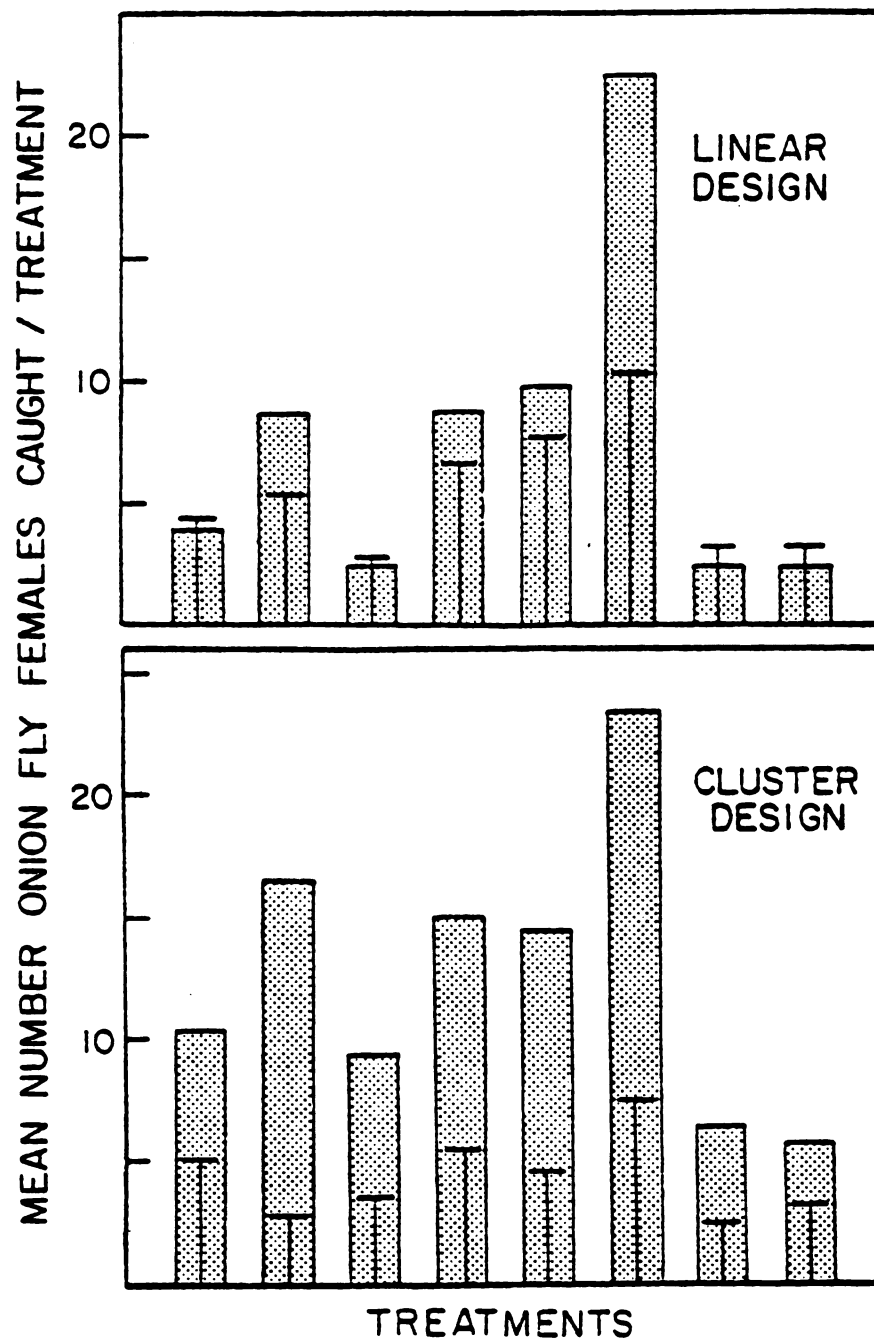


Figure 3. Comparison of the ratio of treatment means and standard deviations (coefficient of variation, $s/\bar{x}$) for two experimental designs resulted in no significant difference according to Fisher's combined evidence test.

The linear design, however, may provide a more accurate measure of the effect of a stimulus. Wide treatment spacing reduces the possibility of treatment plumes mixing, producing a trap catch representative of a single treatment. The close proximity of treatments in the cluster design results in a mixing or an overlap of the treatment plumes. Hence, the responding flies might be caught in a trap baited with a treatment other than the one initiating host-finding responses. Despite the possibility that less optimal treatment catches would be inflated, treatment mean differences for the cluster design were virtually identical with those from the linear design. One interpretation for the results of the cluster design is that the flies, having encountered two or more plumes simultaneously, respond to the optimal host by distinguishing among different blends of host plant volatiles.

A three-way ANOVA of the data from both experimental designs indicated an absence of interaction between designs and treatments, thus permitting the data to be pooled. However, since the results based on the combined data were not different from either of the individual design results, only data from the linear design will be presented here.

Experiment I - *H. antiqua* host-finding responses to healthy and maggot-infested onion plants

The eight treatments elicited widely and consistently differing degrees of host finding by onion flies (Figure 4).

The rotting onions, both seedlings and bulbing plants, caught significantly more *H. antiqua* females than their healthy

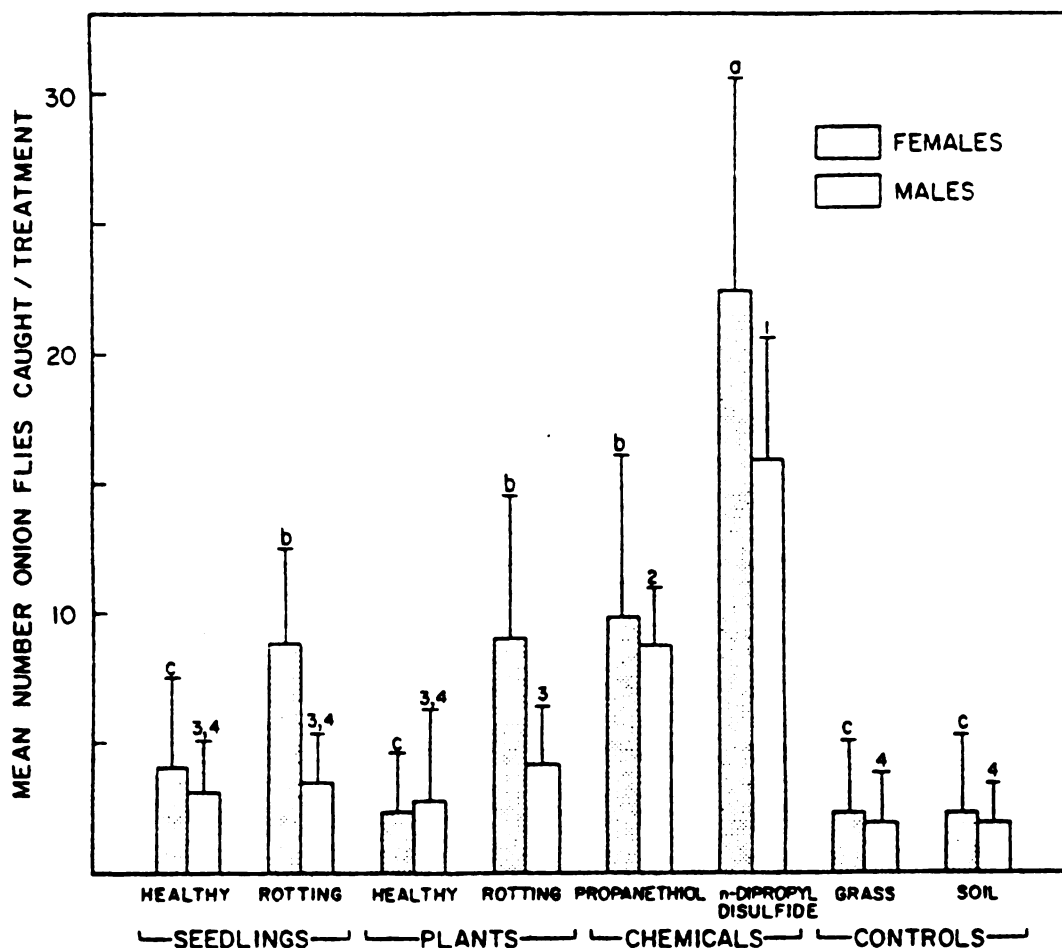


Figure 4. Mean trap catches and 95% confidence intervals for onion fly female and male responses to onion plants and synthetic onion chemicals. Treatments represented by the same letters (female) or numbers (male) are not statistically different as determined by 3-way ANOVA followed by a planned F-test for mean separation on data transformed to $(X + 0.5)^{1/2}$.

counterparts. This significant host-finding response indicated a qualitative and/or quantitative difference in the decomposing onion volatiles as perceived by the female flies. A quantitative difference may be due to damaged onion cells releasing high or proportionately different amounts of onion volatiles, whereas a qualitative difference might result from the additional release of microbial metabolites.

Onion fly females select decomposing onions for oviposition (Kendall 1931, Workman 1958, Loosjes 1976). Because this behavior is prevalent during the second and third generations, it has been postulated that larvae may not be able to penetrate large and healthy bulbs (Perron 1972). Larval survival may thus be dependent on the flies' ability to find decomposing onions. If this host-finding behavior were mediated from a distance by volatile constituents specific to decomposing hosts, then site-specific sampling of all potential hosts would not be necessary. Quick and efficient location of the preferred oviposition site would thus be fostered.

n-Dipropyl disulfide is the major volatile collected from the vapor head space of onions (Carson and Wong 1961, Boelens et al. 1971). In this test, significantly more female and male H. antiqua flies were trapped by n-dipropyl disulfide-baited traps than any other treatment tested. The large trap catch may have been due to the extremely high release rates of this onion chemical, suggesting that the quantitative differences in host volatiles influence host-finding behavior.

Approximately equal numbers of male and female flies were captured in the n-dipropyl disulfide-baited traps. There was a slightly greater male/female ratio in the cluster design (1.2:1), but that ratio was slightly less in the linear (1:1.4), neither ratio varying significantly from 1:1. Loosjes (1976) found a considerably greater male catch (4:1) when this volatile was tested directly in an onion field, whereas Matsumoto (1970) reported a catch of mainly H. antiqua females from n-dipropyl disulfide-baited traps set on fallow land. Although the above three experiments were performed under completely different conditions, there is some indication that the presence or absence of other host plants may alter the female and male response elicited by host chemicals. This possibility is further supported by Loosjes' (1976) nondocumented report that attractants are more effective when removed from the vicinity of onion fields.

Propanethiol (propyl mercaptan), a minor onion volatile (Boelens et al. 1971), promoted less host finding than n-dipropyl disulfide, although propanethiol-baited traps also caught more flies than control traps, and equal numbers of both sexes.

The significant male response to both synthetic onion volatiles and rotting onion bulbs implies, that in addition to oviposition sites, onions may serve as a food source or a mating site. In a similar field experiment with the closely related cabbage root fly, H. brassicae, Eckenrode and Arn (1972)

also reported high male catches in traps baited with the host plant volatile allylisothiocyanate. They postulated that "either the males followed the females to the traps or the mustard oil . . . provides a stimulus which brings the sexes together for mating." Wind tunnel bioassays of cabbage root fly host-finding behavior (Hawkes and Coaker 1976) showed that only gravid females fly upwind in response to host plant volatiles.

In our tests, the catches by traps baited with healthy onions were in all cases statistically indistinguishable from the controls. In the vicinity of an onion field, where volatiles from healthy onions are pervasive, fly visits to any one healthy plant may be random and independent of host volatile-released behavior. Conversely, healthy onion volatiles may be of great importance in mediating location of new host plant communities after dispersal. Long-range host finding of plant communities may be a result of stimuli and corresponding behavioral mechanisms that are very similar to those effecting host finding of particular plants.

Experiment I - *H. platura* host-finding responses to healthy and maggot-infested onion plants

The seed corn fly responses to the treatments were clearly different from those of onion flies; only several of the treatments promoted significantly greater host finding than the controls. A significant number of females responded to both the decomposing seedling and bulbing onion treatments, but no

significant response was demonstrated for the male flies (Figure 5). Although the healthy bulbing plants caught significantly less H. platura females than did their decomposing counterparts, the healthy seedlings elicited a response comparable to that of the decomposing seedlings. The situation with young onion seedlings may be similar to that of germinating bean and corn seeds which are colonized by bacteria that are "attractive" to seed corn flies (Eckenrode et al. 1975).

n-Dipropyl disulfide and propanethiol-baited traps caught significantly more H. platura females than the grass control. However, there was no significant difference between the chemical treatments and the soil control due to a high trap catch by the latter. Overall, n-dipropyl disulfide appears to have considerably greater impact on the host-finding behavior of the onion flies than on seed corn flies. Thirty percent of all onion flies caught were from n-dipropyl disulfide-baited traps, while only 13% of all seed corn flies were caught by traps baited with this treatment.

Experiment I - H. antiqua oviposition

n-Dipropyl disulfide and propanethiol stimulate oviposition in laboratory bioassays (Matsumoto and Thornsteinson 1968, Vernon et al. 1977). However, in our field experiment, no H. antiqua larvae or eggs were found in any of the twelve replicates of n-dipropyl disulfide and propanethiol treatments or the controls. Eggs or larvae were found, however, in all other treatments. Infestation of H. antiqua larvae and/or

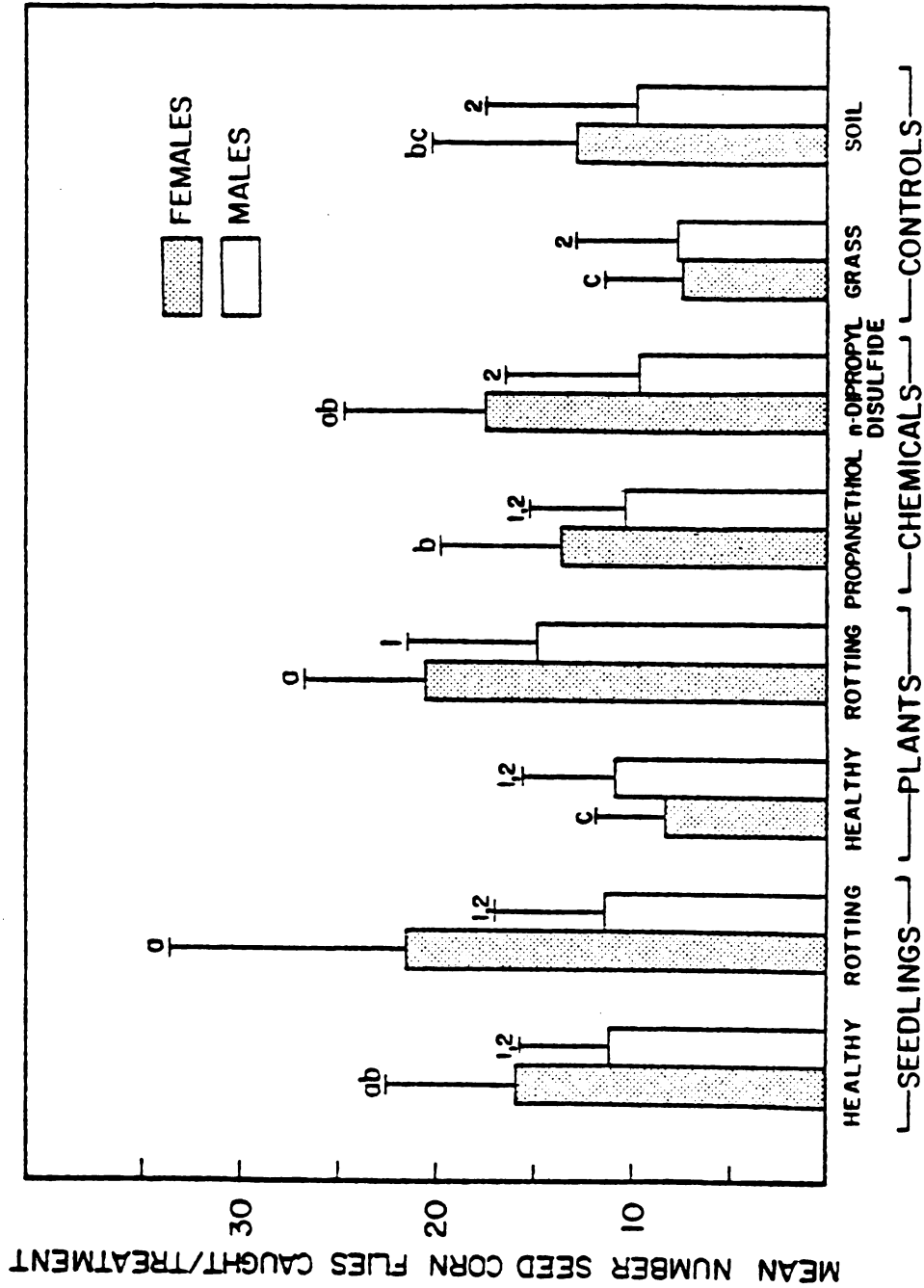


Figure 5. Mean trap catches and 95% confidence intervals for seed corn fly female and male responses to onion plants and synthetic onion chemicals. Treatments represented by the same letters (females) or numbers (male) are not statistically different as determined by 3-way ANOVA followed by a planned F-test for mean separation on data transformed to $(X + 0.5)^{1/2}$.

eggs were found in 33%, 50%, 58%, and 66% of the twelve replicates of healthy onion seedlings, healthy onion plants, rotting onion seedlings, and rotting onion plants, respectively.

Although oviposition may be deterred by the absence of moisture, this was not the limiting factor in our experiment since all treatments were watered thoroughly every second day. Requisite contact chemo-stimulants apparently were missing.

Experiment II - *H. antiqua* host finding of variously damaged onions

During the first two weeks of this experiment, all the traps baited with damaged onions caught significant numbers of *H. antiqua* females (Figure 6). The largest catch of onion fly females occurred in response to the injured plus maggot-infested onion treatment. Larval burrowing may have prolonged the release of onion volatiles as enzymes liberated from continuously damaged cells perpetuated the release of onion volatiles. Thereafter, microbial infestations may have caused additional cell damage creating an environment favorable for larval colonization; microorganisms contain or produce substances that enhance larval survival and accelerate their rate of development (Gorlenko et al. 1956, Friend et al. 1959, 1960, Zurlini and Robinson 1978). The larvae, in turn enhance the microbial infestation by distributing the organisms

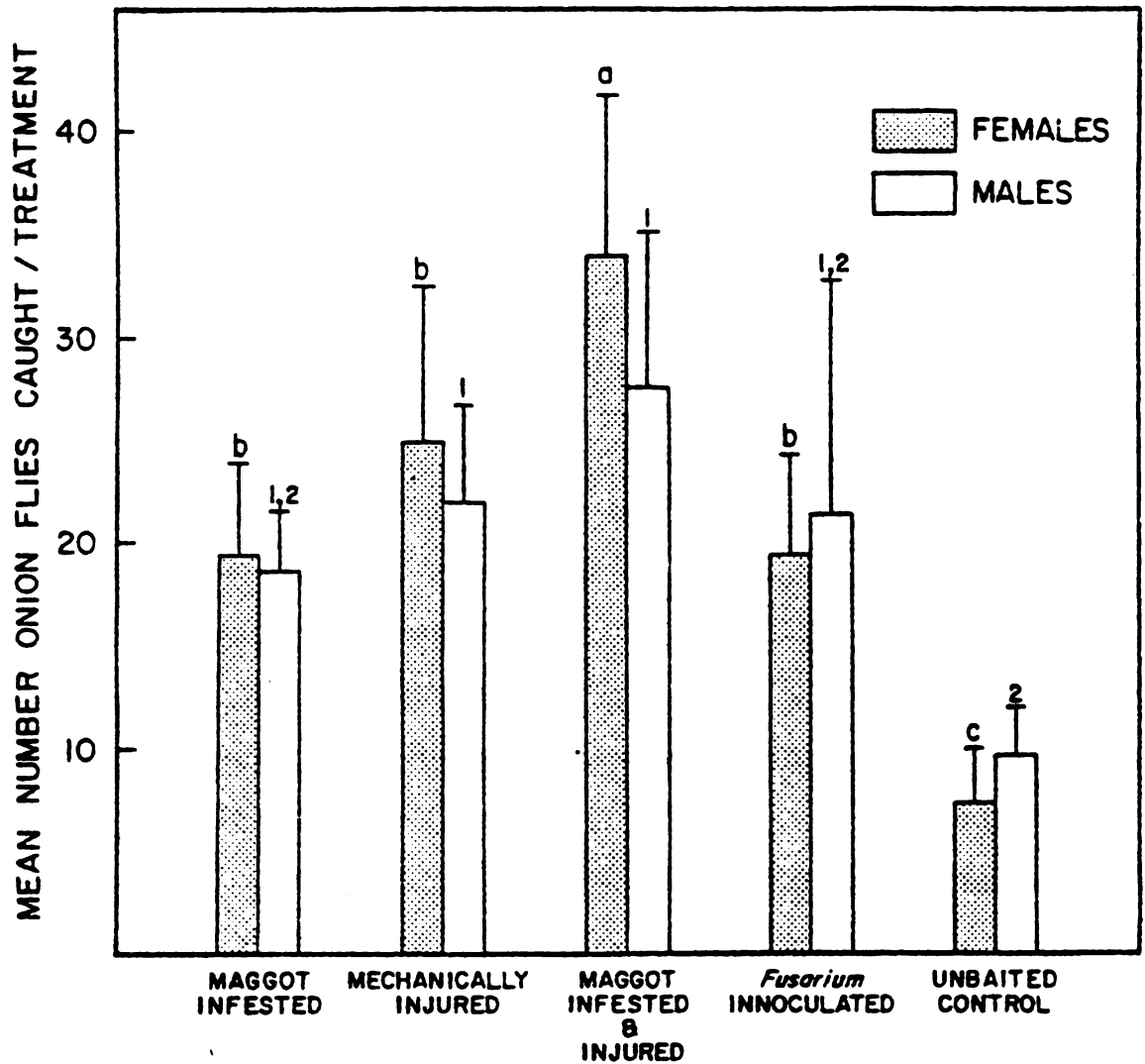


Figure 6. Mean trap catches and 95% confidence intervals for onion fly female and male responses to variously disrupted onions. Treatments represented by the same letter (females) or number (males) are not statistically different as determined by 2-way ANOVA followed by a planned F-test for mean separation of data transformed by $(X + 0.5)^{1/2}$.

throughout their tunnels (Doane 1953). Continuous larval chewing and movement prevents the formation of cork layers and the concurrent partitioning of damaged tissue. The result is a favorable larval habitat demarked by a blend of host and microbial volatiles that stimulate host finding by gravid H. antiqua females.

Male onion flies exhibited a trend of responses very similar to the female flies (Figure 6), but only the injured and injured plus maggot infested onions were significantly different from the control.

The results presented in this experiment are from trap catches over two weeks. A collection after the first week may have produced more distinct treatment differences. The greatest volatile release probably occurred during the days immediately following maggot attack and mechanical injury. During the third week no treatments caught more male and female flies than the unbaited control. By this time the onions had undergone drastic changes. In many treatments, wounds had closed off and severely damaged plants had dehydrated into shrivelled stems. These changes, could have resulted in reduced volatilization of onion and microbial chemicals. The decomposing onion should thus be viewed as a continuously changing microecosystem whose successional stages may elicit correspondingly different responses from associated organisms.

CONCLUSION

Host-finding behavior of both female and male onion flies varied with host plant condition. Male flies responded mainly to stimuli originating from onions (the synthetic onion chemicals and the mechanically damaged onions). The females' host-finding behavior appeared to be governed by chemical cues specific to the decomposing condition as well as by onion volatiles. The decomposing onion, which was preferred by the females over the healthy one, produced volatiles which were qualitatively or quantitatively different from the healthy onion. A quantitative increase of certain volatile components may be of some importance as suggested by the large male and female response to high levels of n-dipropyl disulfide. However, a qualitative change as a result of microbial infestation and volatilization of microbial biproducts may have produced a selective blend of volatiles which increased female host-finding behavior. The apparent adaptive advantage of the females' preference for the decomposing onion would be increased larval survival due to easier onion penetration by the larvae and faster larval development.

CHAPTER 2

Onion Fly Trap Catch as Affected by Release Rates of n-Dipropyl Disulfide from Polyethylene Enclosures

INTRODUCTION

n-Dipropyl disulfide, a major onion volatile found in the head-space of cut onions (Carson and Wong 1961, Boelens et al. 1971), has been identified as an oviposition stimulant and "attractant" for the onion fly, Hylemya antiqua (Meigen) (Matsumoto and Thornsteinson 1968, Matsumoto 1970). In field tests, n-dipropyl disulfide-baited traps caught more flies than traps baited with other onion volatiles (Matsumoto 1970), fresh and rotting onion juice (Loosjes 1976), or healthy and decomposing onion plants (Dindonis and Miller 1980a) suggesting the utility of this compound as a bait for traps monitoring onion fly populations.

The efficacy of a trap bait, however, may depend on the release rate at which the compound is dispensed. For example, significantly more cabbage root fly (Hylemya brassicae Bouché) females flew upwind when presented with an optimal ($104 \text{ mg}\cdot\text{hr}^{-1}$) release rate of allylisothiocyanate (Hawkes and Coaker 1979). Similarly, numerous trapping studies on Lepidoptera show that optimal release rates of pheromone bait are necessary to yield maximal trap catches (Roelofs and Cardé 1977).

In the present experiments, we investigated the effects of five different n-dipropyl disulfide loadings on H. antiqua

trap catches and established a range for optimal catch. Furthermore, polyethylene capsules used for controlled release of the n-dipropyl disulfide loadings were characterized to determine release rates and release rate variability as a function of time and temperature.

MATERIALS AND METHODS

Trap catch as affected by n-dipropyl disulfide loadings

Responses of onion flies as measured by trap catch were tested in the field to five loadings of n-dipropyl disulfide, an onion half, and an unbaited control. n-Dipropyl disulfide was released from size 3 (ca. 300 μ l) BeemTM polyethylene embedding capsules (Pelco Electron Microscopy Supplies, Ted Pella Co., Tustin, CA) in the following manner: 1, 10, and 100 μ l loadings were dispensed as neat compound (>99% pure), the .1 μ l loading was first mixed with hexane which was allowed to evaporate before the capsule was closed, and the largest amount dispensed, (10 x 100 μ l), was achieved by grouping 10 capsules each containing 100 μ l of n-dipropyl disulfide. An empty capsule was used for the control. Capsules were held upright and 2 cm above ground by platforms made from perforated paper cup bottoms. All treatments were inserted into brown paper sleeves to reduce possible degradation due to sunlight. The covered treatments were then secured by wire stakes beneath acetate cone traps (Dindonis and Miller 1980a).

The treatments, replicated five times, were set out June 5 - July 10, 1979, on the border of a muck soil onion field at Stockbridge, Michigan. A linear randomized complete block design was used with treatments spaced 6 m apart and 10 m from the onion field. During each rerandomization, all blocks were shifted 3 m in the same direction to reduce the possibility of inflating a trap catch due to n-dipropyl disulfide odors persisting from a previous treatment; this problem was suspected in previous experiments investigating high loadings of n-dipropyl disulfide. The two highest loadings were renewed every 4-6 days to maintain a visible reservoir within the capsules, whereas the three lowest loadings and the onion half were replaced every 48 hours.

Release rate characteristics of polyethylene capsules

The polyethylene capsules used for controlled release of n-dipropyl disulfide were characterized to determine the release rates of the loadings tested and the release rate profiles as a function of time and temperature.

Fluctuations of release rate over time were quantified gravimetrically. Four capsules filled with neat n-dipropyl disulfide were suspended by a wire within individual 50 ml beakers. A water bath maintained the air temperature within the beakers at $33 \pm 1^{\circ}\text{C}$ as measured by a telethermometer probe placed adjacent to the capsule. Capsules were weighed 3-4 times daily until no visible reservoir remained, at which time weighings occurred once a day until capsules reached their original weight.

The effect of temperature on release rate was quantified similarly. Three capsules containing n-dipropyl disulfide were monitored gravimetrically for each temperature investigated (see Figure 9). For lower temperature determinations ($<38^{\circ}\text{C}$), beakers were held in water baths, whereas GLC ovens were used for determinations at high temperatures ($\geq 38^{\circ}\text{C}$); GLC ovens maintained a more constant temperature at the higher ranges. Capsules were weighed every 2-12 hours; the frequency of weighings was dependent on temperature.

RESULTS AND DISCUSSION

Traps baited with 10 μl , 100 μl , and 10 x 100 μl of n-dipropyl disulfide caught significant numbers of female and male onion flies; although no significant differences occurred among the three (Figure 7). Female flies were also trapped in significant numbers in traps baited with 1 μl . As determined gravimetrically, the release rates from 1 μl and 10 x 100 μl of n-dipropyl disulfide were $60\text{ }\mu\text{g}\cdot\text{hr}^{-1}$ and $9\text{ mg}\cdot\text{hr}^{-1}$, respectively, demonstrating that onion flies are responsive to a wide range of n-dipropyl disulfide emission rates.

Although no one loading produced a distinctly optimal catch, there is a trend towards larger trap catches with increasing n-dipropyl disulfide loads. Increasing the levels emitted may extend the active space, and thus, effect a response

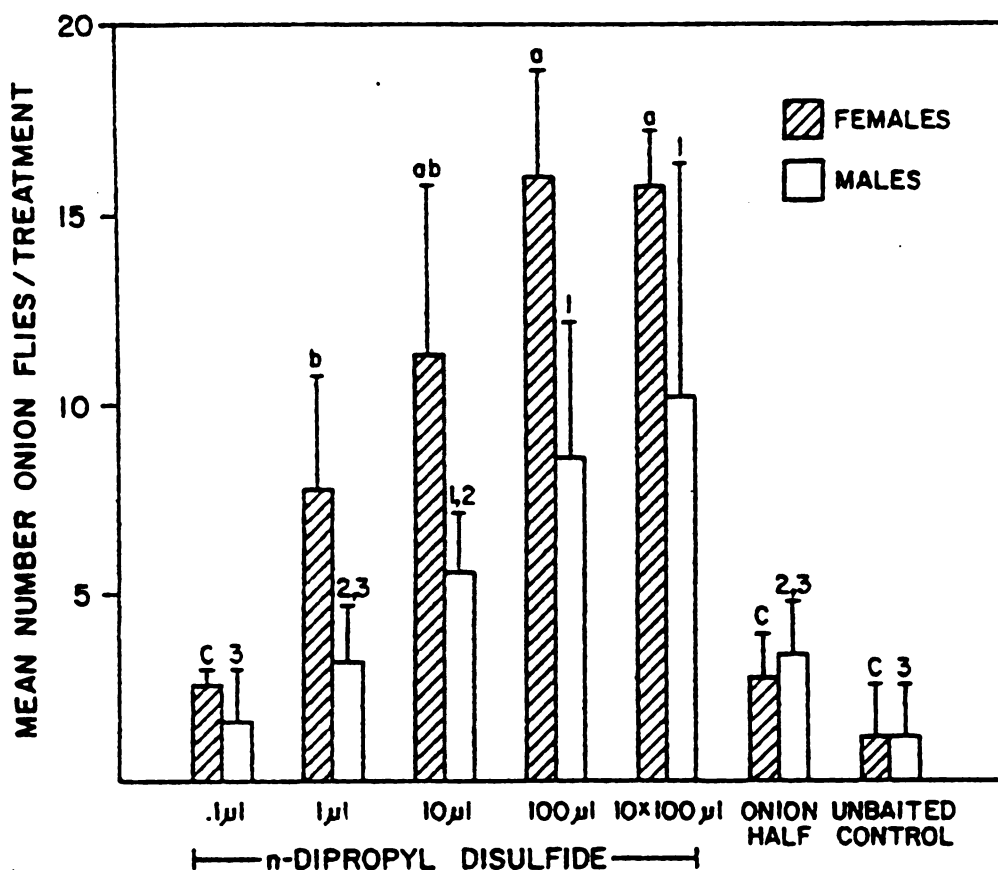


Figure 7. Mean trap catches and 95% confidence intervals for male and female onion fly responses to different loadings of n-dipropyl disulfide and an onion half. Treatments represented by the same letters (females) or numbers (males) are not statistically different based on a 3-way ANOVA followed by a planned F-test for mean separation on data transformed to $(X + 0.5)^{1/2}$.

from more flies. At the 10 x 100 μl loading, however, trap catch plateaued. The effect of extending the distance of communication by the tenfold increase of the release rate from 900 $\mu\text{g}\cdot\text{hr}^{-1}$ to 9 $\text{mg}\cdot\text{hr}^{-1}$ may be negated by an attendant close-range repellancy or arrestment of the incoming flies. For cabbage flies, upwind-oriented flight at 4-5 m from the source is mediated by allylisothiocyanate release rates ranging from 3-300 $\text{mg}\cdot\text{hr}^{-1}$ (Hawkes and Coaker 1979). However, an apparent repellent effect during landing and inhibition of oviposition has been reported for even lower allylisothiocyanate concentrations (Nair and McEwen 1976). Similarly, Matsumoto (1970) reported that onion fly oviposition is inhibited by sand dishes treated with 10 μl of n-dipropyl disulfide, whereas this and greater loadings produced significant trap catches in our experiment. Thus, higher release rates of a single compound may elicit long-range flight but deter landing at the source. Alternatively, high concentrations of the stimulus may induce flight arrestment before the source is reached (Farkas and Shorey 1974).

Neither the traps baited with .1 μl of n-dipropyl disulfide nor the onion half caught more flies than the control. Apparently the concentration of the stimulus from these treatments was subthreshold or effective for only a short time.

Although more seed corn flies than onion flies were caught in the n-dipropyl disulfide-baited traps, no seed corn fly catch by n-dipropyl disulfide was significantly greater than

control. However, a significant catch was obtained in the sliced onion baited traps; a mean of 65.2 and 38.0 seed corn flies, 2/3 of which were females, were caught in onion-baited and control traps, respectively.

Release rate characteristics of polyethylene capsules

Polymeric encapsulation is reported to be an effective dispensing system for controlled release of volatile compounds (Campion et al. 1978, Marks 1976). The mechanism of transport and release rate characteristics of polymeric delivery systems are reviewed by Lewis and Cowsar (1977), Kydonieus (1977), and Rogers (1977). Laminated polymeric vessels and some polyethylene enclosures have been characterized as having constant release rates for an extended period. These findings closely parallel the results obtained in the present investigation.

Under isothermal conditions, the polyethylene capsules utilized in this study released n-dipropyl disulfide at a constant rate (Figure 8). Latency for the onset of a steady emission rate was approximately two hours, during which time disulfide was emitted at $0.55 \text{ mg}\cdot\text{hr}^{-1}$. Thereafter, the rate averaged $0.9 \text{ mg}\cdot\text{hr}^{-1}$ for about eight days, decreasing only after no visible reservoir remained. At this point, the release rate decreased until $\leq .06 \text{ mg}\cdot\text{hr}^{-1}$ was being emitted; this occurred within 44-72 hours at 22°C and 24-32 hours at 40°C .

The release rate of n-dipropyl disulfide increased exponentially as a function of temperature (Figure 9). For temperatures commonly encountered in the field, $18^{\circ} - 40^{\circ}\text{C}$,

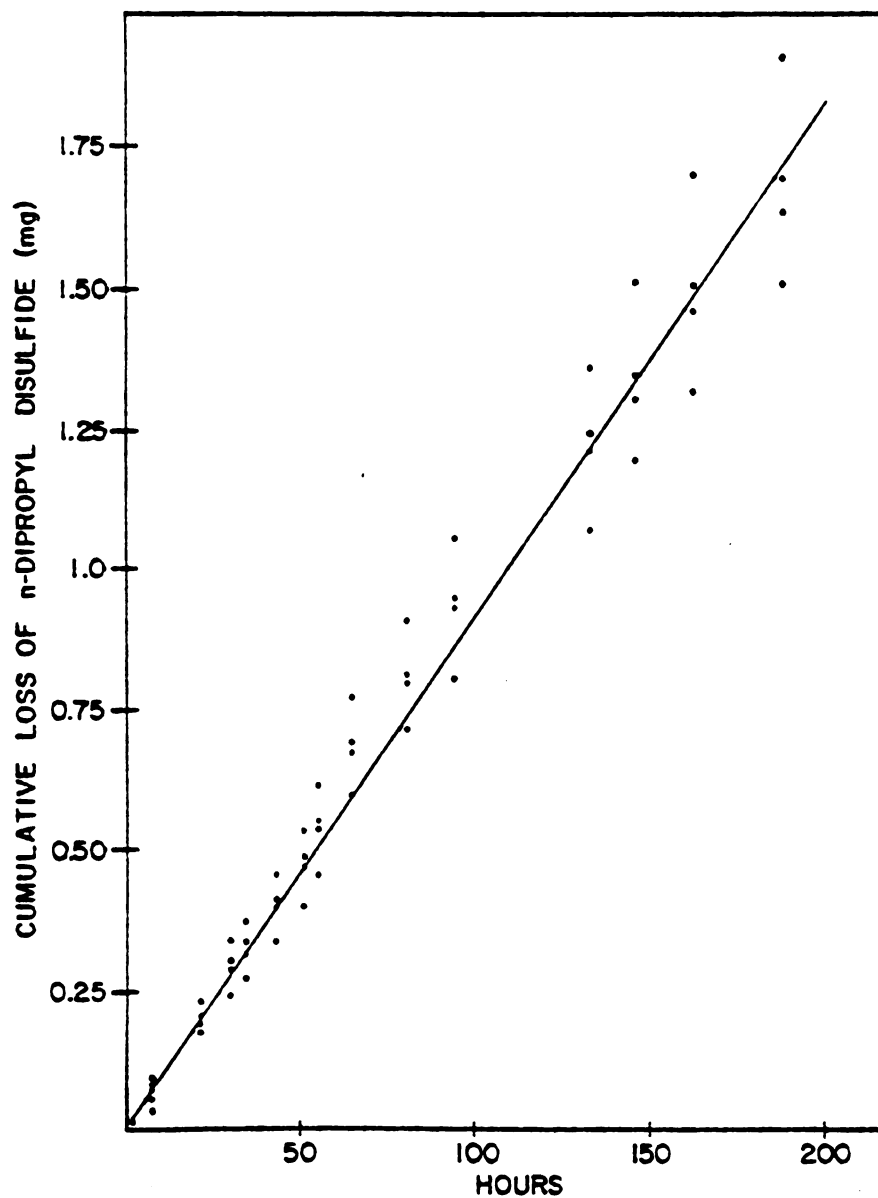


Figure 8. Release rate profile of n-dipropyl disulfide from polyethylene capsules as a function of time at 22° C.

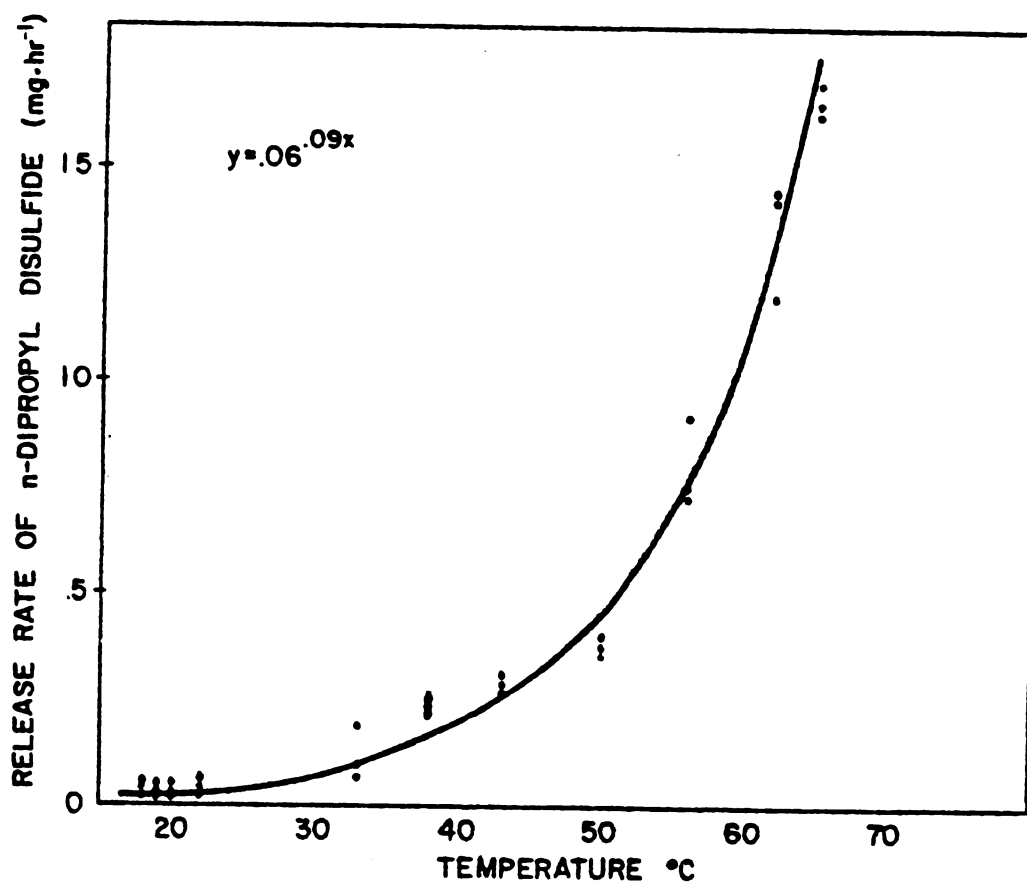


Figure 9. Release rate profile of n-dipropyl disulfide from polyethylene capsules as a function of temperature.

the release rates ranged from $.29 \text{ mg}\cdot\text{hr}^{-1}$ to $2.0 \text{ mg}\cdot\text{hr}^{-1}$. The emission rates at temperatures exceeding 65°C varied extremely, probably due to changes in the polymeric material; at temperatures $\geq 69.5^{\circ}\text{C}$ the capsules emit biproducts from a parting layer formed as a result of the casting process (pers. comm. T. Turnbull, Ted Pella, Inc.) and at ca. 80°C the capsule walls begin collapsing.

These results demonstrate that polyethylene embedding capsules can be utilized as an efficient and easily managed controlled release system for volatile compounds similar to n-dipropyl disulfide. The advantages of this system include a constant release rate over an extended period of time with relatively small deviations in release rates due to temperature fluctuations (within the range of naturally occurring temperatures). These characteristics coupled with the relatively infrequent need for chemical replenishment underline the utility of these capsules as a means of dispensing chemicals under field conditions.

CHAPTER 3

Onion Fly and Little House Fly Host Finding Selectively Mediated by Decomposing Onion and Microbial Volatiles

INTRODUCTION

In field tests conducted in commercial onion fields, both female and male onion flies, Hylemya antiqua (Meigen), were attracted to point sources of damaged onions (Dindonis and Miller 1980a, 1980b). Moreover, the rotting process seemed to enhance the attractiveness of onions (Dindonis and Miller 1980a, Loosjes 1976). A major volatile of crushed onions, n-dipropyl disulfide has been identified as a host-finding stimulant for H. antiqua (Matsumoto 1970). This chemical trapped more onion flies than onion plants (Dindonis and Miller 1980a) or onion juice (Loosjes 1976); however relatively high release rates of n-dipropyl disulfide (ca. $1 \text{ mg} \cdot \text{hr}^{-1}$) were required to achieve these catches.

The available data suggest that the chemical nature of the optimal host-finding stimulus for H. antiqua may be: a particular onion volatile, a mixture of onion volatiles, a mixture of onion volatiles in combination with volatiles from associated microorganisms which cause onion rot, or a particular microbial volatile(s). Quantitative changes in each proposed signal could also be expected to affect host finding.

This paper reports experiments which helped characterize the optimal host-finding stimulus for H. antiqua. Attraction to various combinations of microbial biproducts and onion

volatiles was tested to determine the relative effectiveness of volatile blends. Another experiment assessed onion fly attraction to microorganisms removed from their onion substrate, and concurrently compared the attractiveness of freshly cut and decomposing onion bulbs.

MATERIALS AND METHODS

Experiment 1: Attractancy of blends of onion and microbial volatiles

n-Dipropyl disulfide was dispensed from BeemTM polyethylene enclosures charged with 100 ul neat material (purity 99%). According to preliminary investigations (confirmed by later experimentation, Dindonis and Miller 1980c) the release rate of this system effected maximal H. antiqua catch to n-dipropyl disulfide-baited traps. The BeemTM capsules were not suitable for release of some of the other compounds due to their high volatility. The objective was to achieve a continuous release of each chemical; however, no attempt was made to maintain equal release rates. Hence, 6 ml of all other compounds were dispensed from 8 ml glass scintillation vials capped with polyethylene stoppers. These dispensers could hold a large reservoir of chemical while the hard polyethylene prolonged release by retarding diffusion through the cap. Vials containing ethanal were embedded cap upward in the soil to reduce further the release rate and pressure of this highly volatile compound. All other vials were placed horizontally within cylindrical

brown paper sleeves to reduce chemical degradation by direct sunlight. Onion halves were similarly covered to retard dehydration. During the experiment, the treatments were rerandomized three times, onion halves replaced every fourth day and the chemicals, which could always be detected by the human nose, were replenished whenever necessary to maintain a visible reservoir.

The relative attractancy of nine microbial and onion volatiles was tested during September 29 to October 13, 1978, in Hudsonville, Michigan. The treatments were placed beneath acetate cone traps (Dindonis and Miller 1980a) spaced 3 m apart on the border of a muck soil onion field. The four replicates were arranged in a linear randomized complete block design.

Six metabolic biproducts of soil inhabiting microorganisms (Pelczar et al. 1972): n-butanol, 2,3-butanediol, n-butyric acid, acetyl methyl carbinol (85% aqueous), hexanoic acid, propionic acid and two volatiles common to onions (Whitaker 1976) and microbes (Pelczar et al. 1972), ethanal and isopropanol, were tested individually and collectively. In addition, the oviposition stimulant and attractant, n-dipropyl disulfide was dispensed along and in combination with the chemicals. A freshly cut onion half and a control trap baited with an empty dispensing vial completed the treatments.

Experiment 2: Attractancy of microbial cultures, freshly cut, and decomposing onions

An onion field in Stockbridge, Michigan, heavily infested with H. antiqua was used as the test site during July 5-18, 1979. Treatments covered with acetate cone traps were placed on the border of the field in a linear randomized complete block design with a 6 m intertreatment spacing. The treatments were replicated five times and rerandomized every fourth day at which time all blocks were moved 3 m in the same direction to guard against contamination due to odors persisting from a previous treatment.

The eight treatments investigated were: an agar plate with and without n-dipropyl disulfide, an agar plate inoculated with microorganisms from decomposing onions also tested with and without n-dipropyl disulfide, n-dipropyl disulfide alone, a freshly cut onion half, a decomposing onion half and an empty plate as a control.

The rate of H. antiqua larval development is accelerated by the presence of bacteria transmitted by the larvae (Friend et al. 1960, Zurlini and Robinson 1978). Thus, it was hypothesized that the volatiles specific to this bacterial infestation might be involved in the optimal host-finding stimulus for the female flies. Hence, the decomposing onion treatment was prepared by placing 15 field-collected second-fourth instar onion fly larvae on the cut side of an onion half. The infected onion halves were pressed into nonsterilized muck soil and watered daily. Onions conditioned for four days

were used to inoculate the agar plates and then were placed in the field as the decomposing onion treatment.

Potato dextros agar in 50 mm plastic Petri dishes was inoculated with decomposing onion microorganisms by pressing the cut side of the conditioned onion half into the agar medium for three seconds. One onion half was used for every five plates. The inoculated plates were incubated at 21°C for 48 hours.

Before deploying the plates in the field, the Petri dish covers were replaced with two layers of brown paper toweling tautly secured by a tight-fitting plastic ring. This cover allowed volatiles to emanate from the microorganisms and agar while excluding foreign microorganisms as evidenced by the absence of visible colonization of the agar plates during the experiment.

n-Dipropyl disulfide was released at ca. $900 \mu\text{g}\cdot\text{hr}^{-1}$ from size 3 BeemTM polyethylene embedding capsules (Dindonis and Miller 1980b). The capsules were suspended 1.0 cm above the agar and microbial plates by a wire attached to the plate.

Treatments were placed within paper sleeves and secured beneath traps as in the previous experiment. To maintain a relatively constant volatile release rate, the microbial and agar plates were replaced every 24 hours, the decomposing and cut onions every 48 hours, and n-dipropyl disulfide was replenished as necessary to maintain a visible reservoir.

RESULTS

Experiment 1: Attractancy of blends of onion and microbial volatiles

With the exception of n-dipropyl disulfide, no trap baited with a single compound caught more flies than the control trap (Table 1). However, the combination of the eight chemicals (without n-dipropyl disulfide) elicited a significantly greater female response than any of the chemicals tested individually, excepting n-dipropyl disulfide. Moreover, the combination of volatiles plus n-dipropyl disulfide produced a female catch significantly greater than either the combination or n-dipropyl disulfide alone. Male response was similar, although fewer significant treatment differences were evident (Table 1).

Experiment 2: Attractancy of microorganism cultures, freshly cut and decomposing onions

H. antiqua. The greatest trap catch of female and male onion flies occurred in traps baited with decomposing onion halves (Table 2). Fewer but yet significant numbers of female flies were also caught by all traps containing n-dipropyl disulfide and healthy onion halves. Male flies responded similarly to n-dipropyl disulfide. However, with the addition of microbial plates, n-dipropyl disulfide-baited traps caught no more males than control; furthermore, the microbial cultures alone caught significantly fewer males than the unbaited control trap. Female response to the microbial culture was indistinguishable from that to the control.

Table 1. Onion fly responses to microbial biproducts, onion volatiles, and their combination.

Treatments	Mean Number Onion Flies Caught per Treatment ¹		
	Female	Male	Total
1. Ethanal	13.3 ^d	11.0 ^c	24.3
2. n-Butanol	14.3 ^d	9.8 ^c	24.1
3. 2,3-Butanediol	9.8 ^d	8.8 ^c	18.6
4. n-Butyric acid	21.3 ^{cd}	9.8 ^c	31.3
5. Acetyl methyl carbinol	9.3 ^d	7.5 ^c	16.8
6. Hexanoic acid	8.0 ^d	6.8 ^c	14.8
7. Isopropanol	15.0 ^d	12.3 ^c	27.3
8. Propionic acid	8.0 ^d	10.3 ^c	18.3
9. Combination of 1-8	32.8 ^{bc}	16.5 ^{abc}	49.3
10. n-Dipropyl disulfide	43.3 ^b	25.8 ^{ab}	69.1
11. 9 + 10	73.5 ^a	30.8 ^a	104.3
12. Onion half	27.0 ^{bc}	18.6 ^{abc}	45.5
13. Control	16.5 ^{cd}	14.3 ^{bc}	30.8

¹Treatments followed by same letters within columns are not statistically different as determined by 2-way ANOVA followed by a planned F -test for mean separation of data transformed to $(X + 0.5)^{1/2}$.

Table 2. Fly responses to microorganisms removed from onion substrate, and to freshly cut and decomposing onions.

Treatments	Mean Number Flies				
	Caught Per Treatment ¹				
	<u>Hylemya antiqua</u>		Total	<u>Fannia canicularis</u>	
Females	Males	Females		Males	
1. Decomposing onion half	32.8 ^a	25.2 ^a	58.0	0.4 ^c	0
2. Freshly cut onion half	13.4 ^{bc}	13.4 ^{bc}	26.8	0.2 ^c	0
3. n-Dipropyl disulfide	18.4 ^b	13.8 ^b	32.2	.0 ^c	0
4. Agar plate	12.4 ^{bcd}	8.4 ^{cd}	20.8	0.2 ^c	0
5. 3 + 4	18.0 ^b	10.8 ^{bc}	28.8	.0 ^c	0
6. Microorganism culture	8.4 ^{cd}	4.4 ^e	12.8	43.6 ^b	0
7. 3 + 6	14.0 ^{bc}	7.2 ^{de}	21.2	72.0 ^a	0
8. Control	7.6 ^c	8.0 ^d	15.6	.0 ^c	0

¹Treatments followed by same letters within columns are not statistically different as determined by 2-way ANOVA followed by a planned F-test for mean separation of data transformed to $(X + 0.4)^{1/2}$.

F. canicularis. In contrast to the low catch of onion flies, traps baited with the microbial plates caught large numbers of female F. canicularis (Table 2). Moreover, the catch to the microbial plates was further elevated by the addition of n-dipropyl disulfide which by itself caught no F. canicularis. Virtually no females were trapped by any of the other treatments tested, and no male flies were caught in any of the treatments.

DISCUSSION

H. antiqua

Although additional investigation is necessary, the present experiments have aided our attempt to characterize the chemical composition of an effective host-finding stimulus for H. antiqua. The hypothesis that a blend of chemicals, rather than a single key chemical is the more effective host-finding stimulus is supported by the significantly larger catch by the combination of eight chemicals (Experiment I) compared to any of the chemicals tested individually. Further support is provided by the even greater catch to the combination of the eight chemicals and n-dipropyl disulfide, and the significantly greater onion fly response to the decomposing onion treatments than to optimal release rates of n-dipropyl disulfide. Previous investigations demonstrated that onion flies were equally responsive to a wide range of n-dipropyl disulfide release rates, 150 µg

hr^{-1} $-9 \text{ mg} \cdot \text{hr}^{-1}$ (Dindonis and Miller 1980c), further suggesting that once above threshold, quantitative differences of a stimulus may be of less importance than qualitative differences.

An onion half, renewed every four days, did not catch more flies than the control, whereas an onion half replaced every two days did effect a significant catch, suggesting a time-dependent decrease of onion volatiles. The decomposing onion, although cut four days before exposure, effected the greatest male and female catch, strongly suggesting that the microbial activity on onions produced a blend of volatiles even more stimulating than volatiles from a freshly damaged onion.

The import of microbial activity in releasing onion fly behavior has recently been demonstrated by Ellis et al. (1979) who found that reduced oviposition occurred on onion seedlings grown in a relatively sterile medium. Volatile analysis of onions grown in sterilized sand shows an absence of alkyl sulfides (Coley-Smith and King 1969). Furthermore, soil bacteria are capable of enzymatically cleaving onion precursors which may be exuded by the roots, producing alkyl disulfides, such as n-dipropyl disulfide (Coley-Smith and King 1969). Although damaged onion cells emit high levels of sulfur compounds, the characteristic odor of a healthy onion may be due in part to the onion's microbial inhabitants. Hence, microorganisms are strongly implicated in production of volatiles stimulatory to the onion fly.

The microorganisms removed from their onion substrate and allowed to flourish on agar medium did not enhance onion

fly host-finding behavior. This may indicate that microbial volatiles alone are not stimulatory. However, the disparate carbon sources and the different physical nature of the two media may have altered the expression of certain metabolic pathways producing slightly differing biproducts. Furthermore, the microorganisms emitting the attractive volatiles may have been selected against by the culture medium or they may have been outcompeted by other microorganisms. That the inoculated plates were actually releasing volatiles was substantiated not only by our perception of an odor (not unlike a rotting vegetable) but also by a large trap catch of F. canicularis.

F. canicularis

The little house fly is a serious pest around poultry farms and has been identified as a transmitter of the Newcastle disease virus (Rogoff et al. 1975). This fly is reported to breed in a variety of decaying animal and plant matter, including onions (Chillcott 1960). However, in the present study, practically no F. canicularis were caught by traps baited with the decomposing onions which were attractive to H. antiqua. Possibly, a more severely decomposed onion may be necessary for a suitable oviposition site. Conversely, traps baited with the microbial plates caught high numbers of F. canicularis, but no onion flies. Although of decomposing onion origin, the microbial plates must have produced a volatile profile different from that of decomposing onions and highly specific for F. canicularis.

The behavioral response of onion flies to microbial volatiles appears to be limited to onion-microorganism associations whereas the muscid fly may respond to microbial volatiles produced from non-onion substrates. However, the response of F. canicularis was significantly greater to the microbial plate and n-dipropyl disulfide combination than to the microbial plates alone, indicating that F. canicularis, like the onion fly, may perceive and respond preferentially to a stimulus composed of a specific blend of chemicals. A blend may produce an odor stimulus profile characteristic of a particular host-plant condition which, as evidenced by the insect's behavioral response, communicates various degrees of acceptability. Furthermore, a multi-component signal potentially conveys more information not only about the physiological condition of the host but also may allow more rapid adaptation to host genetic changes.

CHAPTER 4

Chemically Stimulated Anemotaxis — A Behavioral Mechanism for Host Finding by Onion Flies

INTRODUCTION

As a result of recent investigations on host-finding mechanisms of adult phytophagous insects, it is now accepted that distant host-plant odors may evoke directed movements toward the volatile source. Evidence documenting the long-range host-finding mechanism of flying insects, however, exists for very few insects. In wind tunnel bioassays cabbage root fly, Hylemya brassicae (Bouché), females exhibited positive anemotaxis in response to host-plant volatiles (Hawkes et al. 1978). Upwind flight stimulated by host-plant odors has also been reported in Drosophila melanogaster, although supportive data were not presented (Kellogg et al. 1962). Anemotaxis is the only experimentally substantiated behavioral mechanism which explains directed locomotion toward a distant odor source.

Our previous work on onion fly, Hylemya antiqua (Meigen), responses to host-plant condition (Dindonis and Miller 1980) and other reports of onion volatiles elevating trap catches (Matsumoto 1970, Loosjes 1976), suggested that onion fly host location is mediated by long distance odor cues. The present research establishes host volatile-stimulated anemotaxis as a behavioral mechanism by which onion flies locate their host plants, Allium spp.

MATERIALS AND METHODS

Field observations

Direct observations of onion fly behavior in response to three treatments, sliced onions, barnyard grass (Echinochloa sp.), and bare soil as a control, were conducted August 3, 4 and 9, 1978, in Hudsonville, Michigan. Treatments were randomized within linear blocks which were placed on the border of a muck soil onion field and arranged perpendicularly to the prevailing wind direction. All baits were covered with acetate cone traps (Dindonis and Miller 1980a). This arrangement allowed assessment of the trapping efficiency of these traps. Briefly, the 38 cm high and 30 cm diam traps consisted of a cone topped with a cylinder containing ethylene glycol (30% aqueous) for ensnaring the flies. Trap skirts were positioned 5 cm above ground, allowing air to circulate beneath the traps and carry treatment volatiles downwind. Two observers monitored each block of treatments from randomly designated positions outside of the observation area. The data recorded included the number of male and female flies landing within a 0.5 m radius of the trap, the number which approached and entered the trap, a detailed description of the approach, and the number caught by the trap. The flies' positions and movements with respect to the wind were also noted.

Wind-directed traps

Field observations led to the description of onion fly behavior as affected by wind-borne host-plant volatiles. However, quantification of fly movements with respect to the wind was hampered by erratic, albeit infrequent, shifts in wind direction. Thus, a trap was designed to quantify accurately the number of flies approaching the host bait and the direction of their approach with respect to wind direction.

The trap (Figure 10) consisted of a 0.5 cm mesh metal screen cylinder (48 cm high x 30 cm diam), attached by a ball bearing to a vertical metal pole, which was supported by a 40 cm x 40 cm plywood platform. A 25 cm x 60 cm oblong vane, constructed from plastic and painted flat-black, was secured above the trap (Figure 11). The weight of the vane was counter-balanced by weights hung on the opposite side of the cylinder. The cylinder rotated freely, responding to even slight changes in wind direction. Sliced onions were placed on the center of the platform as bait. Bird TanglefootTM covering the screen entrapped flies approaching the bait, marking their approach with respect to ground and wind direction.

On August 3, 1978, three onion-baited and three unbaited wind-directed traps were set out in a paired design on a plant-free patch of muck soil adjoining an infested onion field at Hudsonville, Michigan. Within 48 hours, certain areas of the screen cylinder were covered with flies to the extent that trapping efficiency was impaired. At this time the experiment was terminated and every 9 cm² area of each cylinder was censused.

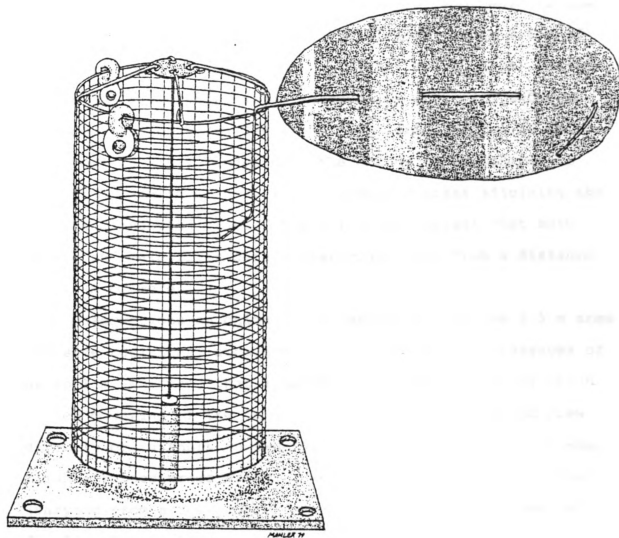


Figure 10. Wind-directed trap for quantifying volatile-mediated approaches of onion flies with respect to wind direction and ground.

RESULTS

Field observations

During 14 hours of field observations, the movements of 105 female and 34 male onion flies were recorded. Of the total 105 females observed, 68 (65%) landed within 0.5 m of the sliced onions, whereas only 16 (15%) and 21 (20%) landed within the observation sites surrounding grass and unbaited control, respectively (Table 3). Similarly, of the 34 males observed, 22 (65%) landed near the onions in comparison to nine (26%) and three (9%) which landed in areas adjoining the grass and unbaited traps. These results suggest that both female and male flies detect host-plant odor from a distance of 0.5 m or more.

Observations on onion flies landing within the 0.5 m area and some additional accounts of fly responses at distances of up to 1 m indicated that a substantial number of flies which landed downwind of the onions turned into the wind and flew directly or in short successive flights to the sliced onions. Flights interspersed with 1-3 landings of irregular duration (varying from a few seconds to eight minutes) were observed for 54% of the flies initially sighted at distances of 0.4 - 1.0 m. Seventy percent of the flies sighted within 0.4 m of the onion flew directly to the bait without landing. The majority of the flights were ca. 15-50 cm long ($x = 35$ cm) and ca. 5-15 cm above ground. Casting across the wind axis

Table 3. -- Field observations on onion fly responses to variously baited traps.

Bait	No. of flies landing within 0.5 m of trap			No. of flies entering trap after landing within 0.5 m of trap			% of flies entering trap after landing within 0.5 m		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
sliced onion	68 ¹	22	90	38	11	49	56	50	54
grass	16	9	25	2	2	4	13	22	16
soil	21	3	24	7	0	7	33	0	29

¹All data from sliced onions are greater than respective data for grass control and unbaited traps as determined by 2-way ANOVA.

was not observed; the line of flight did not appear to deviate from the direction set originally.

A greater percentage of both female and male flies approached and entered the onion baited traps than the grass baited and control traps (2-way ANOVA, $p < 0.001$) (Table 3).

Wind-directed traps

The density of ensnared flies on the baited traps was greatest on the downwind side (Figure 11) within an area corresponding to the diameter of the volatile plume as approximated from measurements of titanium tetrachloride-generated smoke plumes (Miller and Roelofs 1978). The catch within 90° of the vertical axis bisecting the downwind side of the cylinder and within 12 cm of the ground was significantly greater (paired t-test, $p \leq 0.05$) for baited traps than the catch on the corresponding area of the control traps (Figure 11).

The three onion-baited traps ensnared 370 males and 193 females as compared to 102 males and 62 females caught on the unbaited traps.

DISCUSSION

Reports on insect-plant interactions frequently employ Dethier's et al. (1960) classification of behavior to describe the stimuli and the corresponding behaviors. This classification, however, is beset with numerous problems. As pointed out by Kennedy (1978), terms such as "attractant" and "arrestant" do not refer to the different kinds of reactions involved in

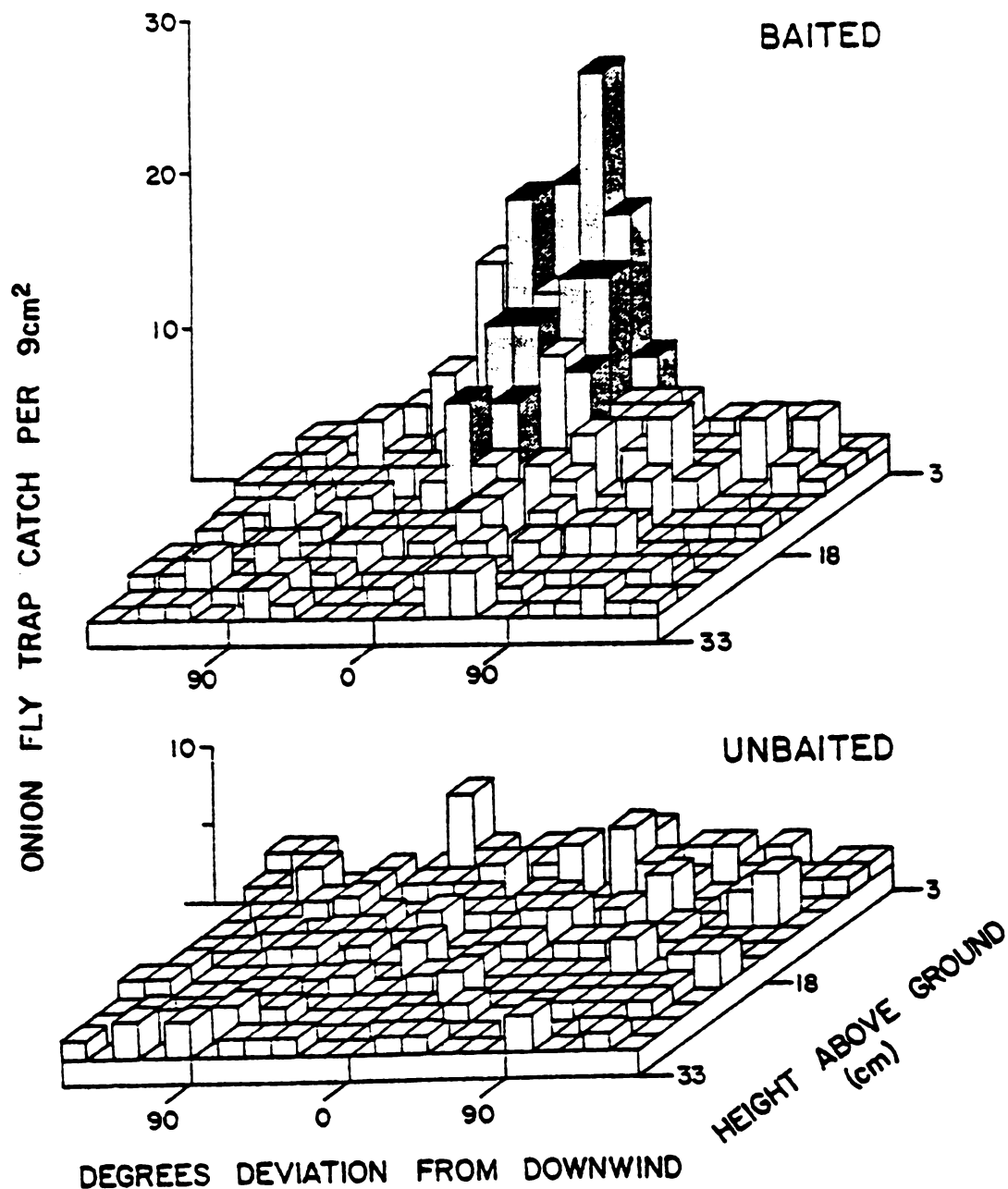


Figure 11. Catches of onion flies on each 9 cm² area of the wind-directed traps. Catches represented by hatched bars are significantly greater than catches on unbaited traps as determined by a paired t-test.

the insect's displacement. Furthermore, these terms suggest that a single stimulus activates the behavior when multiple stimuli may actually be involved (Kennedy 1978). In addition, Dethier's et al. (1960) terms are often misused as a result of inferring behaviors from end results such as trap catch rather than from observations or behavioral bioassays. Various studies on onion fly/host plant interactions can be criticized in this regard. Although the host-finding behavior of onion flies has not been investigated directly, the literature often refers to "attractants," implying that identified onion volatiles and other closely related compounds elicit directed movements toward the chemical source. Those assays, however, evaluated trap catch or oviposition and thus quantified the end result rather than described the behavioral mechanisms by which these results were achieved.

Studies investigating the behavioral mechanism allowing location of distant odor sources have predominantly implicated anemotaxis, although the idea of a chemotactic component to in-flight orientation has not been entirely dismissed (see Farkas and Shorey 1972, Kennedy 1977, 1978). Anemotaxis has been well documented in many lepidopteran species responding to sex pheromones (Roelofs and Cardé 1977) and in a few phytophagous insects as a long range host-finding mechanism, i.e. Drosophila melanogaster (Kellogg et al. 1962) the desert locust, Schistocerca gregaria (Forsk.) (Haskell et al. 1962,

Kennedy and Moorhouse 1969), and the cabbage root fly (Hawkes and Coaker 1976, Hawkes et al. 1978).

This investigation of onion fly host-finding behavior also identified anemotaxis as the principal mechanism involved. As recounted in the field observations, onion volatiles elicited oriented flight, apparently directed upwind. The upwind component of flight was confirmed by the trap catch on the wind-directed traps.

The flights toward the bait were short and frequently interspersed with landings. The onion flies did not zigzag across the plume axis as has been commonly observed for Lepidoptera following sex pheromone plumes. Horizontal and/or vertical casting by Lepidoptera allows relocation of the plume if the odor stimulus is interrupted. The short flights of the onion flies may provide an alternative method for relocating the stimulus and/or reassessing the wind direction. Landing may allow the fly to remain longer in the area where it originally perceived the odor; and thus, where there is a greater probability of being restimulated. Similar host-finding behavior was reported in gravid H. brassicae females (Hawkes et al. 1978). In the presence of host-plant volatiles, gravid females demonstrated initial upwind orientation and flight of an average distance of 0.5 m. As with onion flies, no casting across wind was observed. Hawkes et al. (1978) point out that longer flights may result in more frequent departures from the odor plume.

The frequent landings of onion flies may have been influenced by visual cues from the trap and/or the high release rate of onion volatiles emanating from freshly cut onions. As demonstrated for some moths and beetles flying upwind in response to sex pheromones, high concentrations of the pheromone reduces flight speed and stimulates landing before the point source is reached (Farkas and Shorey 1974). Visual cues also induce landing of male moths exposed to sex pheromone (Farkas and Shorey 1974). Hawkes et al. (1978) investigating the host-finding mechanism of cabbage root flies report that a visual stimulus, even one not apparently resembling a plant, will interrupt flight and stimulate landing.

In previous experiments, we found a significant male response to host plants and synthetic onion chemicals (Dindonis and Miller 1980a). Observations of male flight toward onion baits and the large male catch on the baited wind-directed traps confirm that host volatile-stimulated anemotaxis is also displayed by the males. In contrast, H. brassicae males did not respond to host-plant odors when bioassayed in a wind tunnel; orientation was observed only in gravid females (Hawkes et al. 1978).

The male onion fly orientation to host volatiles suggests that onions serve some function in addition to providing an oviposition site. For example, males and females may be attracted to the onion for aggregation of the sexes for mating, as has been documented for apple maggot flies, Rhagoletis

pomonella Walsh (Prokopy 1968). Male and female apple maggot flies are attracted to a visual stimulus simulating an apple which provides both an oviposition site and an assembly site for mating activity. In addition, our field observations suggest that the onion may be a food source. Upon entering the onion baited traps, the flies fed on damaged onion tissue for up to ten minutes.

Despite the need to investigate other stimuli, i.e. visual cues and chemical gradients, as additional factors mediating host finding, our initial experiments have conclusively established that a major component of onion fly host-finding behavior is chemically stimulated positive anemotaxis. Furthermore, onion volatiles can now be properly designated as attractants of H. antiqua.

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