EXPERIMENTAL AND THEORETICAL STUDIES IN INSECT CHEMICAL ECOLOGY: OVIPOSITIONAL BIOLOGY OF *Delia* FLIES AND SIMULATION MODELLING OF INSECT MOVEMENT

By

Paul Adam Weston

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ABSTRACT

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Seedcorn flies, *Delia platura* Rondani, given a choice of several lima bean developmental stages, laid most eggs on germinating beans and emerging seedlings. Presence of above-ground plant structures had no effect on ovipositional stimulation, but presence of breaks in the substrate surface and chemostimuli from germinating beans both increased oviposition appreciably. Larval survivorship was highest on freshly planted and germinating beans, and lower on emerging and upright seedlings, indicating a poor match between host acceptance by adults and suitability of plant growth stages as larval food. When caged for life with moist sand, seedcorn flies laid half as many eggs and took twice as long to begin ovipositing as females caged with germinating beans. Decreased fecundity was due to reduced egg maturation rates; reproductive status seemed to be the primary determinant of oviposition, with resource quality influencing oviposition only for the first four days of deprivation. The onion fly, *D. antiqua* Meigen, showed similar reductions in lifetime fecundity and increased age at first oviposition when deprived of host-plant chemostimuli, but not when deprived of host-plant visual stimuli. In choice tests, both factors influenced oviposition.

Decreased fecundity for onion flies was due to lack of release of ovipostional behaviors rather than decreased rates of egg maturation.

Computer simulations of movement by hypothetical insects revealed that dispersal may be very strongly influenced by magnitude of potential turns available at each step. Decreased velocity with increased chemostimulus intensity increased arrestment and target-finding ability; conversely, decreased velocity decreased arrestment and target finding. Increasing circular variance of turn angles with increasing stimulus intensity resulted in decreased arrestment and target finding, while monotonic changes in turning frequency had virtually no effect on performance. The only changes in circular variance of turn angles or turning frequency that increased arrestment or target finding were those that resulted in straighter tracks when stimulus intensity increased and more tortuous tracks when stimulus intensity decreased. Movers using such algorithms foraged as efficiently as movers equipped with a more sophisticated algorithm (klinotaxis) over a range of target densities. Merely stopping at targets increased foraging efficiency dramatically, but these increases diminished gradually with increasing target density.

In memory of George.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Insect chemical ecology can be broadly defined as the study of those interaction of insects with their environment that are mediated by chemicals. The majority of research in this discipline deals with insect-insect or insect-plant interactions that are mediated by natural products. But it is not only the interaction itself that is of interest to the researcher. Of ultimate interest is the impact of the interaction on the fitness of insects. So a more restricted definition of insect chemical ecology might be: The study of the impact of natural products on the fitness of insects.

For an organism to be fit, a number of biological imperatives must be satisfied. Short-term needs, such as obtaining food or avoiding predators, must be met before long-term needs (i.e. reproduction) can be considered. Short-term needs, in most cases, require spatial displacement by the insect, while long-term needs require that the insect provision gametes with nutrients in excess of its own metabolic needs, and place offspring in locations ensuring ensure larval survival.

In this dissertation I will address the importance of natural products in the processes insects employ to meet their short- and long-term biological needs. I address first the importance of host-plant chemical cues as they impact on host acceptance and reproductive biology of two anthomyiid herbivores. The second section of this dissertation deals with a theoretical study of the impact of chemical

stimuli on the movement of hypothetical insects and the influence of resource density on finding success.

SECTION I

OVIPOSITIONAL BIOLOGY OF THE SEEDCORN FLY, *Delia platura* Rondani, AND THE ONION FLY, *Delia antiqua* Meigen

INTRODUCTION TO SECTION I

Host acceptance by insect herbivores has been extensively studied and discussed. Although sensory modalities other than olfaction and gustation are important in host acceptance, for most phytophagous insects these two sensory channels are pre-eminent (Kennedy 1965). For this reason, much of the work done on host plant acceptance by insect herbivores has focused on plant-specific chemical cues that mediate the host acceptance (see Dethier 1953, Kennedy 1965, Schoonhoven 1968, Stadler 1976, Dethier 1982, and Miller and Strickler 1983 for reviews). Early work on the subject emphasized the importance of plant-specific "token stimuli" in the recognition of host plants (Dethier 1937, 1941; Fraenkel 1959). Insects were envisioned as possessing specialized receptors responsive primarily to the token stimuli, and these receptors were believed to send information to the central nervous system via "labeled lines" (Dethier 1971). Acceptance of host plants was believed to be determined solely by the presence of stimulatory secondary plant compounds. The importance of inhibitory substances was not articulated until 1965 by Jermy.

Kennedy (1958) broadened our perspective of host-plant acceptance by demonstrating the importance of nutrients in the acceptance process. He proposed the "dual discrimination" hypothesis, which stated that while token stimuli may be important in locating hosts, sensory information from nutrients is very important in determining acceptance by the insect.

To explain the wide range of plant compounds to which insects

respond, Dethier (1971) proposed that across-fiber patterning might occur in the insect nervous system. This concept, borrowed from vertebrate physiologists, postulates that the differential responses by a small number of receptor types are integrated in the central nervous system, resulting in response spectra that are essentially unique for each stimulus. Thus, instead of being sensitive only to chemicals specific to their host plants, insects were now envisioned as perceiving a "chemical Gestalt" (Dethier 1982) of the plant being examined.

In 1982, Dethier presented a model to explain the integration of sensory information by the insect. In essence, the model proposes that acceptance or rejection of a host plant depends on the ratio of external excitatory and inhibitory inputs from the peripheral receptors. In addition, the responsiveness of the insect's decision-making center could be modified by internal inhibition. When viewed with this model in mind, the differences in host plant acceptance among insects are seen to be due to differences in: 1) the responsiveness of peripheral receptors; 2) the level of internal inhibition (or excitation); or 3) the decision-making rules used by the insect.

Only recently has the effect of host deprivation on ovipositional acceptance been rigorously investigated. Singer (1982) observed the effects of ovipositional deprivation on females of Euphydryas editha (Lepidoptera). He found that many females initially discriminated between host-plant species (i.e. they attempted to oviposit on some species but not others), but as time since last oviposition increased, females would oviposit on normally unacceptable hosts. Roitberg and

Prokopy (1983) found that avoidance by Rhagoletis pomonella females of fruits to which its oviposition-deterring pheromone had been applied declined as time since last oviposition increased. It seems likely that there might be a neural or hormonal feedback from oviducts of deprived insects as a result of accumulating unlaid eggs, and that the intensity of this feedback might increase as oviposition proceeds. One might envision a situation anlogous to inhibition of feeding in Phormia regina by the presence of food in the crop (Dethier and Bodenstein 1958), except that in this case the presence of eggs in the oviducts would be stimulatory for oviposition.

One appealing model proposed to explain how the internal state of insects influences ovipositional acceptance is that of Dethier (1982), embellished upon by Miller and Strickler (1984) (Figure 1). The model proposes that positive and negative factors, of both external and internal origin, are "weighed" in some decision-making center of the central nervous system (CNS). If the ratio of positive to negative factors exceeds some threshhold value, the insect accepts the resource (i.e., oviposits). The ratio of excitatory to inhibitory stimuli necessary to "tip the balance" in favor of acceptance depends on the position of the acceptance "fulcrum," which is schematically depicted as rolling as the physiological state of the insect changes. It would be informative to investigate how the acceptance threshold of insects changes in the face of host-plant stimulus deprivation. Of particular interest would be a comparative study of the effect of deprivation on host acceptance of several insect species that differ in their host-plant ranges. This first section of this dissertation is an attempt to determine how host-plant acceptance of two closely-related insect herbivores, the seedcorn fly (Delia platura Rondani) and the onion fly

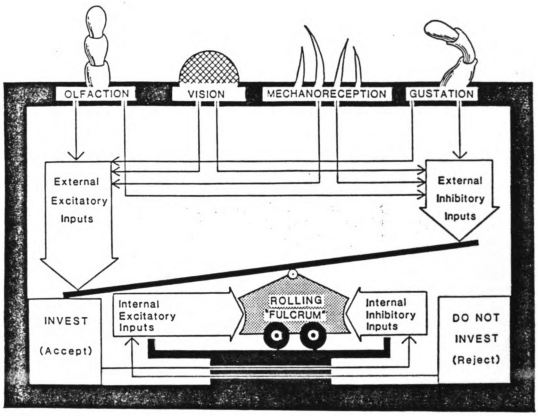

Figure 1. Rolling-fulcrum model of host acceptance of Miller and Strickler (1984). Reprinted by permission of Sinauer Associates, Inc.

(*D. antiqua* Meigen), modulate ovipositional acceptance in the face of host-plant stimulus deprivation. First, however, it was necessary to determine what plant characteristics stimulate oviposition by *D. platura*.

CHAPTER 1

OVIPOSITIONAL RESPONSE OF SEEDCORN FLY (*Delia platura* Rondani) TO DEVELPOMENTAL STAGES OF LIMA BEAN (*Phaseolus lunatis* L.)

Introduction

The seedcorn fly, Delia platura Rondani, is a wide ranging anthomyiid fly whose larvae feed on plants from diverse plant families. Among the more common plant hosts are various bean species, corn, cucurbits, and crucifers (Ristich 1950). In addition, larvae will complete development on decaying organic matter (Miller and McClanahan 1969). Females lay eggs primarily in the vicinity of germinating seeds or organic matter (Barlow 1965, McClanahan and Miller 1969, Yu et al. 1975). Barlow (1965) concluded that females were stimulated to oviposit primarily by olfactory cues associated with germinating seeds and organic matter, and, Eckenrode et al. (1975) showed that microbes associated with germinating seeds are responsible for the production of stimulatory compounds.

Ibrahim and Hower (1979) measured the acceptability of developmental stages of soybean as ovipositional sites for D. platura. They found that emerging seedlings were the most acceptable developmental stage in choice tests, but also found that lima bean (Phaseolus lunatis L.) seedlings stimulated much more oviposition than even the most stimulatory developmental stage of soybean. Since lima beans are much more acceptable as ovipositional sites, it would be very informative to determine if acceptability of lima bean seedlings changes over time in a manner similar to soybean seedlings.

Yu et al. (1975) had previously found that aqueous extracts made from ground, germinating lima bean seeds were not stimulatory for oviposition, even though lima beans that had germinated for 2-3 days

elicited more oviposition than several other large-seeded crops. Apparently, the ovipositional stimuli associated with germinating lima beans are associated more with the rhizosphere of the germinating seed, and not with the seed tissue itself. This is consistent with Eckenrode et al.'s (1975) finding that microbes are responsible for the production of seedcorn fly ovipositional stimuli. Clearly, efforts to isolate and identify seedcorn fly ovipositional stimulants must focus on extracts from the rhizospere of germinating seeds.

Barlow (1965) reported that substrate texture plays a minor role in ovipositional stimulation. He found that oviposition increased as substrate particle size increased up to a critical size, and then decreased. No other effects of substrate physical properties on ovipositional stimulation have been reported.

In this chapter I evaluate the acceptability of developmental stages of lima bean plants as ovipositional sites for the seedcorn fly. I then attempt to pinpoint those characteristics of the most stimulatory developmental stages that elicit oviposition. In addition, I quantify the suitability of developmental stages of lima bean seedlings for seedcorn maggot growth.

MATERIALS AND METHODS

Choice Tests. Flies used in all experiments were from a population that had been in laboratory culture for 1-2 years. Cultures were maintained, and choice tests were conducted, in controlled-environment chambers maintained at 21 ± 2°C and 35 ± 5% RH with a 16:8 (L:D) photoperiod. Ovipositional choice tests were conducted in 50-cm

diam cylindrical cages with floors that rotated at a speed of 4 rph (see Appendix 2 for cage description). Ovipositional treatments were presented in 80-ml styrofoam cups containing 40 ml of fine vermiculite topped with 20 ml of white silica sand. Treatments were evenly spaced around the circumference of cages housing ca. 100 flies of each sex, and removed after 24 h. Eggs were counted after sand was carefully scooped from ovipositional cups and mixed with water to float eggs from the sand. All experiments used a randomized complete block design, and data were analyzed with analysis of variance.

Lima bean ("Fordhook 242") plants in various stages of development were generated by daily planting lima beans in vermiculite in 80-ml styrofoam cups over a period of several weeks. Plants were raised in a glass house and watered daily. The developmental stages chosen for bioassay were: 1) freshly planted seed, 2) 48-h-old germinating seed, 3) emerging seedling, and 4) upright seedling (cotyledons fully exposed). A 2-cm layer of silica sand was placed over the vermiculite to facilitate removal and counting of eggs. A cup of vermiculite and sand was included in the choice test as a negative control. The choice test was replicated fourteen times.

Surrogate emerging lima bean seedlings were made from 3-cm lengths of 5-mm diam. glass tubing bent into a U-shape with both straight ends parallel and in contact. This surrogate was pushed into sand in ovipositional cups with ca. 0.5 cm projecting above the sand surface, so that it resembled a transparent hook of an emerging lima bean seedling. A 2 x 2 factorial arrangement (6 replicates) of surrogates and 48-h germinating beans (planted 1 cm beneath the surface of the sand) was used to determine the relative contributions of the physical and chemical attributes of emerging seedlings to

eliciting oviposition. Thus, the four treatments compared were: 1) moist sand, 2) germinating bean, 3) surrogate seedling, and 4) surrogate plus germinating bean. Factorial analysis of variance was used to analyze the data.

To measure the effect of substrate physical characteristics on ovipositional stimulation, uniform breaks in the substrate were generated by poking holes in the sand surface with a template consisting of a 5-cm diam disk studded with 17 evenly spaced 1-cm-long aluminum rivets. Again, a factorial arrangement of holes and germinating bean (8 replicates) was used to determine the relative ability of these two factors to elicit ovipoistion.

Aqueous extracts of germinating lima beans were generated by placing 110 lima beans in a 2-l Ehrlenmeyer flask containing 2 l of distilled, deionized water. Beans were allowed to germinate for 48 h at 21°C. Eighteen ml of this extract, which represented the amount of extract equivalent to one seed, was pipetted onto the dry sand layer in an ovipositional cup. This extract was tested (5 replicates) for ovipositional stimulation in choice tests with germinating beans and a moist sand control.

Suitability Experiment. The four developmental stages of lima bean plants used in the choice test were tested for their ability to support larval development. Plants were raised in plastic boxes (60 x 40 x 20 cm) containing an 8-cm layer of pea-sized gravel topped with 15 cm of VSP (vermiculite : sphagnum : perlite) potting mix. Holes were drilled at 5-cm spacing on the bottom of the box to allow water passage. The plastic boxes were set inside a 120 x 120 x 20 cm plastic-lined trough having drain holes 5 cm above the trough bottom.

Water continuously trickled into the trough, allowing plastic boxes to be continuously watered from below. The gravel in the bottom of the plastic boxes prevented the potting mix from becoming saturated with water. By watering from below, we avoided having to disturb larvae with routine watering from above. Gravel was placed in the trough around the plastic boxes to prevent algal growth.

Aluminum screen (6 mesh per cm) pyramids were placed over the boxes to trap flies as they emerged. The base of each pyramid was snugly attached to the plastic box with a wooden frame, while the top had a 3-cm diam hole that opened into an acetate cone containing a 1 x 1 x 1 cm chunk of Raid^{TM} solid insecticide. The positivlely phototactic, emerging flies thus entered the cone traps and were killed in a matter of minutes; dead flies fell onto a plaster-of-paris ledge inside the cone. Traps were inspected daily for flies; flies were sexed, dried in an oven at 80^{o}C for 10 days, and weighed.

Planting of lima beans was staggered over time so that plants were in the desired developmental stage on a given day. Thirty plants were raised in each box. Newly eclosed larvae were transferred, one per plant, with a soft, small brush to the base of seedlings for those stages that had portions above the surface of the soil. For those stages having no above-ground plant portions (i.e. freshly planted seed, germinating seed, and soil control), larvae were transferred to the side of a small (3 mm diam) hole poked in the soil above the seeds (imaginary in the case of soil control). One replicate of the experiment was conducted at a time, with a total of three replicates being conducted. Only 10 larvae per treatment were available for the second replicate.

RESULTS

Choice Tests. The acceptability of lima bean plants increased with plant age, with a trend toward peaking at the emerging seedling stage (Figure 2). This five-choice test was not precise enough (in the statistical sense) to reveal whether the decline in the number of eggs received by upright seedlings was significant; therefore, a two-choice test was conducted with emerging and upright seedlings. The two-choice test revealed that emerging seedlings are more stimulatory for oviposition than upright seedlings, receiving more than twice as many eggs (362.4 vs. 161.9, $P < 0.05$). On the basis of these results, the search for seedcorn fly ovipositional stimuli was restricted to germinating seeds and emerging seedlings.

The presence of seedling surrogates made of glass had no effect on seedcorn fly oviposition (Figure 3). The two treatments containing germinating beans received considerably more eggs than the no-bean treatments, and both treatments in each pair received similar numbers of eggs. Thus, there was no interaction between the presence of surrogates and the presence of germinating beans.

The mere presence of holes in the substrate was sufficient to elicit oviposition (Figure 4). Not surprisingly, the presence of germinating beans was more effective than holes at eliciting oviposition. Again, there was no interaction between the two factors.

Aqueous extracts of germinating lima beans were highly stimulatory for oviposition (Table 1). Indeed, the extract appeared to be even more stimulatory than germinating beans, but this difference

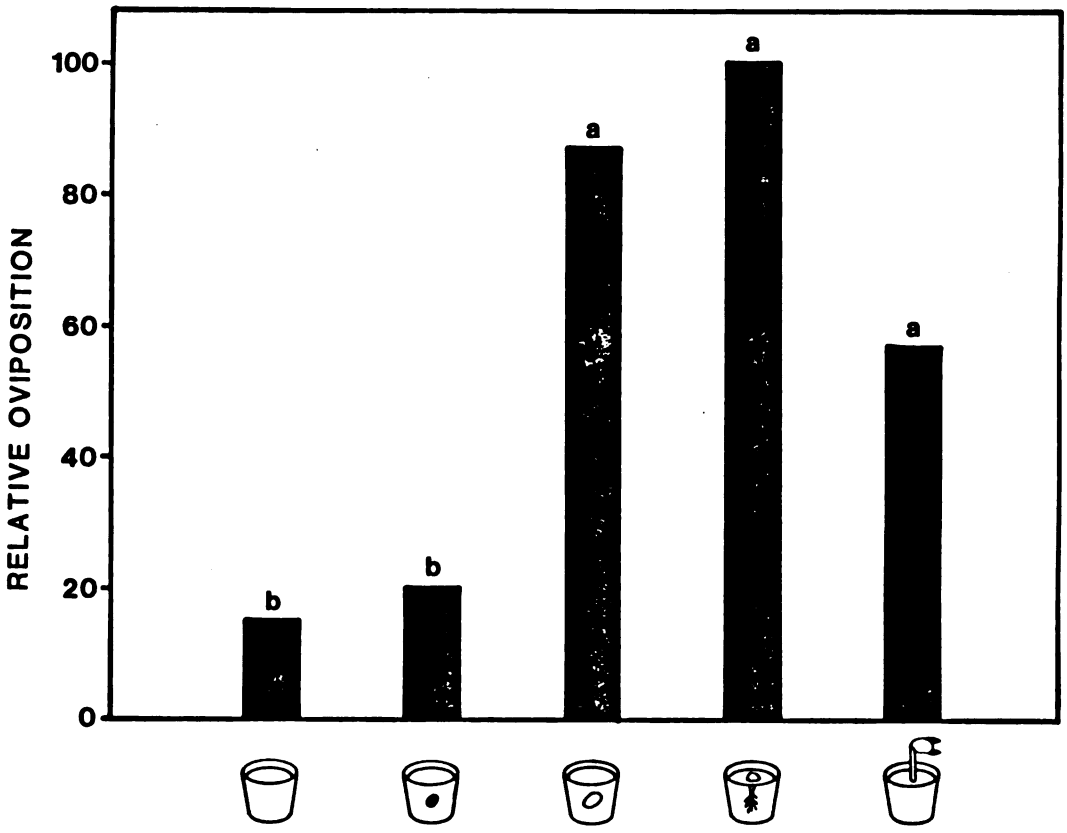

Figure 2. Oviposition by D. platura on developmental stages of lima bean plants. From left to right, treatments are: 1) sand control, 2) freshly planted seed, 3) germinating seed, 4) emerging seedling, and 5) upright seedling. Bars accompanied by the same letter are not statistically different as determined by lsd test (α = 0.001).

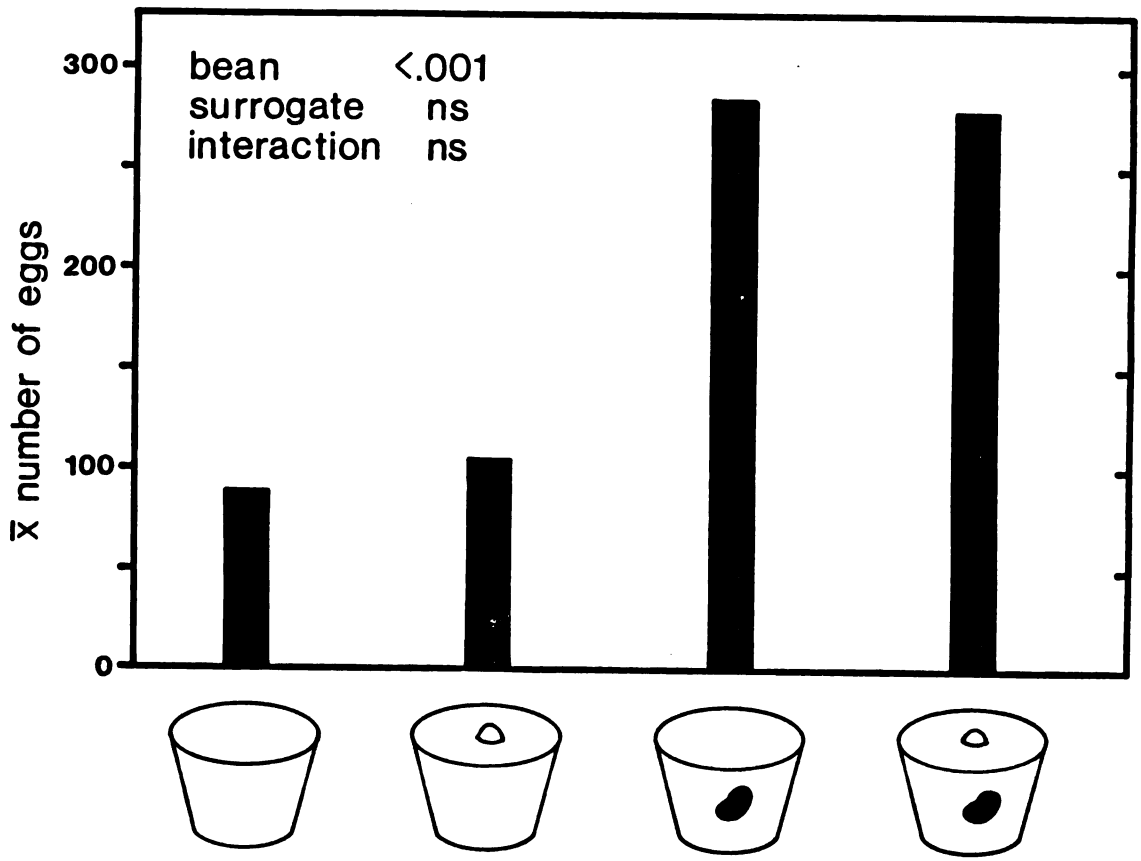

Figure 3. Oviposition by D. platura on factorial arrangement of surrogate seedlings and germinating beans. From left to right, treatments are: 1) sand control, 2) surrogate, 3) germinating seed, and 4) surrogate plus germinating seed. Significance of main effects and interaction is indicated in the box.

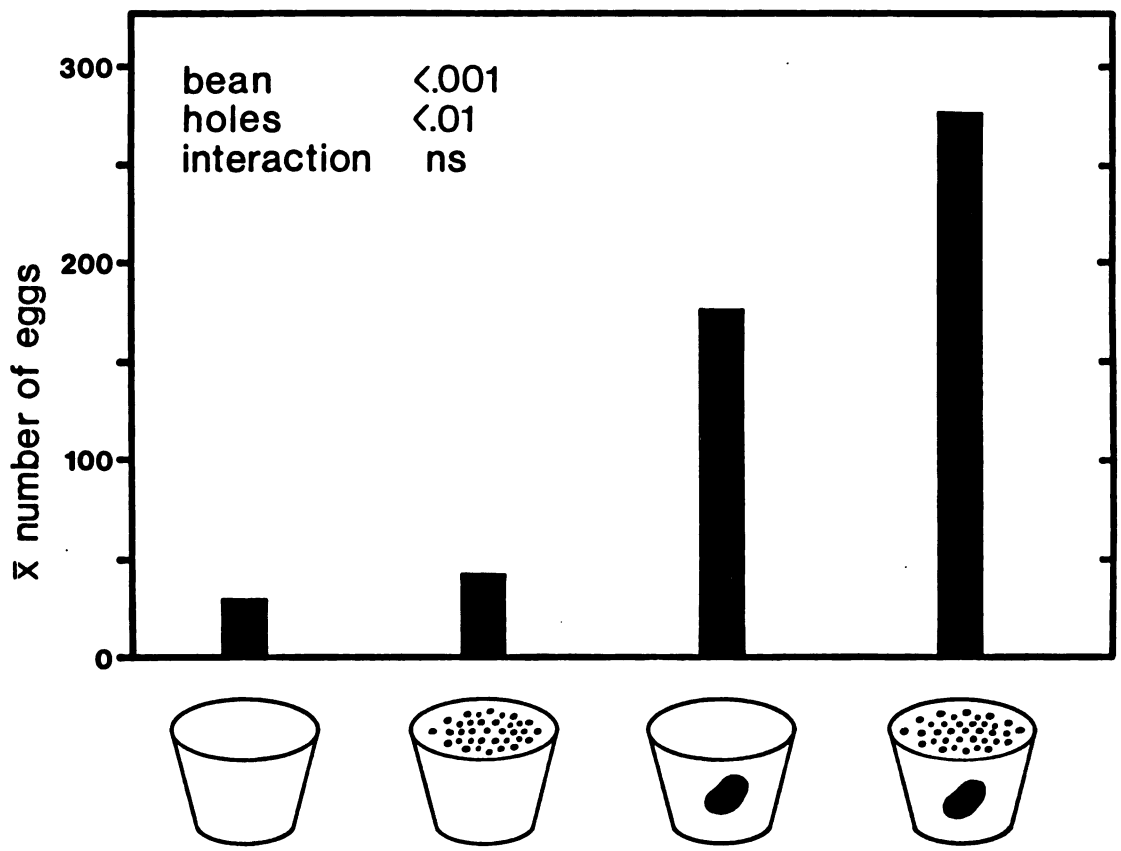

Figure 4. Oviposition by D. platura on factorial arrangement of holes in substrate and germinating beans. From left to right, treatments are: 1) sand control, 2) surrogate, 3) germinating seed, and 4) surrogate plus germinating seed. Significance of main effects and interaction is indicated in the box.

Table 1. Oviposition by *D. platura* on lima bean extract.

Treatment	Mean number of eggs (± SD)[1]
Moist sand	35.8 ± 27.1 b
Germinating seed	85.2 ± 52.0 a
Lima extract	188.2 ± 146.3 a

[1] Means followed by the same letter are not statistically different as determined by lsd test (α =.05) on data transformed to log(x+1).

was not significant.

Suitability Experiment. Survivorship on the various developmental stages of lima bean, which ranged from 13 to 21%, was not significantly different. No larvae survived on the soil control. Male weights did not differ significantly among treatments, but females that fed on fresh and germinating seeds were almost twice as heavy as females that were reared on emerging or upright seedlings (Table 2). In addition, females on the freshly planted and germinating seed treatments took ca. 4 days less to complete development than females feeding on emerging seedlings.

Discussion

As with soybean plants, emerging seedlings are the most stimulatory developmental stage of lima bean plants for seedcorn fly oviposition. This result can be explained in light of Eckenrode et al.'s (1975) finding that microorganisms associated with germinating beans are intimately involved with production of ovipositional stimulants. As seeds germinate, exudates, as well as populations of microbes in the soil surrounding seeds, would be expected to increase up to the time that cotyledons emerge from the soil. It is reasonable to expect that increased microbe populations and/or exudate concentrations would be accompanied by increased production of seedcorn fly ovipositional stimulants. Once the cotyledons have emerged from the soil, however, the primary source of seed exudates is no longer present in the soil, causing a proposed decline in the production of ovipositional stimulants. Thus, it is probably not

Table 2. Suitability of developmental stages of lima bean for *D. platura* growth and survival.

Developmental stage	Larval + pupal duration (days)[1,2]	Female dry weight (mg)	Survivorship (%)
Soil (no seed)	--	--	0 b
Freshly planted seed	19.1 ± 2.5 b	2.08 ± 0.39 a	13.0 ± 9.0 a
Germinating seed	19.5 ± 3.8 b	2.07 ± 0.50 a	18.9 ± 1.9 a
Emerging seedling	23.4 ± 3.9 a	1.04 ± 0.31 b	20.7 ± 1.3 a
Upright seedling	21.6 ± 3.3 ab	1.18 ± 0.48 b	14.4 ± 12.6 a

[1] Values are means ± SD.

[2] Means within columns followed by the same letter are not statistically different as determined by lsd test ($\alpha = 0.01$).

coincidental that both lima bean and soybean seedlings are most stimulatory in the emerging seedling stage.

The results of the surrogate seedling experiment indicate that above-ground plant structures play little or no role in stimulating seedcorn fly oviposition. The slight increase in oviposition observed in the five-choice experiment as plants progressed from germinating seeds to emerging seedlings is most likely due to increased liberation of microbial volatiles, as a result either of increased production of microbial metabolites with time, or to increased release of microbial volatiles as the seedling breaks the soil surface. The color of the emerging seedling has not been ruled out as a potential ovipositional stimulant, but its effect, if present, is trivial since emerging seedlings receive only slightly more eggs than germinating seedlings.

If a seedling creates cracks in the substrate as it emerges, oviposition is increased substantially. This increase is due most likely to the increase in favorable ovipositional locations. Crevices, especially in the absence of host plant cues, are capable of eliciting oviposition (Weston and Miller 1986). Thus, merely providing a place for an ovipositor to be inserted increases the ovipositional acceptability of a given substrate.

By far the most stimulatory quality of lima bean plants is the chemical constituents in the rhizosphere of the germinating bean. Even in the complete absence of germinating beans, aqueous seed extracts elicit as much oviposition as germinating beans. It is not possible to determine from these experiments whether olfactory or gustatory cues are responsible for ovipositional stimulation, but the data of Barlow (1965) strongly implicate a role for volatile compounds in the stimulation of seedcorn fly oviposition.

It is interesting that the acceptability rankings of developmental stages of lima bean plants were not very closely correlated with the ability of the various stages to support larval development. Flies on the most acceptable developmental stage, emerging seedlings, took the longest to develop and weighed less than flies on other treatments. Likewise, flies on the least acceptable stage, freshly planted seeds, along with flies on germinating seeds, had the highest female weights and shortest development times. Since female weight is correlated with fecundity in other dipteran species (Vogt et al. 1985, Finch and Coaker 1969), larval fitness is apparently not maximized by D. platura on lima bean.

Accepting emerging seedlings may represent a trade-off between resource detectability and fitness maximization. To maximize larval fitness, females would have to place eggs near seeds that are beginning to germinate. However, the cues that indicate the presence of germinating seeds are apparently not detectable until germination is well underway and, furthermore, increase in detectability as seedlings enter less suitable growth stages (i.e. emerging seedlings). It seems that D. platura must invest gametes in a "sure thing" (germinating seeds or emerging seedlings) rather than investing in a resource that is not readily detectable (freshly planted or dormant seeds), even though fitness would be higher if females laid eggs on freshly planted seeds instead of emerging seedlings. Given the detectability constraint, D. platura fitness may actually be elevated under the observed pattern of lima bean acceptability, though not necessarily maximized. Fitness would be maximized if females laid eggs only on pre-emergent, germinating seeds, provided that biotic

mortality factors are evenly distributed across the various developmental stages. The detectability constraint may also be important in other insect/plant associations, and should be considered in the face of apparently non-optimal patterns of ovipositional acceptance. This is not to say that host acceptance patterns by all insects is optimized, but rather, that this potential constraint should be considered when evaluating a particular acceptance pattern for optimality.

CHAPTER 2

INFLUENCE OF OVIPOSITIONAL RESOURCE QUALITY ON LIFETIME FECUNDITY OF *Delia platura*

INTRODUCTION

Much of the work on host-plant acceptance by ovipositing insects has been conducted in choice-test situations. Test insects are generally well-provisioned with food and water, and their acceptance of host plants is assessed by counting eggs laid on plants suspected of differing in their acceptabilities as ovipositional sites. In such settings, insects rarely experience deprivation of ovipositional sites since the environment is structured so that encounters with suitable sites are highly probable even if host-finding is random. In terms of the rolling-fulcrum acceptance model of Miller and Strickler (1984) (Figure 1), the "fulcrum" of such insects does not move far from the zone where relatively large external excitatory sensory inputs are required to elicit ovipositional acceptance. Under these conditions, plants offering poor ovipositional stimuli would be expected to receive fewer eggs than they would in a no-choice situation. As insects deprived of the opportunity to oviposit accumulate matured eggs, their acceptance of potential hosts would be expected to become less strict. This, in fact, has been convincingly demonstrated for Euphydrias editha (Singer 1983). In such an insect, the presence of unlaid eggs might provide excitatory internal inputs to the central nervous system, thus increasing the probability of acceptance of normally unacceptable plants. But how fast does this acceptance threshold change and what are its limits? In addressing these questions, we measured the ovipositional acceptance of individual seedcorn fly, Delia platura Rondani, females caged with

either highly acceptable or minimally acceptable ovipositional stimuli.

The seedcorn fly is a cosmopolitan insect. Females lay eggs, and larvae successfully complete development, on a variety of plant species from a number of plant families as well as on non-living plant material (Ristich 1950). Among the more stimulatory plant species is lima bean, Phaseolus lunatis L. (Yu et al 1975). I have found that germinating beans and emerging seedlings are the most stimulatory of lima bean developmental stages for eliciting D. platura oviposition (see Chapter 1). Thus, germinating lima beans constitute a highly stimulatory standard with which to compare oviposition by flies that are deprived of adequate ovipositional stimuli. In this chapter I quantify the effect of ovipositional deprivation on D. platura lifetime oviposition, rate of egg maturation, and release of D. platura ovipositional behavior.

MATERIALS AND METHODS

Lifetime Oviposition. Flies used here were the offspring of a single fertilized female collected in an onion field in Eaton Rapids, Michigan. Progeny of a single female were used in an effort to reduce experimental error. Larvae from this female fed on lima beans. As adults emerged over a four-day span, they were caged individually with water, dry diet (Ticheler 1971), and two males. Cages were cylinders (15 cm diam x 33 cm) of 6 mesh/cm aluminum screening fitted over the tops of plastic flower pots that had a 15 cm diameter petri dish glued to the mouth to support food and ovipositional resources. The top of

the screen cylinder was fitted with a section of nylon stocking to allow access while preventing the escape of flies. An inverted petri dish was placed over the nylon-covered cylinder to complete the closure.

During the first month, males were replaced as they died to ensure adequate opportunity for mating. The cages were housed in an environmental chamber at 21 ± 2 ^{o} C and 35 ± 5% relative humidity under a 16:8 (L:D) light regime. After all flies had emerged, seven were randomly assigned to the "deprived" group and seven to the control group. The control flies had an 80-ml styrofoam cup containing 60 ml of moist silica sand and a 24 h-old germinating lima bean ("Fordhook 242") placed in their cages as an ovipositional site, while the deprived group received a cup of moist sand alone. Moist sand by itself stimulates oviposition, but receives only ca. one-sixth as many eggs as germinating beans (see Chapter 1). Cups were replaced every day with an identical treatment until the female died. Eggs were floated from the sand and counted; thus lifetime ovipositional records were obtained for each fly. Dead females were preserved in 70% isopropyl alcohol until dissection. Only chorionated eggs were included in the egg counts.

Egg Maturation. Pupae from a laboratory population in culture for three years were weighed and placed in vials containing moist sand. The first 24 females to emerge were caged individually as in the lifetime experiment. Flies emerging on a given day were randomly divided between the deprived and control treatment groups. Since emergence was not synchronous, this "pairing" was necessary to eliminate possible bias in assignment of flies to treatment groups since fly physiology might vary with time of emergence. Males and

ovipositional treatments were added on the day following emergence. Every 24 h, eggs were counted and fresh ovipositional cups provided. On the eighth day following emergence, females were preserved in 70% isopropyl alcohol and the number of chorionated eggs remaining in their abdomens was counted. Two females in the control group died before the eighth day and thus were not included in the analysis.

Ovipositional Release. Flies for the deprived and control treatment groups were obtained as pupae from the laboratory culture. The first 28 flies to emerge were placed in individual cages with food, water, and two males. On the day following emergence, all females were provided with ovipositional cups containing germinating beans in moist sand. As before, ovipositional cups were checked daily for eggs and replaced with fresh ones. Two days after the females assigned to the "deprived" group began ovipositing, they began receiving an ovipositional cup containing only moist sand. Control females continued to receive germinating beans in their ovipositional cups. Females in both groups were sacrificed 12 days after they began ovipositing and were checked for unlaid, chorionated eggs. Flies that laid no eggs (N = 6) or lived less than 11 days after beginning to oviposit (N = 6) were excluded from analysis, leaving 9 flies in the deprived group and 7 flies in the control group. Trend analysis was used to detect whether cumulative oviposition curves departed significantly from linearity. When they did, curves were separated into linear segments using least squares techniques. Slopes of corresponding segments of cumulative oviposition curves for the two treatments were compared with the slope comparison test (Sokal and Rohlf 1981, p. 505).

RESULTS

Lifetime Oviposition. Cumulative lifetime ovipositional curves of deprived and control flies are shown in Figure 5. The mean time to first oviposition was twice as long for deprived flies as for control flies (Table 3). Interestingly, the time from first oviposition until death was essentially equal for the two groups. The daily ovipositional rate of the control flies was twice that of the deprived ones, leading to a two-fold difference in lifetime egg deposition.

Flies deprived of host plants laid 32.3% of their eggs in locations other than the ovipositional cup, compared with only 0.9% for the undeprived flies ($P < 0.001$). Eggs laid off the ovipositional cup were found primarily in the crevice between the water dish and the floor of the cage.

Egg Maturation. Host-deprived and control flies matured eggs at different rates during the pre-ovipositional and early ovipositional periods. The total number of eggs matured (those laid plus unlaid) was more than twice as great in the control flies as the deprived ones (71.4 vs. 31.7, $P < 0.05$, t-test).

Ovipositional Release. Flies assigned to the control and deprived treatment groups laid similar numbers of eggs during the first two days prior to the switch in ovipositional treatment for the deprived group (Figure 6). Following the switch from bean to the sand ovipositional treatment, flies in the deprived group laid eggs at a rate significantly lower than flies that continued to receive beans in their ovipositional cups ($P < 0.05$, slope comparison test; Figure 6).

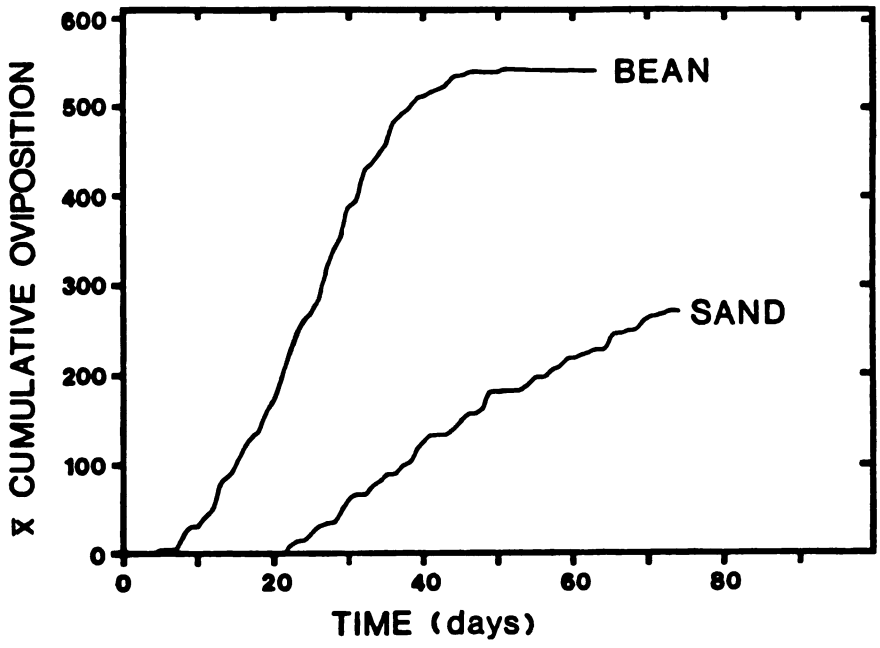

Figure 5. Cumulative lifetime ovipositional curves for D. platura on highly acceptable (bean) and minimally acceptable (sand) resources. Each curve is the mean for seven individuals.

Table 3. Fecundity parameters of *D. platura* in the lifetime oviposition experiment.

Treatment group	Time to first oviposition (days)[1]	Daily ovipositional rate (eggs/day/fly)	Productive life span (days)	Total egg production (eggs/fly)
Control	17.7 ± 5.4	18.6 ± 11.4	30.4 ± 10.5	528.8 ± 327.4
Deprived	32.4 ± 6.8	9.6 ± 5.0	27.3 ± 13.1	266.8 ± 181.6
Significance of Difference[2]	***	*	ns	*

[1] Values are means ± SD.

[2] Significance determined by t-tests. Data transformed, when necessary, to homogenize variances. Levels of significance: * = 0.05, *** = 0.001, ns = not significant.

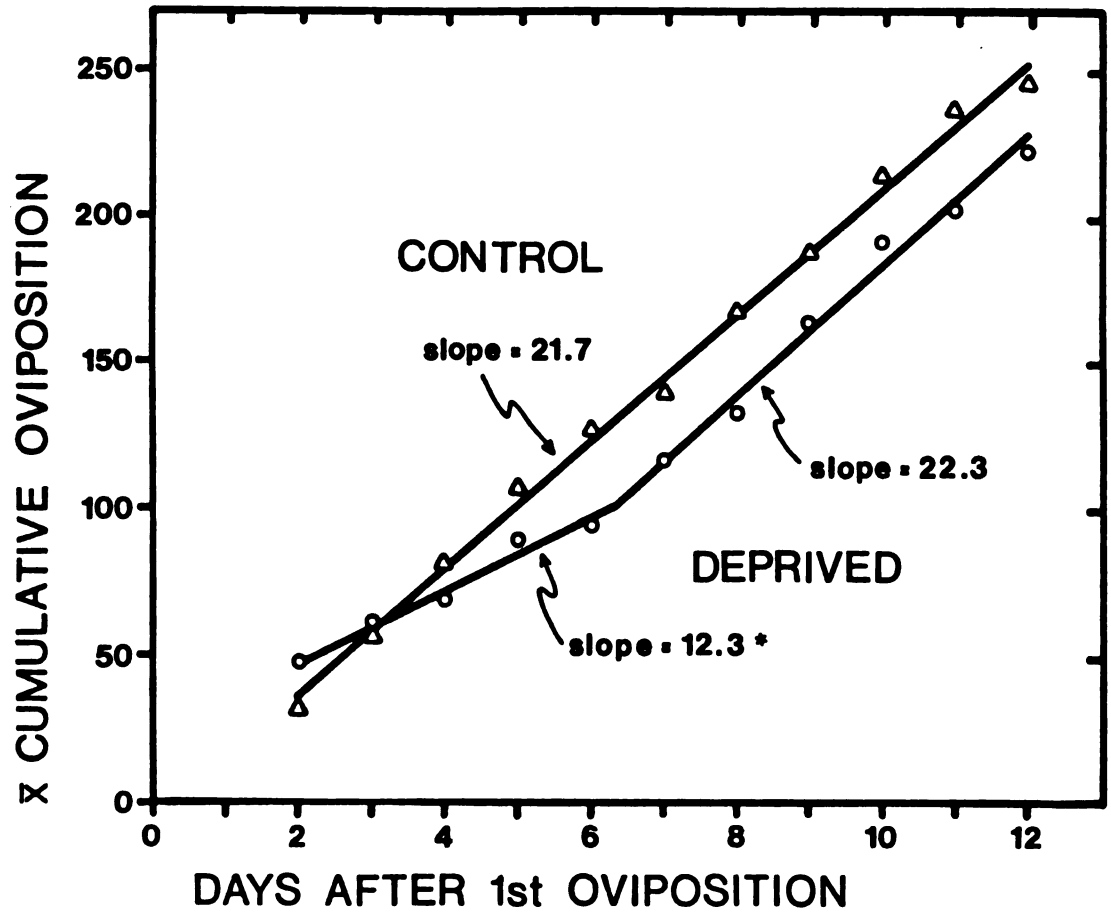

Figure 6. Cumulative ovipositional curves for D. platura in the ovipositional release experiment. Curves are the means for seven individuals (control) and nine individuals (deprived). Slope value accompanied by asterisk is significantly different from the control at $\alpha = 0.05$.

Four days after the ovipositional treatment switch, however, flies in the deprived group resumed laying eggs at the same rate as the control flies ($P > 0.5$, Figure 6). The total number of eggs matured by the two groups (eggs laid plus unlaid) was essentially identical (264.7 vs. 261.0, $P > 0.5$, t-test) when the experiment was terminated at 12 days after first oviposition.

DISCUSSION

The finding that *D. platura* females deprived of highly stimulatory ovipositional sites took longer to initiate oviposition in the lifetime experiment was not unexpected. Several explanations might account for this result. First, lack of appropriate ovipositional cues could result in flies simply not being stimulated to oviposit. Flies might thus retain eggs until the presence of unlaid eggs provides so strong an excitatory stimulus that eggs can no longer be withheld. Another explanation is that absence of appropriate host stimuli results in decreased rates of egg maturation.

The results of the egg maturation experiment support the latter hypothesis. The 50% decrease in egg maturation under deprival conditions could account not only for the two-fold increase in time to first oviposition, but also for the 50% decrease in lifetime egg-laying for the deprived flies. Another piece of evidence supporting this explanation is the result of the ovipositional release experiment. Although deprived flies initially showed a significant decrease in the rate of oviposition, they soon resumed laying eggs at a rate no different from control flies. This result suggests that reproductive developmental status plays a critical role in determining

ovipositional acceptance. These flies were exposed after the treatment switch only to ovipositional stimuli that elicited half the oviposition released by germinating beans in the no-choice lifetime experiment, yet these flies laid eggs at the same rate as the control flies after the initial lag period. Taken with the fact that the deprived flies in the release experiment had developed the same number of eggs as the controls, this result suggests that the quantity of eggs matured is the primary determinant of ovipositional acceptance in a no-choice situation, with minor control exerted on a short-term basis by the quality of the external ovipositional stimuli.

In terms of the rolling-fulcrum acceptance model, unlaid eggs might be acting as internal excitatory inputs to the central nervous system. As eggs accumulate in the oviducts of flies that have had stimulatory ovipositional cues removed, they drive the fulcrum to the point where previously weakly-stimulatory cues are capable of eliciting normal rates of oviposition. The duration of the lag period provides an estimate of the time required for the "fulcrum" to move to a compensatory point, 4 days for D. platura in the present case. The lag period also represents the time during which flies retain their ability to discriminate to some extent against poor oviposition sites in a no-choice situation. Presumably this no-choice discrimination ability is lost when the acceptance threshold is driven sufficiently far by the internal excitatory inputs generated by unlaid eggs. The number of eggs required to change the acceptance threshold can be estimated from the difference in number of eggs retained by the two treatment groups, assuming that flies in both groups matured eggs at the same rate, which certainly seems to be the case since

ovipositional rates were equal after the lag period for the deprived flies. This quantity, ca. 30 eggs, represents the capacity of the insect for withholding eggs in the face of host deprivation.

Further evidence that the threshold for oviposition declines with ovipositional site deprivation is found in the observation that deprived flies in the lifetime experiment laid close to one third of their eggs in locations other than the ovipositional dishes, while control flies laid nearly all (99.1%) of their eggs in the ovipositional dishes. Apparently, in the absence of stimulatory host volatiles, crevices (which increase oviposition into substrates containing germinating beans [see Chapter 1]) become acceptable ovipositional sites by themselves.

The changes in seed fly oviposition in response to removal of stimulatory host plants might represent adaptive compensatory mechanisms to conserve gametes in the absence of host plants suitable for larval development. The compensatory response appears to have two components, one short-term and one long-term. The short-term response appears as the initial decrease in ovipositional rates following removal of the host plant. The long-term compensatory response is the reduction in rate of egg maturation, a phenomenon that has been observed in other insects in response to absence of host stimuli (Pouzat 1978, Robert 1976). Having the ability to adjust ovipositional rates in the face of local host-plant shortages would enable insects to allocate gametes primarily to suitable hosts among a range of potential hosts, while adjusting egg development rates over extended periods of host-plant shortages would reduce the energetic cost of resorbing mature oocytes and then reprovisioning oocytes when the host plant is again abundant.

It is often assumed that the "non-preference" displayed by phytophagous insects for minimally acceptable host plants will break down when the insect is confronted with a situation where more acceptable plants are absent. The results of these experiments provide evidence that this is not necessarily true. The fact that host-plant deprivation resulted in decreased egg maturation suggests that lack of exposure to highly acceptable plants in the field may similarly result in decreased fecundity. This suggestion is worthy of investigation especially considering its implications for the control of oviposition by phytophagous insects in fields planted with non-preferred cultivars of agricultural crops.

CHAPTER 3

INFLUENCE OF HOST-PLANT STIMULUS QUALITY ON LIFETIME FECUNDITY OF THE ONION FLY, *Delia antiqua* Meigen

INTRODUCTION

Host-plant acceptance by insect herbivores is determined not only by physico-chemical properties of the host plant, but also by the physiological and behavioral states of the consuming insect. Thus, the cues associated with host plants eliciting acceptance (i.e. feeding or ovipositing) may have different behavioral effects depending on the insect's prior experience or nutritional state. Many insects are known to become less finicky in accepting plants for feeding or oviposition after deprivation [Dethier 1982, Knoll 1922 (cited in Hinton 1981), Schwarz 1923, Singer 1983]. This decrease in finickiness has been interpreted (Dethier 1982, Miller and Strickler 1984) as a manifestation of a change in the behavioral state of the insect caused by physiological changes induced by lack of normal feeding or ovipositing. In terms of the rolling-fulcrum model of host acceptance (Miller and Strickler 1984)(Figure 1), the fulcrum of deprived insects moves sufficiently far, as deprivation proceeds, that weaker and weaker host stimuli elicit acceptance.

While some insect species begin to accept host plants promiscuously following host deprivation, others will retain eggs and consequently may be less fecund over a lifetime than their undeprived conspecifics, e.g., Acanthoscelides obtectus (Coleoptera: Bruchidae) (Pouzat 1978), Acrolepia assectella (Lepidoptera: Hyponomeutoidea) (Cadeilhan 1965), and Delia radicum (Diptera: Anthomyiidae) (Nair and McEwen 1976). In addition to merely withholding eggs in the absence of acceptable hosts, insects may decrease rates of egg maturation, e.g.,

A. obtectus (Pouzat 1978) and Delia platura (Diptera: Anthomyiidae) (Weston and Miller 1986).

Because insects modulate ovipositional behavior in various ways in response to host deprivation, it is difficult to predict a priori how a particular species will respond to such deprivation. In the face of deprivation, specialist herbivores might be expected to remain more finicky about ovipositing than generalist herbivores since specialists require a more restricted set of stimuli to elicit oviposition. Two closely related (Harris and Howard, unpublished) anthomyiid herbivores, the onion fly (D. antiqua) and the seedcorn fly (D. platura), differ enough in their host-plant acceptance patterns to be considered a specialist and a generalist, respectively; thus, they represent ideal model organisms for addressing the above hypothesis.

D. antiqua is a common pest on onions cultivated in Northern temperate climates (Loosjes 1976). As its common name suggests, ovipositional acceptance and larval development are largely restricted to the onion plant, Allium cepa. Adult females are stimulated to oviposit by shape, color, and chemistry of onion foliage (Harris and Miller 1982). Surrogate onion stems painted to match onion foliage or coated with wax containing onion foliar chemicals elicit more oviposition than moist sand, but when color and chemicals are presented simultaneously, the two stimuli act synergistically (Harris and Miller 1982).

In contrast, D. platura is a generalist insect which completes development on plants from a broad range of families (Ristich 1950). Not surprisingly, D. platura females are stimulated to oviposit by stimuli associated with various plants, most notably by chemostimuli associated with germinating seeds (Eckenrode et al. 1975) and with

decaying organic matter (Barlow 1965). When deprived of germinating seeds over their lifetimes, D. platura females laid half as many eggs as did undeprived females; deprived flies matured eggs at one-half the rate of undeprived flies (Weston and Miller 1986).

In addition to providing the opportunity to compare host acceptance of a host-deprived specialist herbivore with that of a generalist, D. antiqua provides a unique opportunity to investigate the influence of host-plant sensory components on insect fecundity. Surrogate onion seedlings, which are 12-cm lengths of 4-mm diam glass tubing painted green and coated with wax containing synthetic onion foliar chemicals, receive similar numbers of eggs as do onion seedlings (Harris et al. 1986). The physical, visual, and chemical characteristics of surrogates resemble those of onion seedlings, and can be manipulated independently so that surrogates lacking some or all host-plant characteristics can be generated. Thus, confining females with variously modified surrogates can provide insight into the contribution of various host stimuli to host acceptance under conditions of partial or complete host-stimulus deprivation.

In this chapter, I quantify the effect of host-plant stimulus deprivation on D. antiqua lifetime oviposition and egg maturation.

MATERIALS AND METHODS

Flies used in all experiments were from a population that had been in laboratory culture for 5 to 15 generations. Flies were housed in environmental chambers at 23 $\pm$ 2^{o} C under a 16L:8D light regime. Individual flies were obtained by placing pupae individually in 20 ml

glass vials containing moist sand, and collecting adults within 24 h of emergence. Since adult emergence was not synchronous, flies were assigned to treatments in rotation as they emerged to spread variability due to time of emergence across replicates.

Lifetime Oviposition. Newly-emerged female flies were placed in cages containing food, water, and two males. Cages were aluminum screen cylinders fitted over plastic flower pots (See Chapter 2 for full description); food was the meridic diet of Ticheler (1971). Twenty-four h after fly emergence, single ovipositional treatments were placed in each cage. Ovipositional treatments were presented in plastic cups (40 mm diam x 40 mm) containing moist silica sand. Host plant stimuli were presented on surrogate stems (Harris et al. 1986). Green paint mimicked foliar color, while n-dipropyl disulfide (Pr_2S_2, Eastman Kodak, Rochester, NY) mimicked plant foliar chemicals. The following combinations of surrogate color and chemical cues were used: 1) clear surrogate (no color or chemical), 2) clear surrogate plus Pr_2S_2 (no color), 3) green surrogate (no chemical), and 4) green surrogate plus Pr_2S_2. The green surrogates plus Pr_2S_2 contained the optimal combination of host plant stimuli, and thus served as the positive control. Since the ability of Pr_2S_2 to stimulate oviposition when presented in this formulation reaches a maximum 24 h after preparation, surrogates were allowed to air for 24 h prior to use. Surrogates were used for only two consecutive days since ovipositional stimulation declined appreciably thereafter (Harris et al. 1986). A fifth ovipositional treatment, consisting of a plastic cup containing moist sand, was the negative control. Twelve flies were assigned to each treatment group.

Ovipositional cups were removed daily and checked for eggs; sand

from ovipositional cups was stirred with an excess of water, and the eggs were removed by flotation. Cages were also inspected for eggs since flies in some treatment groups had a tendency to lay eggs in locations other than ovipositional cups, particularly in crevices. In addition to measuring daily oviposition over the lifetime of each fly, I measured age at first oviposition as well as lifespan. An additional measure, the reproductive life span, was calculated as the difference between the age at which flies laid their last egg(s) and the age at which they laid their first. Finally, rates of maximum oviposition were calculated as the number of eggs laid per day for the 20 day intervals when oviposition was maximal for each fly.

Since the four treatments using surrogates formed a 2 x 2 factorial arrangement of visual and chemical stimuli, the fecundity measures from these four treatment groups were analyzed using factorial ANOVA. Flies laying < 60 eggs (N=13), were considered abnormal and excluded from the analysis. These flies were uniformly distributed among treatments (χ^2 = 3.2, P>0.5). In addition, two flies escaped and were not included in estimates of total fecundity, but were included in measurement of time to first oviposition. The influence of Pr_2S_2 on the skewness of cumulative oviposition curves was judged by comparing the average daily egg production for the two groups exposed to Pr_2S_2 with the average of the two groups not similarly exposed

Egg Maturation. Newly-emerged females were placed individually in cages with food, water, and two males. Twenty-four h later, cages were provided with ovipositional cups. Half of the flies received cups containing a green surrogate stem coated with Pr_2S_2-paraffin (the

undeprived group), while the others received cups of moist sand alone (the deprived group). Ovipositional cups were replaced daily and checked for eggs. After a predetermined exposure to ovipositional treatments, females were sacrificed and dissected. Mature eggs (stage 10 of Thuneissen 1973) remaining in the ovaries were counted, and summed with eggs laid in the ovipositional cups. This experiment was repeated three times, using exposure periods of 4, 5 and 6 days. Ten females were used in each treatment group for each exposure period, the exposure period of 6 days being replicated twice.

Choice Test. The ovipositional acceptability of the five treatments in the lifetime experiment were compared in a choice test. Ovipositional cups were placed in a cage (80 x 60 x 60 cm) housing several hundred flies. Food and water were placed in the center of the cage, and treatments were placed ca. 10 cm away in a circular array. Eggs were counted after 24 h. Fifteen blocks were conducted over time.

RESULTS

Lifetime oviposition. The mean cumulative ovipositional curves (Figure 7) reveal two basic groupings of treatments: those with Pr_2S_2-containing surrogates and those without. Evident from these curves is the higher rates of oviposition for the two treatment groups exposed to Pr_2S_2, in addition to greater total oviposition.

Detailed analysis of the fecundity parameters supports this initial impression (Table 4). Total oviposition was significantly different among treatments, as were time to first oviposition, and maximum egg-laying rates. Factorial ANOVA of the four treatment groups incorporating surrogates revealed that the presence of Pr_2S_2

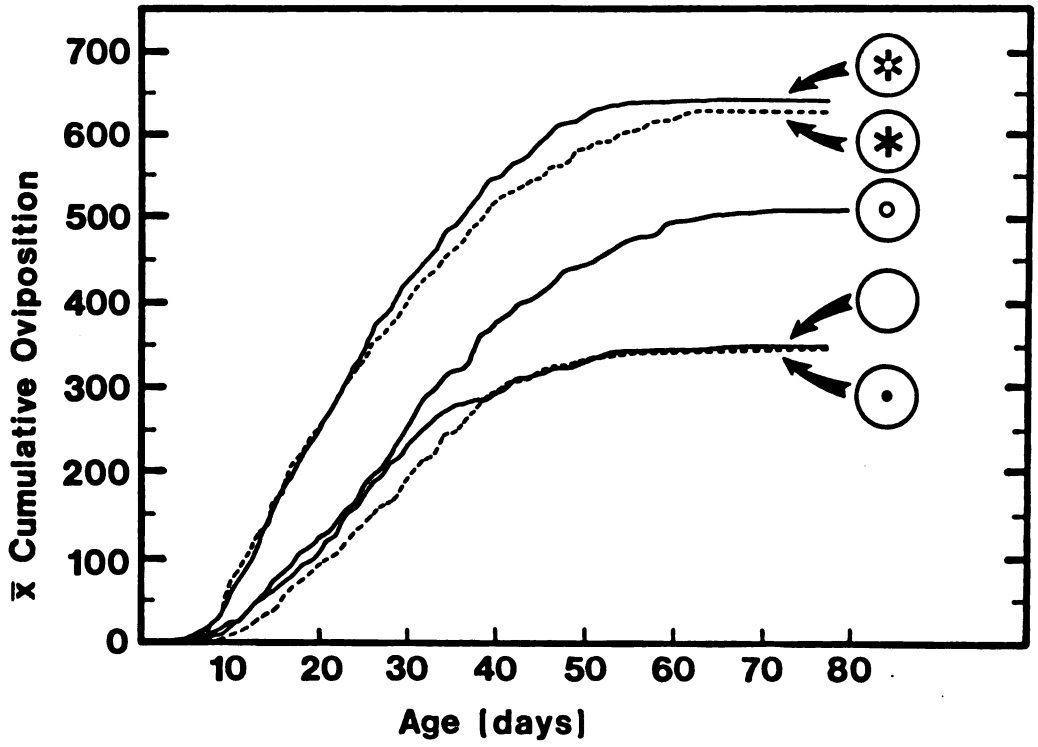

Figure 7. Mean cumulative ovipositional curves for D. antiqua in the lifetime experiment. Treatments are indicated by symbols: large open circle = sand control, open small circles inside large circle indicate clear surrogates, filled small circles indicate green surrogates, and stars in center indicate presence of dipropyl disulfide. Each curve is the mean of from 9 to 11 individuals.

Table 4. Lifetime fecundity parameters of D. antiqua exposed to artificial onion ovipositional cues varying in quality.

Treatment group	Days to first oviposition[1,2]	Total oviposition[3] (eggs/female)	Maximum Ovipositonal rate (eggs/day)	Percentage of eggs laid in ovipositional cups vs. other locations[4]
Sand	11.9 ± 6.1	337.0 ± 154.8	15.2 ± 2.1	20.8 ± 18.0 b
Clear surrogate	12.2 ± 4.5	519.7 ± 249.8	18.1 ± 2.9	77.3 ± 23.8 b
Clear + Pr_2S_2	8.2 ± 1.7	640.4 ± 291.9	20.3 ± 2.4	98.6 ± 2.1 a
Green surrogate	11.9 ± 4.0	355.2 ± 239.0	18.6 ± 3.0	95.8 ± 4.1 a
Green + Pr_2S_2	7.8 ± 1.4	633.4 ± 348.4	22.9 ± 2.8	99.1 ± 1.8 a
Significance level for:				
Color	ns	ns	ns	
Chemical	0.0005	0.03	0.007	
Interaction	ns	ns	ns	

[1] Values are means ± SD.

[2] Data transformed to log(x+1) to satisfy homogeneity of variances.

[3] Analysis of covariance, using reproductive life span as the covariate.

[4] Means followed by the same letter are not significantly different as determined by Kruskall-Wallis Test, experimentwise error rate = 0.25.

significantly decreased the age at first oviposition, and significantly increased total oviposition and maximum ovipositional rate. Flies laid significantly more eggs in places other than in ovipositional cups when neither host color nor host chemicals were present. Life span (grand $\bar{x}$ = 59.8 days) and reproductive life span (grand $\bar{x}$ = 42.4 days) were not significantly different among treatment groups.

For many individuals, a two-day cycle of ovipostion was observed. Not uncommonly, individuals alternated between laying 50-55 eggs one day and none the next (Figure 8). This periodicity was most evident for flies exposed to Pr_2S_2. The overall patterns of daily oviposition were slightly different for the five treatment groups; flies in the Pr_2S_2 groups showed a sharper, earlier peak in oviposition, while flies in the other groups exhibited a more gradual increase in daily oviposition (Figure 9). This difference was judged not significant, however ($P > 0.1$, Kolmogorov-Smirnov two-sample test).

Egg maturation. Flies exposed to green surrogates containing Pr_2S_2 showed a tendency toward slightly higher numbers of eggs matured in early reproductive life than flies exposed to sand alone, but this difference was not signigicant ($P = 0.17$, factorial ANOVA). Not surprisingly, flies in both treatment groups showed a significant increase in the number of eggs matured over time ($P < 0.0001$, Figure 10. When this experiment was repeated with onion seedlings vs. moist sand at an exposure period of 5 days, the proportional decrease in eggs matured by the sand treatment group (10%) was similar to that observed with surrogates vs. moist sand (7%).

Choice Test. Flies laid most eggs on green surrogates containing

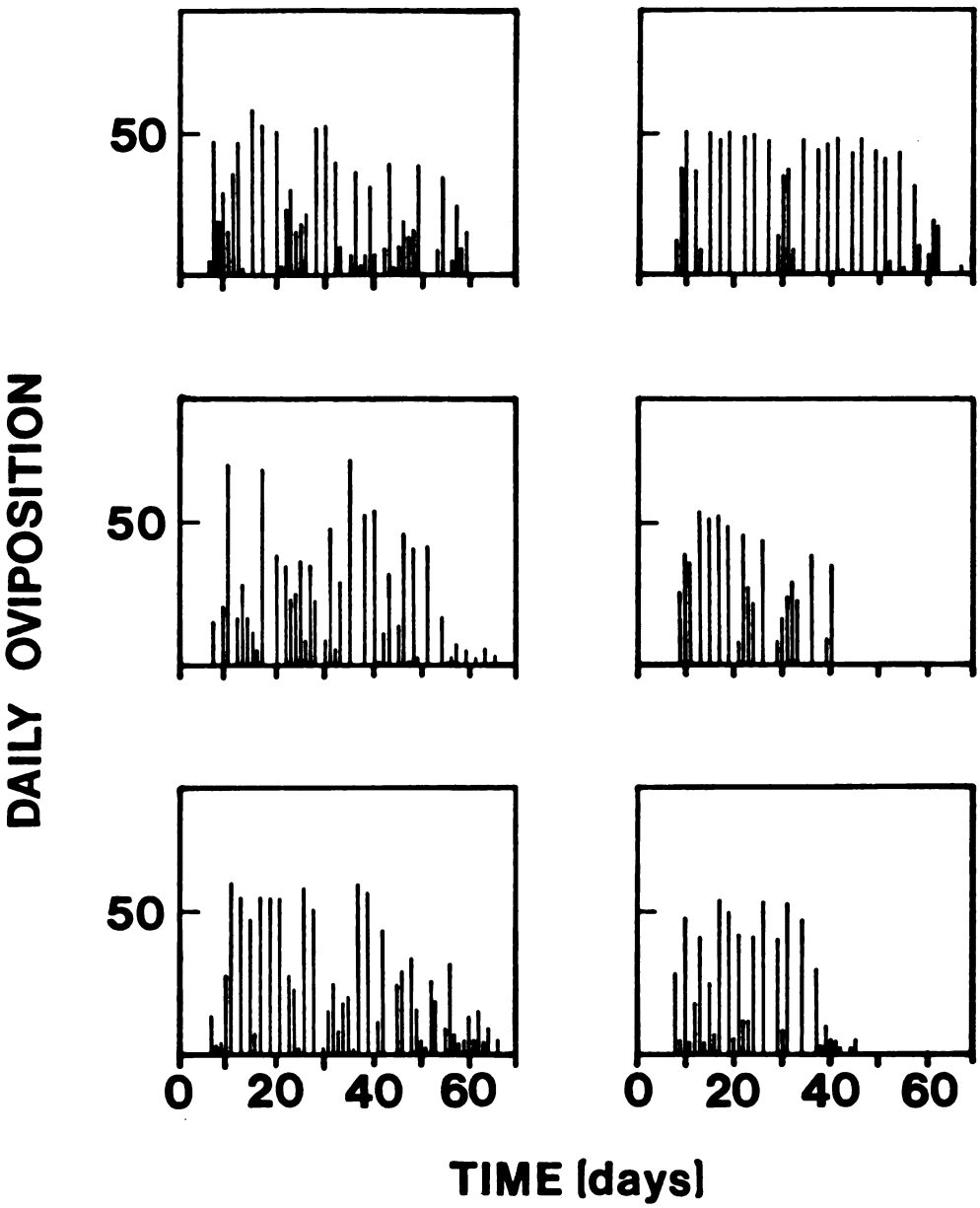

Figure 8. Representative lifetime ovipositional records from D. antiqua exposed to full complement of host stimuli (green surrogate plus dipropyl disulfide).

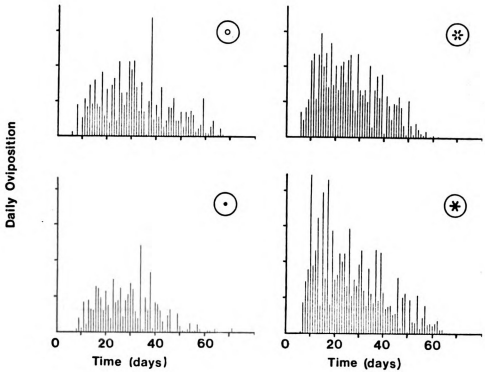

Figure 9. Summated daily ovipositional records for D. antiqua in treatment groups exposed to surrogates in the lifetime experiment. Symbols indicate treatments as in Figure 7.

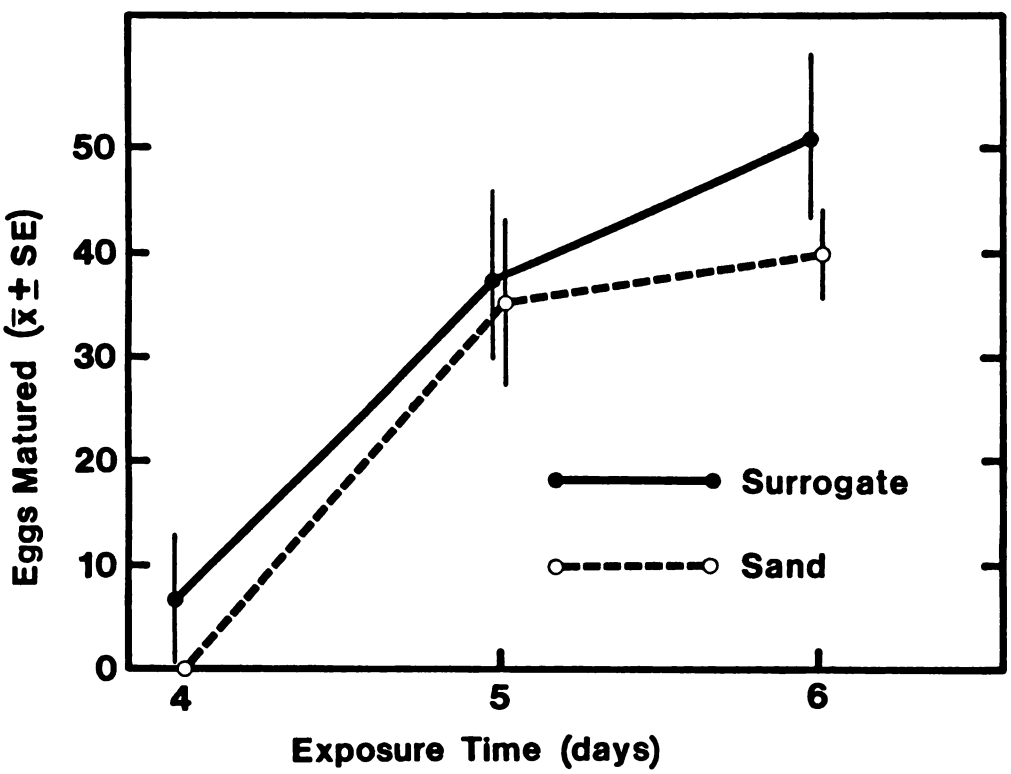

Figure 10. Numbers of eggs matured over time for D. antiqua exposed to optimal surrogate in sand vs. sand alone. Points at days 4 and 5 are the means of 10 individuals, while points at day 6 are the means of 20 individuals.

Pr_2S_2 (Figure 11). This treatment received more than twice as many eggs as the next most accepted treatment, clear surrogates plus Pr_2S_2. The remaining treatments received a trivial percentage of the eggs laid.

DISCUSSION

The results of the lifetime ovipositional experiment indicate that *D. antiqua* fecundity can be influenced by ovipositional resource quality. In particular, the absence of the ovipositional stimulant Pr_2S_2 can result in long-term decreases in rate of egg-laying and total oviposition. The decrease in egg-laying rate does not appear to be the result of decreased rates of egg maturation as a direct result of lack of exposure to host-plant ovipositional stimuli, as is the case for *D. platura* (Weston and Miller 1986). In *D. platura*, rates of oviposition and egg maturation were decreased in the absence of host plant stimuli; thus, reduced fecundity could be explained be reduced rates of egg maturation. Since egg maturation was not appreciably influenced by exposure to host stimuli in *D. antiqua*, we must conclude that differential oviposition in the lifetime experiment was the result of differences in the degree to which ovipositional behaviors were released in gravid females.

In spite of the differences in effect of host-plant stimuli on ovipositional biology of *D. antiqua* and *D. platura*, there are similarities in how these two species responded to host deprivation. When female *D. platura* with normally-developed reproductive systems were suddenly deprived of access to host stimuli, they exhibited reduced ovipositional rates for four days, and then resumed laying

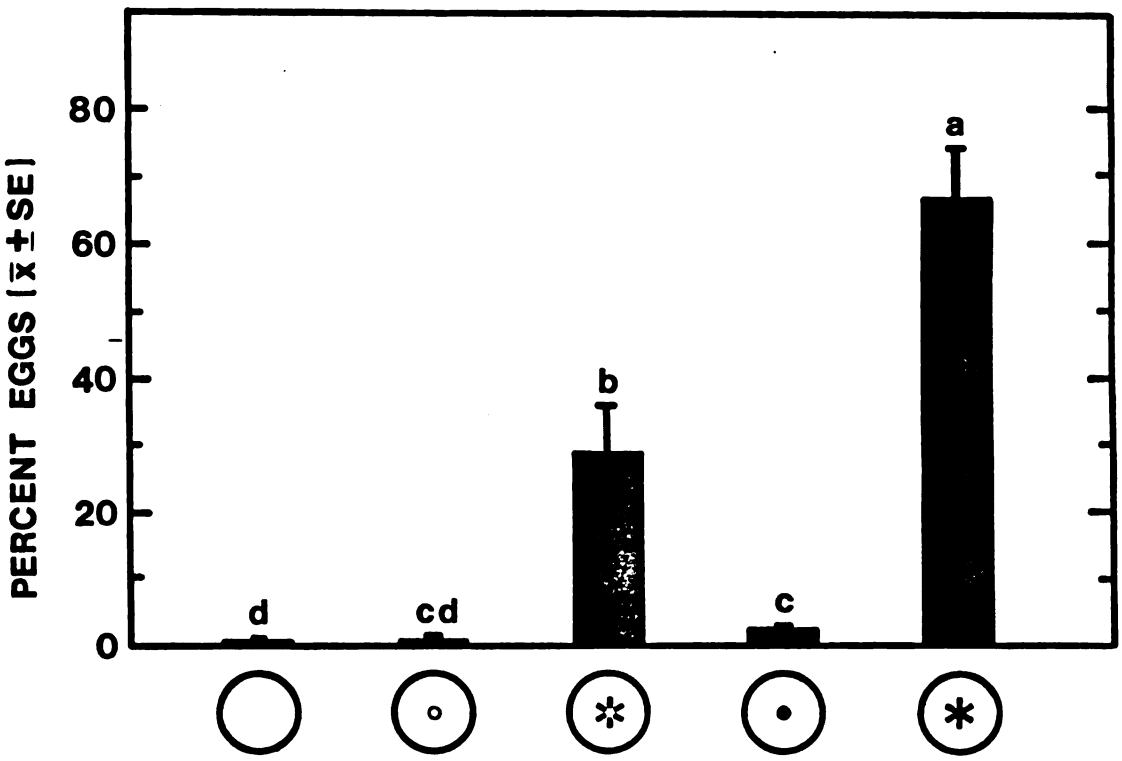

Figure 11. Oviposition by D. antiqua on surrogates varying in stimulus quality (choice test). Symbols refer to treatments as in Figure 7. Bars accompanied by the same letter are not statistically different as determined by lsd test applied to arcsin-transformed means (α = 0.01) following randomized complete block ANOVA.

eggs at rates equal to those of flies continuously exposed to host stimuli (Weston and Miller 1986). The duration of this discrimination phase was similar for *D. antiqua*, which delayed ovipositing by four days when deprived of Pr_2S_2 (Table 4). Both of these fly species have the ability to retain eggs to some extent in the absence of host stimuli, but this ability is limited. After four days, it seems that too many eggs have accumulated for the female to withold eggs any longer. In terms of the rolling fulcrum model, this accumulation of eggs pushes the fulcrum far enough that previously marginally acceptable resources become fully acceptable as ovipositional sites. Surprisingly, *D. radicum*, another anthomyiid herbivore fairly closely related to these two species, does not show any relaxation of host-plant discrimination ability under deprival conditions. When deprived of host plants, *D. radicum* females laid no eggs during their lifetimes (Nair and McEwen 1976). It is not clear whether this result was due to lack of priming or lack of ovipositional-behavior release by host stimuli.

Although surrogate color had no effect on *D. antiqua* lifetime fecundity, it did influence the placement of eggs by ovipositing females. This perhaps resulted because flies exposed to green surrogates may have a tendency to spend more time in the vicinity of ovipositional cups than flies exposed to no host-plant cues. Harris and Miller (1983) found that yellow wavelengths of light elicit alighting, stem walks, and ovipositor probing by *D. antiqua*. Although the 1983 experiments were conducted in choice tests, and flies were presented chopped onion in ovipositional cups, it seems likely that color stimuli alone would also help to localize ovipositional

behaviors in no-choice situations. From the results presented here, it appears that the primary stimulus eliciting D. antiqua oviposition in long-term, no-choice situations is host chemostimuli, with either host color or chemostimuli guiding egg placement.

It is interesting that the relative numbers of eggs laid on the five ovipositonal treatments in the lifetime (no-choice) experiment differ markedly from those in the choice experiment. In the choice test, the ratio of eggs laid on green and clear surrogates containing Pr_2S_2 was approximately 2:1 as opposed to essentially 1:1 in the no-choice situation. This disparity might be the result of flies in the choice situation being able to move to alternate surrogates after being stimulated to oviposit by Pr_2S_2. Since color plays a role in egg placement, flies stimulated to oviposit by Pr_2S_2 on a clear surrogate may not immediately lay an egg but rather move around until the appropriate combination of visual and chemical stimuli are present (i.e., on the green, Pr_2S_2-containing surrogate). In the no-choice test, flies do not have the option of moving to other surrogates, so perhaps, after being stimulated to oviposit by Pr_2S_2 on clear surrogates, they oviposit as readily as on green surrogates containing Pr_2S_2 since no better combination of host stimuli is available.

The cyclic pattern of oviposition observed in the lifetime experiment is similar to that reported by Vernon and Borden (1979). They found the mean maximum daily egg production to be 52 eggs, which coincides very well with our range of 50-55 eggs per day. The highest total egg production by a female in our experiment (1070 eggs) is considerably higher than the highest previously reported for D. antiqua (706 eggs, Allen and Askew 1970).

Contrary to expectation, the specialist D. antiqua appears to be

no more finicky upon host deprival than the closely related generalist, D. platura. Although both species exhibited roughly two-fold decreases in fecundity when deprived of host-plant stimuli, the mechanisms underlying these decreases in fecundity are quite different. Host-deprived D. platura exhibited reduced fecundity owing to a lack of priming of reproductive processes by host stimuli, while host-deprived D. antiqua exhibit reduced fecundity owing to a lack of release of ovipositional behaviors by host stimuli. It would be very interesting to determine if this pattern of host stimuli having a priming role in the reproductive processes of a generalist, but having a releaser role in the behavior of a specialist, is a common phenomenon among insect herbivores or is merely coincidental.

SECTION II

SIMULATION MODELLING OF INSECT MOVEMENT IN RESPONSE TO CHEMICAL STIMULI

INTRODUCTION TO SECTION II

The spatial displacement of animals has interested naturalists for centuries, but it is only within the past hundred years that serious attempts have been made to interpret the movements of animals without anthropomorphic bias. The pioneering work of Loeb, followed by the movement-behavior classification system of Kühn (1919), represented the beginnings of the modern era of the study of animal movement. Loeb (1918), borrowing from the work of botanists studying directed growth of plants, proposed his "tropism" theory of animal movement, which explained animal movements as the result of unequal muscle tension of symmetrical muscles as a consequence of unequal stimulation of sense organs by external stimuli. Although we now know this theory is far too simplistic to account for many movement responses, it was the first attempt to explain animal movements mechanistically. Kühn (1919), building upon the tropism theory, developed the first classification system of movement responses of animals to external stimuli. Kühn reserved the term "tropism" for the the growth responses of non-motile organisms, and advocated use of the term "taxis" for movement responses of motile organisms. Kühn was the first to distinguish between responses made with, and those without, respect to a stimulus gradient. Although terminology has been modified somewhat, this classification system still represents our basic conceptual framework for viewing movement behavior.

Terminology has always been of concern to scientists studying animal movement. Although terminology may intimidate those unfamiliar

with the study of animal behavior, it does play the vital role of facilitating communication. Further, jargon is an inescapable attribute of literature dealing with animal behavior. The commonly-used terminology system of movement behavior, developed by Fraenkel and Gunn (1940) as a modification and extension of Kühn's system, is fairly logical and is not difficult to learn. Essentially, each term for a movement response is a compound word, the root describing whether the movement is oriented with respect to a stimulus gradient (taxes, sing. taxis) or not (kineses, sing. kinesis), and the prefix describing some detail of the nature of the response. The prefixes are derived from Greek. Thus, orthokinesis refers to a response made without respect to the direction of a stimulus gradient (kinesis), and in which the animal moves in essentially a straight path (ortho: straight, direct). Thus, this term describes a response characterized, not by turning, but by a change in the rate of locomotion. Additional modifiers may be used to describe the direction of change; thus, positive orthokinesis describes a movement response characterized by an increase in the rate of movement in response to an increase in the intensity of a stimulus.

The most obvious way to study insect movement behavior is to record tracks of insects in defined arenas and subsequently to analyze the tracks for quantifiable changes in path characteristics (e.g. rates of locomotion or turning). If correlations are found between changes in path characteristics and changes in external stimuli, one can determine which movement mechanism is used by the insect in a particular situation. An alternative approach to understanding movement behavior is to reverse the situation; provide a "mover" with defined response mechanisms to external stimuli and

observe the resulting path characteristics in defined arenas. This allows one to determine the theoretical limits to response mechanisms -- not which mechanisms are possessed by real animals, but rather, the ability of inferred mechanisms to accomplish that which they are believed to do. The beauty of this latter approach is that precise control over the "experiment" is possible; the stimulus field can be precisely defined, and the behavioral capabilities of the mover are exactly known. An ideal way to conduct this type of study is via simulation modelling -- using computer programs that simulate the behavior of a system. Assumptions are made to limit the system to a workable size, and rules for responding to changes in factors external to the system (inputs) are provided. For the purposes of studying insect movement, the system under consideration is the hypothetical insect (mover): its sensory apparatus, its response rules to external inputs, and its locomotory abilities. The relevant output of this system is the resultant two-dimensional displacement of the mover. Alterations of response characteristics and external inputs provide the opportunity to investigate how insects or other organisms move adaptively in their environments.

CHAPTER 4

COMPUTER-SIMULATED MOVEMENT OF HYPOTHETICAL ORGANISMS RESPONDING KINETICALLY TO CHEMICAL STIMULI

INTRODUCTION

Spatial maneuvers made in response to external stimuli are fundamentally important in determining how moving organisms increase their proximity to valuable resources and increase their distance from unfavorable regions in their environments. Several different systems of classifying locomotory responses to stimuli have been proposed (Kuhn, 1919; Fraenkel and Gunn, 1961; van der Steen and ter Maat, 1976; Bell and Tobin, 1982), but they all acknowledge a distinction between direct and indirect movements. In direct movements, new directions are taken with a particular orientation to the stimulus gradient. Movement responses in this class of behaviors have been termed "taxes" (singular = taxis). In contrast, indirect movements are characterized by turns made with no particular orientation to the stimulus gradient. Indirect movements have been termed "kineses" (singular = kinesis). This class of movement responses includes not only modifications to turning frequency or severity (klinokineses) but also changes in the rate of movement (orthokinesis). Exactly how these indirect movements result in directional displacement of organisms is not clear. That organisms do use kinetic mechanisms to approach or avoid sources of chemical stimuli has been well documented (Berg and Brown, 1972; Havukkala, 1980; Bursell, 1984; among many others), but the elements essential for finding or avoidance have not been fully elucidated.

A major stumbling block hindering progress in elucidating the operation of kinetic and tactic response mechanisms is that more than

one mechanism may be operating at a given moment. Most research on guidance mechanisms has relied on analysis of tracks made by real moving organisms (Berg and Brown, 1972; Miller and Brokaw, 1970; Havukkala and Kennedy, 1984; White et al. 1984; among others). Hence, the investigator has little or no control over the variety of mechanisms that may be operational in a given organism at a given moment. No one working with real organisms has precisely determined how adjustments of single movement parameters affect the overall movement patterns of organisms, although van Houten (1978) has come closest by studying the spatial maneuvers made by Paramecium mutants deficient in one of several locomotory abilities. Clearly, greater control over the responses of test organisms to external stimuli would facilitate determining the essential components of kinetic response mechanisms under a variety of environmental conditions.

Computer simulation allows this problem to be addressed theoretically. Models can be designed to represent "organisms" that can be precisely controlled and observed in environments defined by the researcher. The effects of adjusting movement parameters, alone or in any combination, can be observed and quantified. Precision in these experiments is exceptional since: i) the experimenter has precise control over the external stimuli in the organisms' environment, ii) the behavior of the simulated organism is precisely known at all times, and iii) copious replications of an experiment can be generated by merely increasing the execution time.

Several researchers have reported on the use of simulation methods for investigating response mechanisms in real and hypothetical organisms. Green (1977) simulated the movement of nematodes in

response to simulated chemical gradients, as did van Houten and van Houten (1982) for Paramecium. In 1969, Rohlf and Davenport investigated the effects of changing several movement parameters on the distribution of hypothetical organisms moving under simple movement models. More recently, Bornbusch (1984) quantified the effect of varying the sizes of the foward and reverse turning fields on the finding efficiency of hypothetical organisms responding to chemostimulation with longitudinal klinotactic responses. Bovet (1983), using a fairly sophisticated movement model, explored the relationship between the standard deviation of turn angles and searching efficiency of hypothetical foragers. The effect of turn angle "concentration" and "move length" on the efficiency of host finding by simulated organisms has been analyzed by Cain (1985).

I present here the results of computer simulated experiments quantifying: i) the effect of movement parameters on dispersal, and ii) the effect of modifying various movement parameters, in response to chemical stimuli, on arrestment and finding by hypothetical organisms.

METHODS AND RESULTS

Description of the Model. Movement of a hypothetical organism was simulated with a computer program (Figure 12) written in Microsoft Advanced Basic.[1] The program was run on an IBM Personal Computer equipped with a graphics monitor. The path of the "organism" could be

[1]Author will consider requests for listing of the computer program by individuals who wish to conduct similar research. Author can be contacted at: 1725 Brook Park Dr., Lexington, KY 40502 (606)-271-1092.

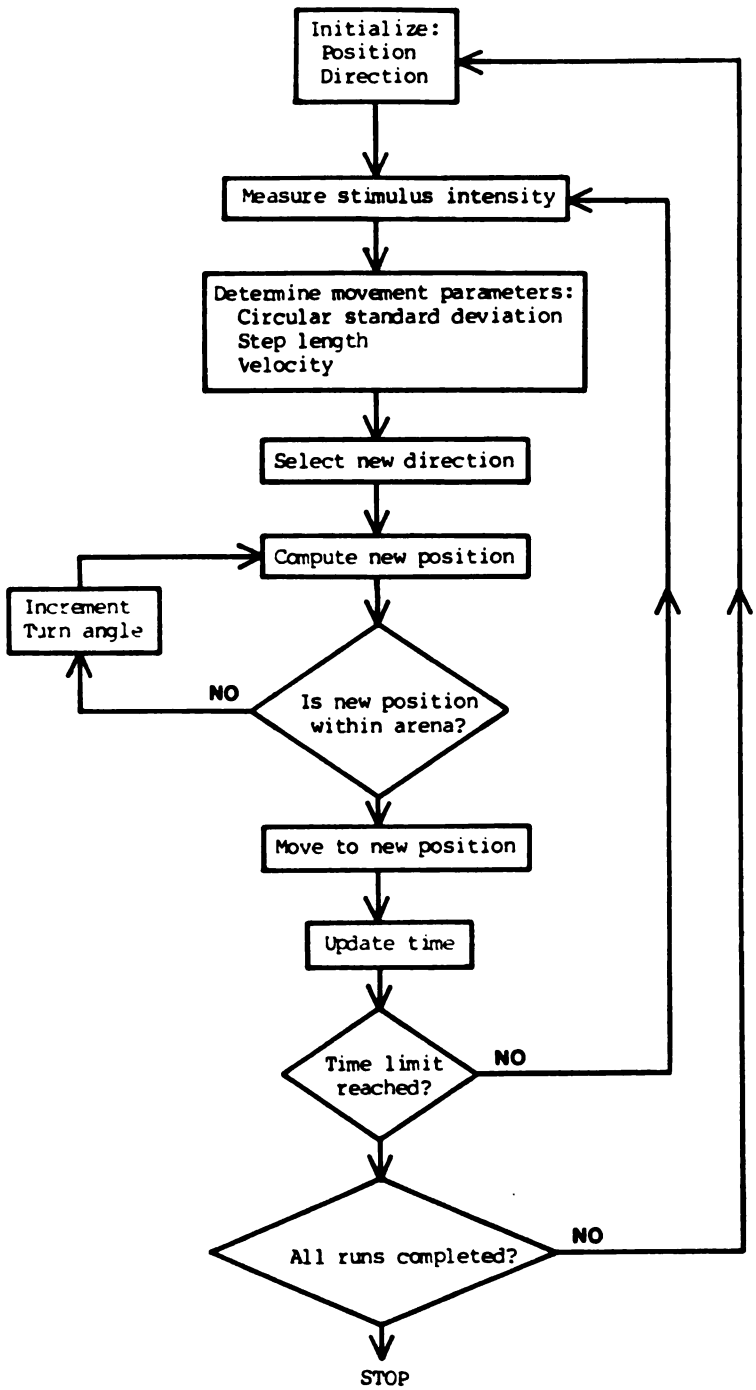

Figure 12. Flow chart of movement simulation program.

displayed on the monitor, when desired, by lines connecting the consecutive positions of the organism (mover). Hard copies of the tracks were made on a dot matrix printer using the GRAPHICS utility of MS-DOS version 2.0.

From its initial position, the organism was moved a distance of 2 units (the arbitrary default step size chosen for all the simulations) in a specified direction. All subsequent positions were determined by first selecting a new direction from a normal distribution of turn angles centered at the current direction and having a standard deviation of 8^{o}. The organism was then moved 2 units in the specified direction. The magintude of the step length was chosen to be 0.01 of the diameter of the arena used for the efficiency experiments (described further on). The time required to move each step was computed by dividing the step length by the velocity of the mover, and this quantity was added to the time already elapsed. The default velocity was chosen such that each step took 1 time unit to execute. A run was terminated when the elapsed time exceeded a predetermined limit.

The model described above was termed "restricted random" movement. While a truly random motion model would allow the organism to take steps in directions chosen from a uniform distribution from -180^{o} to 180^{o}, the directions of successive steps are generally much more highly correlated than this for moving organisms, particularly walkers. The restricted random model is random in the sense that turn angles are chosen at random from a specified distribution, and step size and the distribution of potential turn angles are not under the influence of external "stimuli." This type of motion falls under the category of "correlated walks", in which the options available to the

walker at each step depend in some fashion on the previous steps (Barber and Ninham, 1970). The values of 8^{o} for the circular standard deviation of turn angles and 2 units for the step size were chosen to yield movement that appeared to resemble that of walking insects. One would expect the choice of values for these parameters to markedly influence the movement and dispersal ability of an organism. Below I explore the effects of varying circular standard deviation and turning frequency on dispersal.

Effect of movement parameters on dispersal. Increasing the circular variance of turn angles or decreasing the step size (which is the same as increasing the turning frequency) of an organism moving with constant velocity both have the effect of increasing the rate of change of direction [r.c.d. in the terminology of Fraenkel and Gunn (1961), measured in degrees per unit time]. Therefore, increases in r.c.d. would be expected to decrease the maximum distance reached by a moving organism. This hypothesis was tested by setting the cybernetic organism loose in the middle of a boundless arena and letting it run under the restricted random model for 1000 time units. The maximum distance reached by the mover in the alloted time was recorded for each of 20 trials under various combinations of step size and circular variance.

As expected, the maximum distance achieved did decrease with increased turn severity (increased circular variance) and increased turning frequency (decreased step size) (Figure 13). This decline was precipitous for values of circular standard deviation (c.s.d.) up to 15^{o}, but beyond 45^{o} further increases in c.s.d. had relatively little effect on dispersal. This result can be understood by considering the

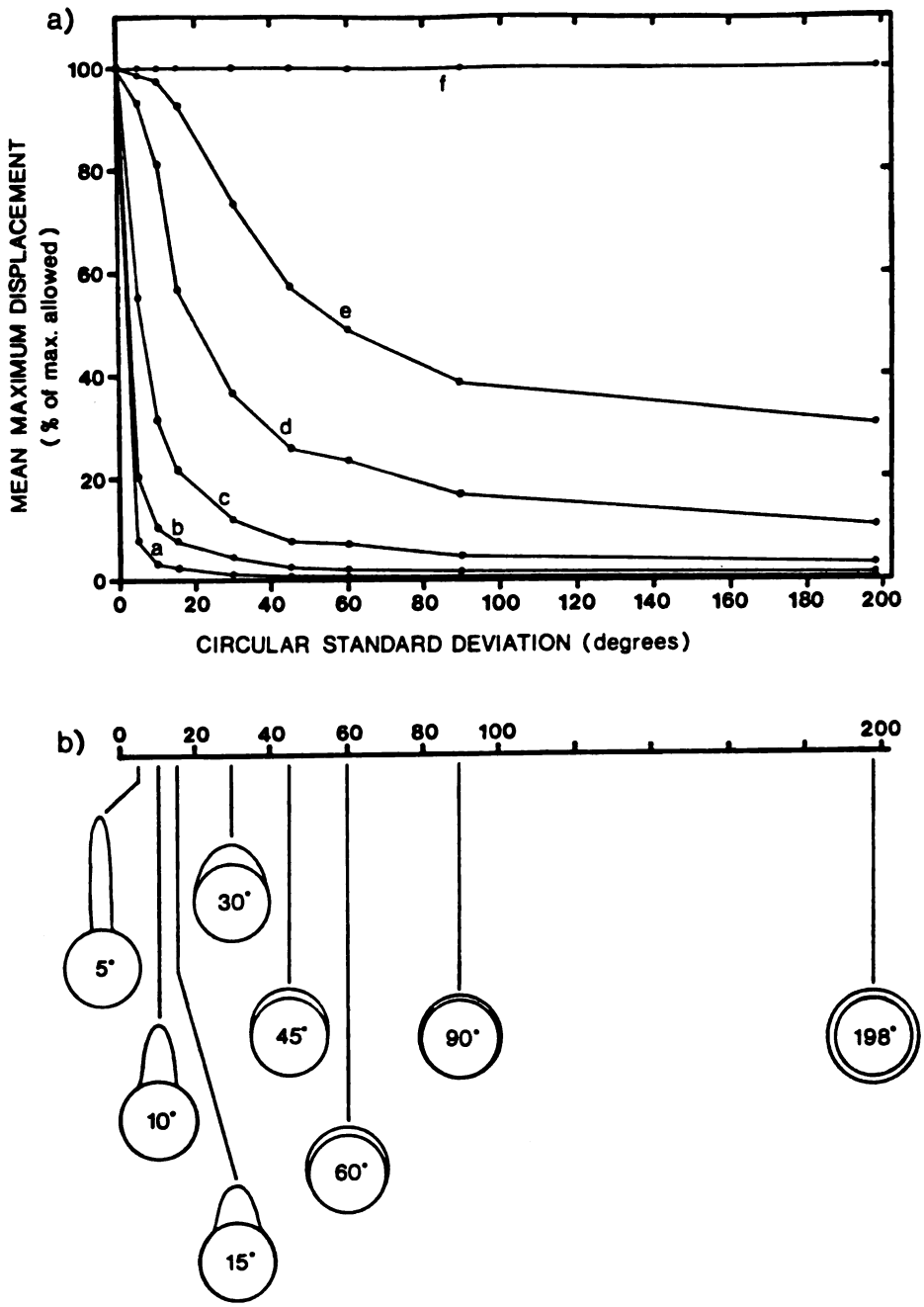

Figure 13. a) Relationship of maximum displacement of mover vs. circular standard deviation for various values of turning frequency. Line (a) = 10 turns per distance unit. Each line above represents a 10-fold increase in turning rate over the one below it. Line (f) = 0.0001 turns per distance unit (no turns made since maximum displacement allowed was 10,000 distance units).
b) Wrapped normal distributions of turn angles available to the mover under the various values of circular standard deviation used in the dispersal experiment.

shapes of the turn angle distributions (Figure 13b); for values of c.s.d. of 45^{o} or less, no more than 5% of the turns available at any step lie to the rear of the mover. As the c.s.d. increases beyond 45^{o}, however, an increasing proportion of possible turns may be selected from behind the mover. This increased turning potential apparently resulted in increased backtracking and, thus, a decreased tendency to make continued progress in any one direction. For the largest values of c.s.d. the portions of the distribution curves beyond $\pm 180^{o}$ were added to those segments from the opposite direction, thus forming wrapped normal distributions (Batschelet, 1965). For the distribution with a c.s.d. of 198^{o}, the resulting wrapped normal distribution formed a uniform circular distribution, meaning that new directions from all compass points were equally likely (Figure 13b). Of course, movers selecting turn angles from this broad distribution showed the greatest tendency to remain near the starting point.

The decrease in dispersion with increasing turning frequency can be understood by considering the stochastic nature of the direction selection algorithm. With an increase in the number of possible turns in a given dispersal distance, the maximum displacement must decline owing to the fact that each step taken increases the probability that deviations from straight line movement will occur. Naturally, the maximum displacement would be achieved if all turn angles were equal to zero (straight-line movement). The differences in maximum displacement achieved under various values of turning frequency are enhanced as the distribution of potential turn angles becomes narrower, judging from the shape of the displacement vs. c.s.d. curves (Figure 13).

What would happen if a moving organism changed its movement

parameters in response to changes in chemostimuli in its environment? One can speculate on what movement patterns might result, but it is difficult to conduct this sort of "armchair" experiment without bias. To obtain unbiased estimates of how movement patterns will change under various alterations in movement parameters, one can providethe computerized "organism" with rules for responding to external stimuli and measure the "performance" of the organism in various environments. The next section describes the experiments used to measure exactly what happens to movement by organisms utilizing various mechanisms in response to changes in external stimuli.

Comparison of Various Kinetic Response Mechanisms. The central questions addressed here are: i) how is an organism's effectiveness in increasing time spent in favorable areas of an environment and decreasing time spent in unfavorable areas influenced by exercising indirect (kinetic) controls such as modifying circular variance of turn angles, turning frequency, and velocity? and ii) how is a mover's effectiveness in finding a point source of stimulation (e.g. food or a mate) influenced by exercising these kinetic controls? I use "find" here in the sense of Miller and Strickler (1984): "to behave so as to establish and maintain proximity with something, sensed by the finder's nervous system, that was previously apart and of undetermined location." The two problems presented (i and ii) are fundamentally different since the first involves remaining in or avoiding a particular location once the organism encounters it, and the second involves indirect mechanisms that promote movement of the organism from a region of low stimulus intensity to region of higher intensity. The first problem is one of arrestment and the second, target-finding.

The models used to measure the effects of kinetic responses to chemical stimuli in the arrestment and target-finding problems were modifications of the restricted random movement model described earlier. Movement was now restricted to a circular arena 200 units in diameter. If a new position lay outside the arena, the organism was not moved there but instead the turn angle was incremented or decremented (at random) by 90^{o} until the new position was within the arena boundary. Movement not under the influence of chemical stimulation utilized the default movement parameter values of 8^{o} for circular standard deviation and 2 distance units for step size.

In response to changes in stimulus intensity, three movement parameters were varied, alone and in combination: circular variance of turn angles, step size, and velocity. Each of these parameters could vary directly, inversely, or not at all in response to changes in stimulus intensity. For direct variation, each parameter was allowed to increase linearly with stimulus intensity, reaching maximum values of 90^{o} circular standard deviation, 4 distance units step length, and 4 distance units/time unit velocity. Inverse responses were also linear, with circular standard deviation decreasing to a minimum of 1^{o}, step length to 1 distance unit, and velocity to 1 distance unit per time unit. The range of velocity values was selected after scanning the literature for data of real organism movement, while the range of step lengths was chosen by intuition. A limited sensitivity analysis of step lengths was conducted to determine how influential the lower limit of this parameter was on responses that decreased step length. In addition to these monotonic changes in parameter values, circular standard deviation of turn angles could change according to another mechanism, wherein its value was decreased

to 1^{o} if stimulus intensity increased, and increased sharply (to 90^{o}) if the intensity decreased. This mechanism will be referred to as the "-|,++|" state of circular variance (read "decrease c.s.d. with increasing stimulus strength, sharply increase c.s.d with decreasing stimulus strength"). A similar program for step size was also employed, wherein step length was decreased abruptly to 0.5 unit when a lower stimulus intensity was encountered. This is equivalent to increasing turning frequency sharply with decreasing stimulus intensity, and thus is referred to as the "++|" state for turning frequency. Additionally, turning frequency was allowed to increase gradually with increasing stimulus intensity following the linear algorithm presented earlier, and to increase abruptly by decreasing step length to 0.5 unit with decreasing stimulus intensity (the "+|,++|" state of turning frequency). These last two algorithms are similar to the "temporal comparison and modulation of turning frequency" model of Bell and Tobin (1982), said to be employed by bacteria, with the exception that my implementations do not permit the turning frequency to decrease (i.e. step size to increase) with increasing stimulus intensity.

In addition to measuring the influence of these various parameters on "performance" in the two movement problems, the effect of sensory adaptation on performance criteria was also measured. Sensory adaptation can be defined as a decrease in the responsiveness of sensory neurons after extended exposure to a stimulus (Barlow and Mollon, 1982). I simulated sensory adaptation by causing receptor sensitivity to decay exponentially with the integrated exposure to the stimulus. Stimulus exposure was reset to zero whenever the organism

left the active space of the stimulus. The decay constant chosen caused receptor sensitivity to attenuate by one half after the organism was exposed to the amount of stimulus that would be perceived after five steps were taken in the maximum stimulus intensity region. The value for the decay constant was based on estimated values of sensory adaptation for Dendroceolum taken from Ullyott (1935) and Stasko and Sullivan (1971).

Within the bounds shown in Table 5, each parameter was allowed to vary with all possible values of the others, yielding 120 distinct models. The models will be referred to by four digit numbers, one for the state of each movement parameter and one for the state of sensory adaptation (whether on or off). For example, model 1201 would increase circular variance and decrease turning frequency with stimulus intensity, velocity would remain unchanged, and sensory adaptation would be operational. In the discussion of results, x's in the model designations mean that the value of that particular parameter made no difference to the result under discussion.

A. The arrestment problem. Chemostimulation in the arrestment problem was simulated by providing a constant, positive concentration in one portion of the arena and a concentration of zero in the other (Figure 14a). Between these two regions was a linear gradient distributed across a band 40 units wide straddling the midline of the arena. Performance of the various models was judged by comparing the percentages of time spent in the stimulus half of the arena with that for the restricted random model. Since the stimulus occupied not only the stimulus half of the arena but also the region between the midline and the lower limit of the stimulus gradient, the amount of time expected in the stimulus region for a randomly moving control is not

Table 5. Possible changes in state of movement parameters in response to increased simulated chemostimulation.

Circular Variance		Turning Frequency		Velocity		Adaptation	
0)	0	0)	0	0)	0	0)	Off
1)	+	1)	+	1)	+	1)	On
2)	-	2)	-	2)	-		
3)	-↑,++↓	3)	++↓				
		4)	-↑,++↓				

0 = no change, + = increase, - = decrease in given parameter
↑ = increase in stimulus intensity
↓ = decrease in stimulus intensity

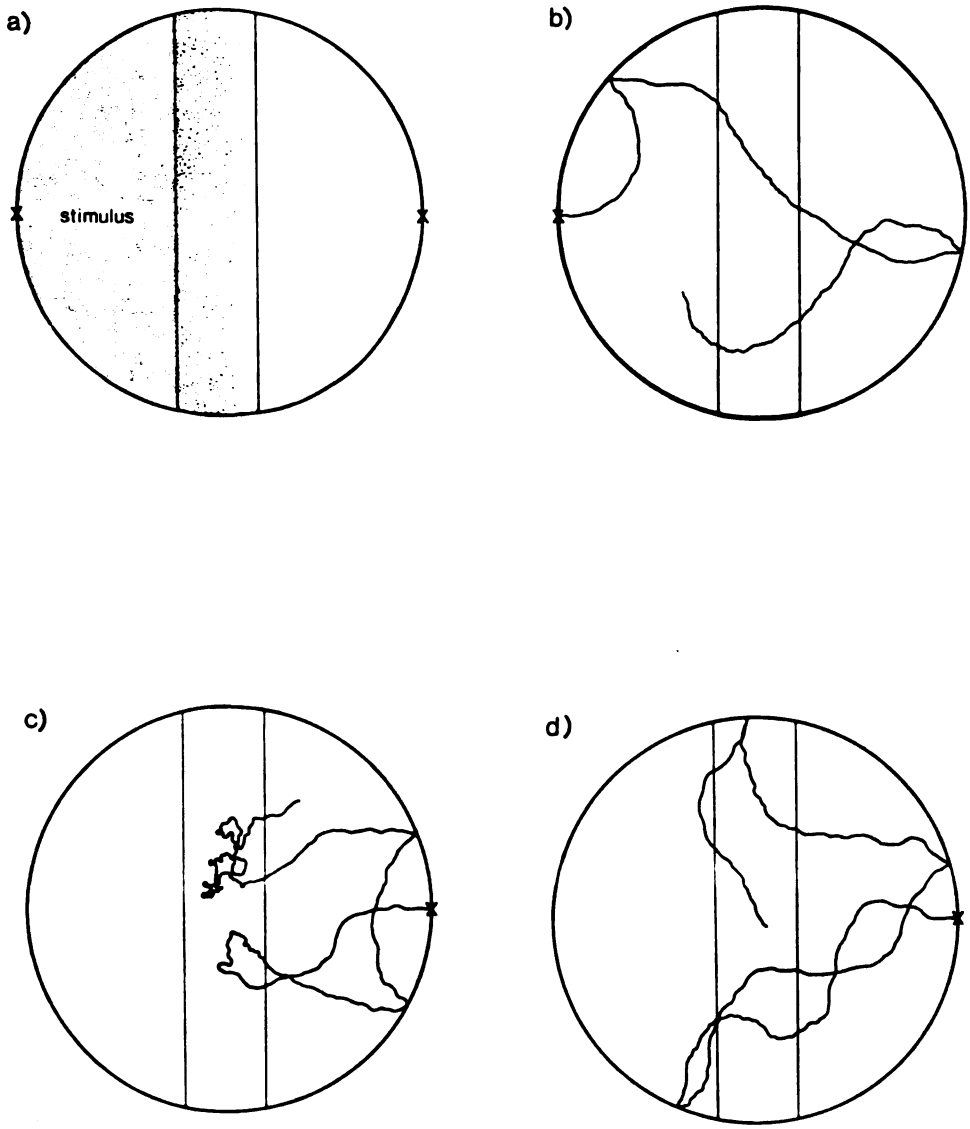

Figure 14. a) Arena for the arrestment problem.
b-c) Tracks made by mover using various response mechanisms in the arrestment problem. Time limit for each track not necessarily equal. "X" denotes starting point. b) Restricted random movement (model 0000). c) Increased circular variance with stimulus intensity (model 1000). d) Increased turning frequency with stimulus intensity (model 0100).

one-half, but 0.628 instead. Each model was allowed to run for 1000 time units, and was replicated 40 times. Performance was influenced, sometimes drastically, by the location of the starting point; therefore, half of the releases were from the stimulus side, and half from the no-stimulus side. The "organisms" were released from the points marked "X" along the arena wall in Figure 14a. In each case, the initial heading was toward the opposite half of the arena. To increase accuracy of the performance estimate for the randomly moving control, this model was run 120 times from both release sites.

A(1). Release site: stimulus half. The restricted random model (0000) spent almost exactly the percentage of time in the stimulus region as expected from the percentage of area occupied by stimulus (63.6% compared to the theoretical value of 62.8%, Table 6). Figure 14b shows the initial segment of a track made by a mover operating under the restricted random model.

The most noteworthy models in this problem were those that allowed circular variance to decrease with stimulus intensity and to increase sharply with decreasing stimulus intensity (models 3xxx). All models in this group remained in the stimulus region 100% of the time. The only other models with such high performances were those increasing circular variance with stimulus intensity and allowing turning frequency to increase sharply with decreasing stimulus intensity (13x0, 14x0), and the model showing maximum angular velocity and reduced linear velocity in the stimulus region (1120). When the lower limit for step size modification was increased to 1 unit from 0.5 unit, the only models in the 13x0 and 14x0 groups to spend 100% of their time in the stimulus region were those that reduced linear

Table 6. Performance of kinetic models in the arrestment problem. Release site: stimulus half of arena.

C T V A	P%	C T V A	P%	C T V A	P%	C T V A	P%
0 0 0 0	63.62	1 0 0 0	94.69	2 0 0 0	66.23	3 0 0 0	100.00
0 0 0 1	66.09	1 0 0 1	52.58	2 0 0 1	65.29	3 0 0 1	100.00
0 1 0 0	59.98	1 1 0 0	99.72	2 1 0 0	64.03	3 1 0 0	100.00
0 1 0 1	62.18	1 1 0 1	46.26	2 1 0 1	61.49	3 1 0 1	100.00
0 2 0 0	58.95	1 2 0 0	79.66	2 2 0 0	59.10	3 2 0 0	100.00
0 2 0 1	65.92	1 2 0 1	50 65	2 2 0 1	60.10	3 2 0 1	100.00
0 3 0 0	74.31	1 3 0 0	100.00	2 3 0 0	66.01	3 3 0 0	100.00
0 3 0 1	76.83	1 3 0 1	76.23	2 3 0 1	70.80	3 3 0 1	100.00
0 4 0 0	75.58	1 4 0 0	100.00	2 4 0 0	65.28	3 4 0 0	100.00
0 4 0 1	79.91	1 4 0 1	75.34	2 4 0 1	75.01	3 4 0 1	100.00
0 0 1 0	50.27	1 0 1 0	75.52	2 0 1 0	48.59	3 0 1 0	100.00
0 0 1 1	58.01	1 0 1 1	47.71	2 0 1 1	59.59	3 0 1 1	100.00
0 1 1 0	54.16	1 1 1 0	94.73	2 1 1 0	46.33	3 1 1 0	100.00
0 1 1 1	61.79	1 1 1 1	51.44	2 1 1 1	59.07	3 1 1 1	100.00
0 2 1 0	45.98	1 2 1 0	64.04	2 2 1 0	43.50	3 2 1 0	100.00
0 2 1 1	57.58	1 2 1 1	45.49	2 2 1 1	59.78	3 2 1 1	100.00
0 3 1 0	62.49	1 3 1 0	100.00	2 3 1 0	56.00	3 3 1 0	100.00
0 3 1 1	70.90	1 3 1 1	75.39	2 3 1 1	69.81	3 3 1 1	100.00
0 4 1 0	63.45	1 4 1 0	100.00	2 4 1 0	55.72	3 4 1 0	100.00
0 4 1 1	73.96	1 4 1 1	74.54	2 4 1 1	71.17	3 4 1 1	100.00
0 0 2 0	75.59	1 0 2 0	96.90	2 0 2 0	73.76	3 0 2 0	100.00
0 0 2 1	69.37	1 0 2 1	49.40	2 0 2 1	62.95	3 0 2 1	100.00
0 1 2 0	78.79	1 1 2 0	100.00	2 1 2 0	74.97	3 1 2 0	100.00
0 1 2 1	62.62	1 1 2 1	53.15	2 1 2 1	64.65	3 1 2 1	100.00
0 2 2 0	75.14	1 2 2 0	90.55	2 2 2 0	66.03	3 2 2 0	100.00
0 2 2 1	64.31	1 2 2 1	56.52	2 2 2 1	63.42	3 2 2 1	100.00
0 3 2 0	85.01	1 3 2 0	100.00	2 3 2 0	76.31	3 3 2 0	100.00
0 3 2 1	72.62	1 3 2 1	71.06	2 3 2 1	71.77	3 3 2 1	100.00
0 4 2 0	79.97	1 4 2 0	100.00	2 4 2 0	80.93	3 4 2 0	100.00
0 4 2 1	79.51	1 4 2 1	75.67	2 4 2 1	75.40	3 4 2 1	100.00

C = circular variance, T = turning frequency, V = velocity, A = adaptation, P% = performance (percent of time spent in stimulus half of arena). Numbers for each parameter refer to the state of that parameter (see Table 5 for details).

velocity in the stimulus region (1320 and 1420, data not shown). The remaining models showing increased arrestment relative to the restricted random model fell into three groups: 1) fourteen of the fifteen models allowing velocity to decrease with stimulus intensity (xx20), 2) fourteen of the fifteen models allowing circular variance to increase with stimulus intensity (1xx0), and 3) twenty-seven of the thirty-six models allowing turning frequency to increase sharply with decreasing stimulus intensity (x3xx and x4xx).

Under several models, the mover spent less time in the stimulus region relative to the control. Eight of these models (10x1, 11x1, 1201, and 1211) employed increased circular variance with stimulus intensity and sensory adaptation, while the remaining six (0x10, 2x10, where x = 0, 1, or 2) allowed velocity to increase in the stimulus region and employed no sensory adaptation. Sensory adaptation generally reversed the effect of movement parameter modifications on arrestment; sensory adaptation generally reduced arrestment in those models showing greater arrestment than the control, while it increased arrestment in those models showing reduced arrestment relative to the control.

A(ii). Release site: no-stimulus half. Naturally, the performances of movers when released from the no-stimulus half of the arena were generally lower than when released from the stimulus half (Table 7). The models showing the greatest arrestment were again those employing the -|, ++| algorithm for circular variance (3xxx). Sixteen of the remaining twenty-two models displaying increased arrestment utilized decreased velocity with increased stimulus intensity (xx20) and/or sharply increased turning frequency with decreased stimulus intensity coupled with increased circular variance

Table 7. Performance of kinetic models in the arrestment problem. Release site: no-stimulus half of arena.

C T V A	P%	C T V A	P%	C T V A	P%	C T V A	P%
0 0 0 0	60.56	1 0 0 0	45.54	2 0 0 0	60.45	3 0 0 0	92.76
0 0 0 1	65.40	1 0 0 1	35.30	2 0 0 1	58.61	3 0 0 1	93.49
0 1 0 0	58.84	1 1 0 0	44.87	2 1 0 0	59.32	3 1 0 0	93.74
0 1 0 1	59.17	1 1 0 1	39.04	2 1 0 1	58.92	3 1 0 1	94.49
0 2 0 0	58.02	1 2 0 0	47.30	2 2 0 0	64.74	3 2 0 0	93.77
0 2 0 1	63.26	1 2 0 1	43.14	2 2 0 1	59.25	3 2 0 1	94.26
0 3 0 0	68.56	1 3 0 0	88.57	2 3 0 0	67.63	3 3 0 0	94.09
0 3 0 1	68.66	1 3 0 1	63.42	2 3 0 1	66.32	3 3 0 1	94.05
0 4 0 0	65.97	1 4 0 0	86.50	2 4 0 0	70.79	3 4 0 0	92.92
0 4 0 1	72.71	1 4 0 1	57.96	2 4 0 1	68.44	3 4 0 1	91.34
0 0 1 0	47.74	1 0 1 0	35.82	2 0 1 0	49.07	3 0 1 0	92.76
0 0 1 1	56.15	1 0 1 1	29.95	2 0 1 1	59.31	3 0 1 1	93.14
0 1 1 0	46.73	1 1 1 0	32.36	2 1 1 0	48.13	3 1 1 0	95.28
0 1 1 1	53.36	1 1 1 1	30.55	2 1 1 1	59.86	3 1 1 1	92.74
0 2 1 0	47.51	1 2 1 0	38.39	2 2 1 0	47.60	3 2 1 0	94.92
0 2 1 1	61.43	1 2 1 1	35.89	2 2 1 1	60.44	3 2 1 1	94.27
0 3 1 0	59.62	1 3 1 0	87.01	2 3 1 0	53.30	3 3 1 0	93.79
0 3 1 1	70.42	1 3 1 1	65.13	2 3 1 1	64.73	3 3 1 1	94.24
0 4 1 0	57.42	1 4 1 0	81.92	2 4 1 0	53.60	3 4 1 0	91.97
0 4 1 1	70.87	1 4 1 1	64.73	2 4 1 1	64.67	3 4 1 1	92.64
0 0 2 0	70.65	1 0 2 0	59.03	2 0 2 0	74.76	3 0 2 0	93.83
0 0 2 1	64.01	1 0 2 1	43.35	2 0 2 1	63.42	3 0 2 1	90.65
0 1 2 0	76.04	1 1 2 0	49.01	2 1 2 0	74.50	3 1 2 0	94.11
0 1 2 1	64.85	1 1 2 1	39.34	2 1 2 1	60.69	3 1 2 1	92.73
0 2 2 0	73.09	1 2 2 0	61.94	2 2 2 0	70.81	3 2 2 0	93.58
0 2 2 1	60.67	1 2 2 1	41.35	2 2 2 1	61.10	3 2 2 1	93.24
0 3 2 0	79.93	1 3 2 0	87.85	2 3 2 0	76.90	3 3 2 0	92.64
0 3 2 1	70.87	1 3 2 1	59.47	2 3 2 1	69.27	3 3 2 1	91.58
0 4 2 0	79.04	1 4 2 0	83.91	2 4 2 0	76.39	3 4 2 0	91.91
0 4 2 1	72.35	1 4 2 1	61.73	2 4 2 1	67.35	3 4 2 1	93.99

Column headings same as in Table 6.

with stimulus intensity (13x0 and 14x0). Interestingly, five models incorporating sensory adaptation showed modest increases in arrestment (models 0401, 0311, 0411, 0321, and 0421). These models all relied on abrupt increases in turning frequency with decreasing stimulus intensity. When the lower limit for step size was restricted to 1 unit rather than 0.5 unit, this modification of turning frequency no longer resulted in appreciable arrestment (data not shown).

Unlike the situation when the organisms were released from the stimulus half of the arena, increases in circular variance with stimulus intensity resulted in arrestment. In fact, many of the models which increased circular variance with stimulus intensity and were arrested when released from the stimulus side of the arena now showed decreased arrestment relative to the control. Figure 14c illustrates how increasing circular variance with stimulus intensity decreases the probability that the mover will penetrate the stimulus gradient into the region of high stimulus intensity. Essentially, the closer the mover gets to the high end of the stimulus gradient, the more likely it is to make turns that will turn it toward the low stimulus end.

Most of the models showing decreased arrestment were those that increased circular variance with stimulus intensity (1xxx) and/or those that increased velocity in the stimulus region (xx10). Increased circular variance did, however, enhance the degree of arrestment of those models that increased turning frequency with increasing stimulus intensity (13x0, 14x0) relative to when circular variance was not changed (03x0, 04x0).

Decreased circular variance with stimulus intensity had essentially no effect on arrestment. Variability in arrestment by

models decreasing circular variance and not employing sensory adaptation (2xx0) could be accounted for almost entirely by modifications to linear velocity.

Finally, simple changes in turning frequency had almost no effect on arrestment. The ineffectiveness of increasing turning frequency on displacement can be seen by comparing the track in Figure 14d (model 0100) with the restricted random control (Figure 14b).

B. The target-finding problem. For the target-finding problem, the source of chemostimulation was a circle of radius 5 units in the center of the previously-described arena. A circular stimulus gradient radiated from this circle, with the stimulus intensity decreasing linearly with distance from the "source." The stimulus intensity reached zero beyond 40 units from the center of the source. A linear gradient was used since mathematical models predict that stimulus intensity decreases linearly with distance from a source (Okubo, 1980), even in confined areas (Mankin et al. 1980). The area within this 40-unit radius circle can be considered the active space of the stimulus, outside which the organism could not detect the stimulus. Within the active space, the mover was given as great an ability to detect differences in stimulus intensity as the precision of the computer (10 - 100 ppb). The performance criterion used was the number of "target hits" (contact with the 5 unit radius stimulus source) in twenty trials after being released from the wall of the arena. As in the arrestment problem, the restricted-randomly moving control was run for 120 times to increase the accuracy of the performance estimate.

The restricted random model had 40% success in finding the target. A portion of track made by a "random" mover is shown in Figure 15a. As with the arrestment problem, the best performers were those

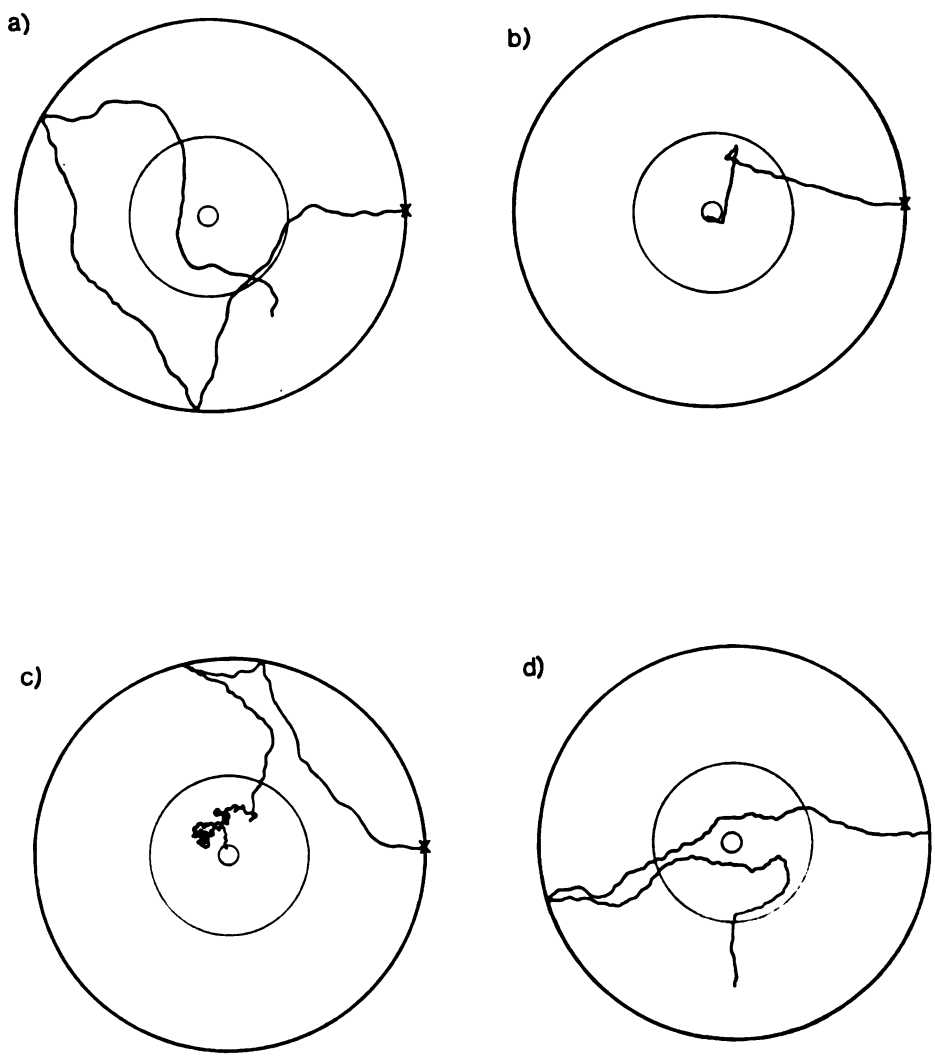

Figure 15. Tracks made by mover using various response mechansisms in the target-finding problem. Time limit for each track not necessarily equal. "X" denotes starting point. Small circle in center is the target (source), next larger circle is the boundary of the active space.
a) Restricted random movement (model 0000).
b) Decreased circular variance with increasing stimulus intensity and sharply increased circular variance with decreasing stimulus intensity (model 3000).
c) Increased circular variance with stimulus intensity and sharply increased turning frequency with decreasing stimulus intensity (model 1300).
d) Sharply increased turning frequency with decreasing stimulus intensity (model 0300).

Table 8. Percent of encounters with stimulus source in the target-finding problem.

C	T	V	A	P%	C	T	V	A	P%	C	T	V	A	P%	C	T	V	A	P%
0	0	0	0	40	1	0	0	0	30	2	0	0	0	30	3	0	0	0	100
0	0	0	1	50	1	0	0	1	20	2	0	0	1	55	3	0	0	1	100
0	1	0	0	20	1	1	0	0	20	2	1	0	0	40	3	1	0	0	100
0	1	0	1	40	1	1	0	1	15	2	1	0	1	50	3	1	0	1	100
0	2	0	0	30	1	2	0	0	25	2	2	0	0	35	3	2	0	0	100
0	2	0	1	50	1	2	0	1	35	2	2	0	1	55	3	2	0	1	100
0	3	0	0	35	1	3	0	0	95	2	3	0	0	50	3	3	0	0	100
0	3	0	1	50	1	3	0	1	50	2	3	0	1	60	3	3	0	1	100
0	4	0	0	45	1	4	0	0	80	2	4	0	0	55	3	4	0	0	100
0	4	0	1	50	1	4	0	1	60	2	4	0	1	55	3	4	0	1	100
0	0	1	0	55	1	0	1	0	30	2	0	1	0	25	3	0	1	0	100
0	0	1	1	25	1	0	1	1	25	2	0	1	1	65	3	0	1	1	100
0	1	1	0	50	1	1	1	0	25	2	1	1	0	70	3	1	1	0	100
0	1	1	1	25	1	1	1	1	20	2	1	1	1	55	3	1	1	1	100
0	2	1	0	50	1	2	1	0	35	2	2	1	0	40	3	2	1	0	95
0	2	1	1	55	1	2	1	1	35	2	2	1	1	50	3	2	1	1	100
0	3	1	0	25	1	3	1	0	95	2	3	1	0	35	3	3	1	0	100
0	3	1	1	50	1	3	1	1	70	2	3	1	1	45	3	3	1	1	100
0	4	1	0	35	1	4	1	0	90	2	4	1	0	40	3	4	1	0	100
0	4	1	1	75	1	4	1	1	85	2	4	1	1	65	3	4	1	1	100
0	0	2	0	50	1	0	2	0	30	2	0	2	0	30	3	0	2	0	100
0	0	2	1	70	1	0	2	1	30	2	0	2	1	35	3	0	2	1	100
0	1	2	0	40	1	1	2	0	15	2	1	2	0	45	3	1	2	0	100
0	1	2	1	45	1	1	2	1	20	2	1	2	1	70	3	1	2	1	100
0	2	2	0	45	1	2	2	0	35	2	2	2	0	30	3	2	2	0	100
0	2	2	1	40	1	2	2	1	30	2	2	2	1	45	3	2	2	1	100
0	3	2	0	55	1	3	2	0	80	2	3	2	0	30	3	3	2	0	100
0	3	2	1	50	1	3	2	1	60	2	3	2	1	50	3	3	2	1	100
0	4	2	0	50	1	4	2	0	70	2	4	2	0	35	3	4	2	0	100
0	4	2	1	60	1	4	2	1	60	2	4	2	1	50	3	4	2	1	100

Column headings same as in Tables 6 and 7.

going straight with increasing stimulus intensity and employing increased circular variance as stimulus intensity decreased (models 3xxx) (Table 8, Figure 15b). All but one of these models were 100% successful in finding the target. Eight of the remaining twelve models showing increased arrestment allowed circular variance to increase with stimulus intensity and allowed turning frequency to increase abruptly with decreasing stimulus intensity (models 13x0, 14x0, 1311, and 1411). Figure 15c is a track of a mover employing model 1300. The importance of increasing turn severity with stimulus intensity in this case can be seen by comparing this track with that in Figure 15d, which was made by a mover that could only increase turning frequency with decreasing stimulus intensity (model 0300). The remaining four models scoring higher than the control showed no readily identifiable pattern to account for their increased performance, but three of them (0411, 0021, and 2121) utilized sensory adaptation.

DISCUSSION

These simulations have shed light on the processes by which kinetic responses increase or decrease arrestment or aid organisms in finding resources. That decreased velocity increases the time spent in a "stimulus region" is intuitively expected and has been well-discussed by Fraenkel and Gunn (1961) and Davenport et al. (1960). Of greater novelty are the effects of turning parameter modifications that influence arrestment and target finding. Contrary to commonly held beliefs, it appears that merely increasing turning frequency in response to increasing sensory stimulation does not result in

arrestment in a region of high stimulus intensity. Although increasing turning frequency can decrease displacement, as shown by the results of the dispersal experiment, strong arrestment does not occur when such increases are made in response to increasing stimulus strength. Rather, increases in angular velocity with stimulus intensity appear to result in avoidance of high stimulus intensity regions if encountered from a low stimulus intensity region. If an organism does find itself suddenly in a region of high stimulus intensity at some distance from regions of lower stimulus intensity, increased angular velocity will result in arrestment. This result is in agreement with Cain's (1985) conclusion that decreased turn angle concentration (increased angular velocity) results in increased searching success when resource density is high (high probability of encountering stimulus).

The results ofthese simulations suggest that the only modifications to turning parameters alone that will increase arrestment when an organism enters a stimulus region from a low stimulus region is to decrease angular velocity while stimulus intensity is increasing, and to increase angular velocity if stimulus intensity decreases. On an intuitive level it is easy to understand why this algortithm is so effective. Going straight upon encountering increasing stimulus intensity will obviously result in increasing the proximity of the organism to the stimulus source. On the other hand, increasing angular velocity as stimulus intensity decreases is effective in minimizing displacement away from the source, owing to the effect of reduced displacement with increased angular velocity, as illustrated in the dispersal simulations (Figure 13). Since this algorithm was effective in maximizing performance in the target-

finding and both arrestment problems, it appears to be broadly effective and thus might be selected for in organisms not capable of more sophisticated response mechanisms (i.e., taxes). This algorithm does involve temporal comparisons of stimulus intensities and thus requires some form of "memory," but complex neural networks are not necessary for the minimal type of memory required. Temporal comparisons of stimulus intensities could be accomplished by comparing simultaneous outputs from as few as two types of receptors differing in their temporal response characteristics. That this type of algorithm can be implemented by simple organisms is supported by the fact that bacteria have been observed to employ it (Bell and Tobin, 1982).

Knowing the extent to which sensory adaptation operates in real organisms is important for determining how the various response mechanisms investigated will function in actual animals. This need becomes acute when considering that the model with the lowest scores in the arrestment problem, regardless of the starting point, utilized sensory adaptation (models 1xx1), but when sensory adaptation was not operational, these models were among the highest scoring ones when the mover was released from the stimulus region (Table 7). If sensory adaptation is an unavoidable consequence of neural architecture, then only those models employing a facsimile of sensory adaptation approximate real organisms. Unfortunately, it is not clear to what extent sensory adaptation does operate in real animals. It should be noted that sensory adaptation was not necessary for the avoidance of stimulus regions in these simulations, indicating that the model of klinokinesis proposed by Frankel and Gunn (1961) is but one possible

klinokinetic mechanism for avoidance of high stimulus regions. Rohlf and Davenport (1969) found sensory adaptation to be necessary for displacement of simulated organisms in gradients, but this may be due to their admittedly somewhat arbitrary implementation of sensory adaptation. Whenever their simulated organism encountered increasing stimulus intensity, they assumed it would perceive the maximal stimulus intensity possible instead of the stimulus intensity corresponding to the organism's position within the stimulus gradient. When I allowed stimulus intensity to change abruptly along the midline of the arena instead of increasing gradually along a gradient, I also found that organisms increasing turning severity with increasing stimulus intensity tended to remain in the no-stimulus region only when sensory adaptation was operational (data not shown). The conclusion that sensory adaptation is not necessary for displacement in linear gradients has recently also been reached by Havukkala (1986).

Perhaps the most significant finding from these simulations is the enormous effect of changes in circular variance of turn angles and turning frequency on dispersal, particularly for low values of circular variance. It would seem that these parameters could be fairly easily controlled by an organism. For walkers, turning frequency could easily be controlled by regulating the swing of the legs or the distance moved between turns. Changes in circular variance could easily be effected by modulating the movement of the leg(s) on one side of the body relative to those on the other side. Bacteria presumably modulate turning frequency by regulating the intervals between "twiddles" -- episodes of rapid turning -- and could perhaps modulate circular variance by regulating the time spent twiddling.

Whether or not real organisms use mechanisms identical in all respects to those of the models does not seem to change the overall conclusion: relatively small changes in turning parameters over certain regions of the theoretical range can have major effects on net displacement.

Some caution should be used, however, when attempting to infer the ecological implications of some of the response mechanisms explored here. First, it is unlikely that stimulus gradients as precisely defined as the ones simulated here will exist in nature. Secondly, the ability of most organisms to sense differences in stimulus intensities is probably less than that of the model organisms. The overall result of these departures from realism would result in greater noise in the detection of stimulus intensities but would not be expected to yield highly qualitatively different behavioral results in the various types of environments simulated. Another subtle artificiality of the models is the complete decoupling of the various movement parameters. For instance, it may be impossible for walking organisms to change their turning frequency without changing their linear velocity. The results of the simulations should be regarded as best-case scenarios of the capabilities of the various response mechanisms.

A problem of practical importance surfaces in analyzing movement tracks of real animals in terms of turning frequency and circular variance of turn angles: What constitutes a turn? If we define a "turn" as a deviation in track heading larger than some threshold angle, as is often done, then turns of small magnitude are eliminated from the analysis. We are then forced to accept distributions of turn angles that are biased against turns in the smallest turn angle

interval, or even distributions that are no longer normal but are instead bimodal, with zero probability of turns in the region below the threshold angle. Since, as these simulations have shown, the distribution of turn angles around 0^{o} can have pronounced effects on net movement, it appears that great care must be taken when determining the distribution of turn angles for a moving organism.

In addition, the definition of a "turn" itself needs attention. Should a turn be defined in terms of displacement resulting from one fundamental unit of propulsion (e.g. a step) relative to that from the previous unit, or, is a turn comprised of a series of fundamental propulsion units biased in one direction? The former definition is comparable to the functional turn used inthese models, and the endpoints of such units represent points at which potential turning "decisions" can be made. Do organisms have "programs" that initiate turns only at certain decision points, or do they have some higher level program which orchestrates turning over several basic propulsion units (or simultaneously over multiple propulsion units as in insects)? The answer to this question is fundamental to our understanding of how organisms move and to making decisions on how best to analyze movement tracks.

In conclusion, these simulations were intended to provide greater theoretical insights into the kinetic responses of moving organisms. Hopefully, results from this type of computer analysis will serve as a useful guide to the puzzles of locomotory ecology, which I define as the study of how organisms regulate their displacement in a heterogeneous world and the consequences of that regulation or lack thereof.

CHAPTER 5

INFLUENCE OF TARGET DENSITY OF FORAGING EFFICIENCY OF HYPOTHETICAL ORGANSIMS UTILIZING COMPUTER-SIMULATED MOVEMENT ALGORITHMS

INTRODUCTION

A fundamental process determining the fitness of organisms is the ability to move toward regions containing resources required for survival (e.g. food items or mates), and/or away from regions that are detrimental to survival (e.g. excessively hot or dry regions). The ability to maneuver adaptively requires, by definition, a locomotory apparatus. However, merely having the ability to move will not ensure survival without the ability to sense those qualities of the environment that are correlated with the existence of resources or detrimental factors. Thus, a sensory system governing locomotion is necessary for organisms to move adaptively in most environmental settings. The sensory channel most universally utilized in the animal kingdom is chemoreception. A wide variety of sensory and locomotory systems has evolved in organisms, as has a variety of response rules dictating how an organism should respond to given chemostimuli. Response systems range in complexity from changes in flagellar beating by bacteria in response to changes in the concentration of certain organic compounds (Adler and Tso 1974), to the directed movement toward or away from sources of chemostimuli by insects as a result of unequal stimulation of antennae and differential movement of legs on opposite sides of the body. Obviously, anatomy constrains the degree of sophistication of the response system possible, but qualities of the environment, such as the distribution of favorable or detrimental regions, might also be expected to shape locomotory responses.

The study of resource finding by animals has been approached by

researchers from a variety of biological disciplines, but the various approaches have remained largely discrete. Weston and Miller (1986) have proposed the term "locomotory ecology" to encompass the phenomena of common interest to researchers studying animal movement. They defined locomotory ecology as "the study of how organsims regulate their displacement in a heterogeneous world and the consequences of that regulation or lack thereof."

One popular approach for studying locomotory ecology has been to study the movement of foraging animals, and to determine if the behavior of such individuals maximizes the harvesting of some common currency, such as energy. This approach has come to be known as "optimal foraging theory" (see Schoener 1971 and Pyke et al. 1977 for reviews). While this approach has been useful for gaining insight into some ecological aspects of animal foraging, it has not contributed much to our understanding of the ecological implications of possessing particular behavioral response capabilities to external stimuli. Many attempts have been made to simulate animal foraging behavior (Cody 1971, Smith 1974, Jones 1977a, b, Pyke 1978, Cain 1985), but virtually all of these models have focused on the environment from a gross level, e.g., once hypothetical organisms were within a certain distance of a resource, they were assumed to move directly to the resource. Little attention has been paid to the detailed movement of the simulated organism as it responds to stimuli emanating from the resource. Jones (1977a) and Cain (1985) have incorporated changes in movement parameters, such as velocity and turning, in simulated organisms following contact with resources, but the movement of simulated organisms prior to contact has been largely ignored.

Another approach to studying locomotory ecology has focused more on actual behavioral responses that result in movement toward or away from sources of chemostimuli. This approach had its origin in the early 1900's with the pioneering work of Loeb (1918), who attempted to describe animal movements in mechanistic terms. Kühn (1919) devised a classification scheme to organize movement responses to external stimuli, and in 1940 Fraenkel and Gunn revised this scheme into what has become the commonly accepted conceptual framework for non-anthropomorphically viewing animal behavioral responses to stimuli. Recently, modifications to this scheme have been proposed by Bell and Tobin (1983), who stress the role of internally-stored information and the information-processing capabilities of the animal in determining movement patterns.

Simulation techniques have also been used to study the movement of real and hypothetical organisms in response to chemical stimuli (Rohlf and Davenport 1969, Green 1977, Van Houten and Van Houten 1982, Bornbusch 1984, Havukkala 1986, Weston and Miller 1986). These models allow detailed study of the effects on spatial displacement of altering movement parameters in response to changes in simulated chemical stimuli in the environment. The studies cited above have focused on patterns of displacement or on the finding success of hypothetical organisms in environments containing single resource units.

In this chapter I extend the simulation approach to quantifying the effect of resource density on the relative foraging efficiencies of hypothetical organisms equipped with a variety of behavioral mechanisms for responding to changes in chemical stimuli.

MATERIALS AND METHODS

Movement was simulated using the two-dimensional movement model of Chapter 4. Essentially, movement proceeded by allowing the "mover" to take a series of discrete steps in directions selected from a normal distribution of angles centered at the previous direction. The step length and standard deviation of turn angles were 2 units and 8^{o}, respectively, which resulted in movement resembling that of walking insects. Velocity was held constant at 2 distance units per time unit. Each trial began at a random location within a rectangular arena 320 units long by 200 units wide. Movement was confined to this arena by reflecting the path of the mover back into the arena whenever a new position would take the mover outside. Although the search area was bounded, reflecting the mover back into the arena essentially provided the mover with an infinite universe occupied throughout by a constant resource density since after "bouncing off" the wall the mover encountered essentially what it would have encountered had it entered an adjoining region occupied by targets at the same density as the areana.

Stimulus sources were circles of radius 6 units in fixed locations for each target density. Radiating from each source ("target") was a circular gradient of stimulus extending 40 units from the center of the source. The intensity decreased linearly from 1.00 at the center of each source to zero 40 units away. Linear gradients were used since concentrations of dispersing chemicals are believed to decrease linearly from a source (Mankin et al. 1980, Okubo 1980). This

40-unit radius circle could be considered the active space of the stimulus, outside of which the stimulus could not be detected. Targets were arranged in a hexagonal lattice so that each target was equidistant from its six nearest neighbors. The distances between targets varied with target density, but were chosen so that targets nearest the side walls of the arena were half the inter-target distance from that wall. Using these criteria for selecting target locations, the allowable densities were 1, 2, 3, 4, and 11 targets per arena. At densities higher than eleven per arena, active spaces of adjacent sources overlapped. Target densities with overlapping active spaces were not used to compare efficiencies of the various algorithms since it seemed unlikely in reality that stimulus gradients would be maintained in overlap regions, but would instead rapidly merge into fields of uniform stimulus intensity due to diffusion. However, densities up to 540 targets per arena were used with the restricted random model (described below), which did not respond to stimuli; this was done to verify that this basic model performed correctly. For the restricted-random model, parametric regression analysis was used to inspect the conformity of time spent in the stimulus regions to the percent of the arena occupied by such regions.

Fifty runs of each model were performed at each target density. A run was terminated after 1000 time units. Foraging efficiencies, defined here as the percent of time spent in the target circles, were computed to compare the effectiveness of the various algorithms at each target density. Some models were not allowed to stop when targets were encountered (no arrestment); for these models, time in the target region was registered by computing the time elapsed for each step

taken that ended in the target region. For those models that could stop at targets (arrestment), time in target regions was tallied by summing the arrestment durations for each target encounter.

Movement Algorithms. Four movement algorithms were tested at all target densities. The restricted random model utilized the default movement parameters (step length 2 units, 8^{o} standard deviation for turn angle distribution). The restricted random model served as a non-responsive control with which to compare the effectiveness of the various responses to chemical stimuli incorporated into the other models. This model had no sensory capability and thus did not change movement parameters in response to changes in stimulus intensity. To ascertain the influence that stopping (arrestment) has on foraging efficiency, the restricted random model was run with (model 2) and without (model 1) the ability to stop at targets. When stopping was permitted, the mover was moved to a random location of the arena after the arrestment duration had elapsed. Stopping at targets would be analogous to the mover consuming the resource located at the stimulus source. To evaluate the effect of resource quantity per target on foraging efficiency, two arrestment durations were tested: 50 and 5 time units.

The algorithms compared with the restricted random model were, following the definitions of Fraenkel and Gunn (1940): 1) klinokinesis, 2) transverse klinotaxis, and 3) tropotaxis. Klinokinesis is defined as an increase in rate of change of direction (r.c.d., measured in degrees turned per time unit) with changes in stimulus intensity. Although there exist many ways to change r.c.d. with changes in stimulus intensity, I chose to use the algorithm of Chapter 4 which allows the mover to move essentially in a straight

line when stimulus intensity is increasing, but causes the mover to turn more sharply upon detecting decreasing stimulus intensity by increasing the standard deviation of the distrbution from which turn angles are selected. The directions chosen for individual movement steps are not correlated with the direction of the stimulus gradient, so this algorithm is correctly termed a kinesis. This was the only klinokinetic algorithm that Weston and Miller (1986) found to be consistently effective at allowing hypothetical organisms to find targets or remain in stimulus regions. As with the restricted random model, this model was run with (model 3) and without (model 4) the ability to stop when targets were contacted.

Transverse klinotaxis followed the original definition of klinotaxis proposed by Fraenkel and Gunn (1940). They defined klinotaxis as a mechanism, accompanied by regular deviations from straight line movement, by which an organism moves directly toward or away from a stimulus source. At the points where the deviations occur, the organism is presumed to measure stimulus intensities with a single sense receptor, thus providing information for determining the direction of subsequent movement. I modeled klinotaxis (model 5) by having the mover sample two points lying fifteen degrees to either side of the midline of the mover, at a distance of 1 unit in the foward direction, when the mover was inside the active space. The stimulus intensities at each of these points were compared, and the direction of the sample probe that detected the higher stimulus intensity became the direction for the next step. Each probe added to the time elapsed the amount of time it would take the mover to move the length of the probe and back (1 time unit). When the stimulus

source was contacted, movement was halted for the duration of the arrestment period, and the mover was then moved to a random location on the screen, as described earlier for arrestment.

Positive tropotaxis is a mechanism that results in movement directly toward a stimulus source as a consequence of moving consistently toward the sense receptor, of a pair of receptors, perceiving the higher stimulation. I simulated tropotaxis (model 6) by allowing the mover to measure simultaneously the stimulus intensity at two points in front of it. The points lay fifteen degrees to either side of the current direction at a distance of 2 units. When one "receptor" detected a higher stimulus intensity than the other, the mean direction of movement was biased toward that receptor. At the low end of the stimulus gradient, the bias was 10^{o} in the direction of the higher stimulus intensity, but as the mover approached the stimulus source, the bias was increased linearly, reaching a maximum of 20^{o} in the immediate vicinity of the source. Again, movement was temporarily halted when the source was contacted and the mover was relocated to a random position before the simulation was allowed to continue.

Table 9 summarizes the responses to external stimuli that were endowed in each of the six movement algorithms compared in this study. The simulation program was written in Microsoft Advanced BASIC, and all simulations were performed on an IBM Personal Computer.[1]

[1]Author will consider requests for listing of the computer program by individuals who wish to conduct similar research. Author can be contacted at: 1725 Brook Park Dr., Lexington, KY 40502 (606)-271-1092.

Table 9. Responses of movement models to simulated chemical stimuli.

Model No.	Description	Response to stimuli when inside active space
1	Restricted random	None
2	Restricted random plus arrestment	Stop when stimulus source is contacted. Then relocate to random position and continue.
3	Klinokinesis	Move straight when stimulus intensity increases, turn sharply when stimulus intensity decreases.
4	Klinokinesis plus arrestment	Combined responses of models (2) and (3).
5	Klinotaxis	Sequentially sample stimulus intensity on both sides of body and move toward higher side. Stop, and then relocate, after source is encountered.
6	Tropotaxis	Instantaneously sample stimulus intensity on both sides of body and move toward higher side. Stop, and then relocate, after source is encountered.

RESULTS AND DISCUSSION

Time spent in the stimulus regions by the restricted random model was nearly perfectly correlated with the percentage of the arena occupied by stimulus, as indicated by the very high correlation coefficient (r^2 = 0.9993) and the nearness of the slope and intercept of the regression equation to 1.0 and 0.0, respectively (y = 1.007x + 0.268). This result confirmed that this model, basic to all others in this study, correctly accounted for time spent in various locations of the arena.

To compare foraging efficiencies of the various models, it is helpful to have a measure of efficiency that is expressed relative to the restricted random model not allowed to stop at the stimulus source. One such measure is the ratio of the foraging efficiency of a particular model divided by the foraging efficiency of the restricted random model at the same target density. This quantity estimates the degree to which resources are effectively concentrated in space and/or time by employing a particular algorithm. I term this ratio the gain in foraging efficiency; the electronics term of the same name describes the degree of amplification of a signal. A foraging-efficiency gain of 1.0 indicates that the algorithm has the same efficiency as the restricted-randomly moving control that does not respond to stimuli. All algorithms studied had gains of nearly 1.0 or higher, meaning that they all performed as well as or better than the resticted-random model.

The most efficient algorithm at all target densities and

arrestment durations was tropotaxis (model 6)(Figure 16). This result is not surprising since tropotaxis is the most sophisticated mechanism tested; at each step inside the active space, movement was guaranteed to be up the stimulus gradient, and no time cost was assessed to determine the direction of higher stimulus intensity.

The algorithms with the next highest foraging efficiencies were klinotaxis (model 5) and klinokinesis plus arrestment (model 3). These two models had nearly identical foraging efficiencies at both arrestment durations (Figure 16). Although klinotaxis had higher path directionality toward the stimulus source than klinokinesis once inside the stimulus gradient (Figure 17), it achieved this increased path efficiency at the cost of time spent sampling the chemical gradient. Thus, a less sophisticated, and thus less precise, algorithm (klinokinesis) can be as efficient (by my definition) as a more sophisticated algorithm (klinotaxis). When the arrestment duration was small 5 time units, klinotaxis was marginally more efficient than klinokinesis plus arrestment.

Merely having the ability to stop at a stimulus source (model 2) increases the foraging efficiency of the restricted-random model if the arrestment duration is sufficiently long. With an arrestment duration of 5 time units, which is essentially the time required by the restricted random mover to pass through the source area, the gain in efficiency is negligible, ranging from 0.9 to 1.6 (Table 10). At an arrestment duration of 50 time units, however, the gain in efficiency for model 2 was appreciable, ranging from 9.7 to 13.9 (Table 11). Over the range of densities tested, the gain in foraging efficiency for model 2 was quite uniform for a given arrestment duration, which is

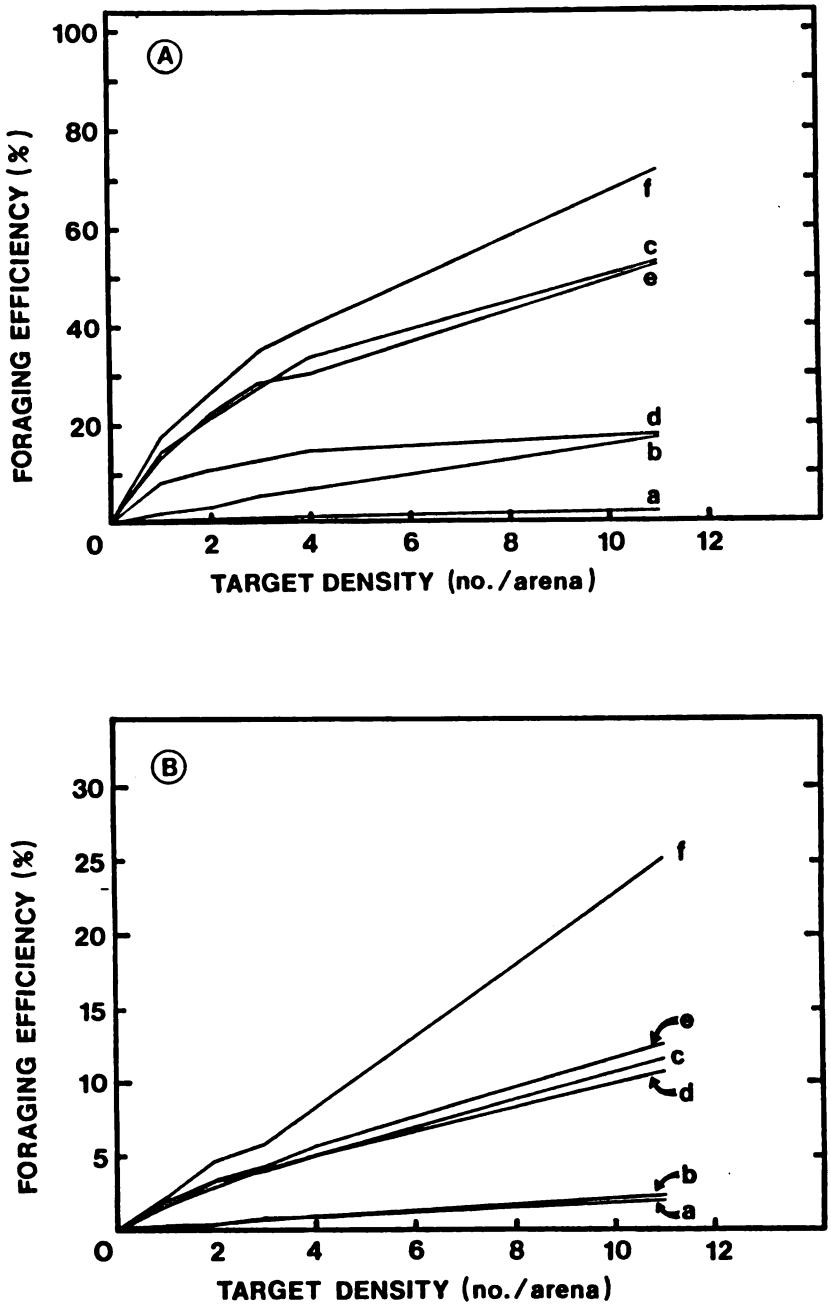

Figure 16. Foraging efficiencies of various movement algorithms as a function of resource density. A) Arrestment duration = 50 time units. B) Arrestment duration = 5 time units. Letters on curves refer to model designations: a) Restricted random movement, b) Restricted random plus arrestment, c) Klinokinesis plus arrestment, d) Klinokinesis, e) Klinotaxis, and f) Tropotaxis.

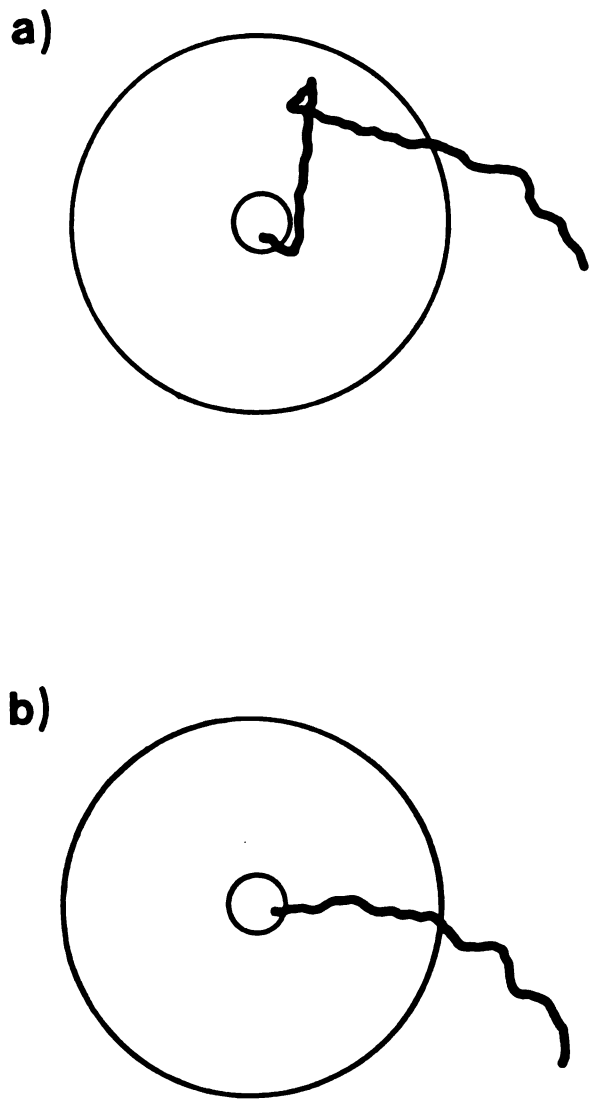

Figure 17. Sample tracks made by selected movement algorithms. a) Klinokinesis. b) Klinotaxis.

Table 10. Gain in foraging efficiency for movement algorithms under various target densities. Arrestment duration = 5 time units.

Target density (no./arena)	Gain					
	Model					
	1	2	3	4	5	6
1	1.0	1.6	8.1	7.8	9.5	10.6
2	1.0	0.9	7.8	7.1	7.8	11.1
3	1.0	0.9	5.6	5.7	5.8	7.8
4	1.0	1.1	6.2	6.2	7.0	10.1
11	1.0	1.1	6.1	5.6	6.3	12.6

Table 11. Gain in foraging efficiency for movement algorithms under various target densities. Arrestment duration = 50 time units.

Target density (no./arena)	Gain					
	Model					
	1	2	3	4	5	6
1	1.0	10.5	72.0	41.2	67.4	81.5
2	1.0	13.9	89.6	44.9	92.1	106.6
3	1.0	13.0	62.5	29.3	64.5	78.9
4	1.0	11.6	55.4	23.2	50.6	65.7
11	1.0	9.7	29.0	9.8	28.7	39.4

corroborated by the fact that foraging efficiency for model 2 was linearly related to target density, but with a steeper slope than model 1, at each arrestment duration. At sufficiently high densities, the gain for model 2 would have to level off as the efficiencies of both models approach the theoretical maximum value of 100%.

The importance of stopping at the stimulus source can be appreciated even more by comparing the gains in foraging efficienies of model 2 (restricted-random + arrestment, arrestment duration = 50) and model 4 (klinokinesis, no arrestment). At one target per arena, klinokinesis had much higher gains in efficiency than restricted random plus arrestment (41.2 vs. 10.5, Table 11), but at 11 targets per arena, restricted random movement plus arrestment was just as efficient at foraging as klinokinesis without arrestment, as indicated by the nearly identical gains (9.8 vs. 9.7). At still higher densities it appears that restricted random movement plus arrestment will perform even better than klinokinesis without arrestment. At low arrestment durations, the advantage accrued to arrestment alone disappears, and klinokinesis without arrestment consistently outperformed restricted random movement plus arrestment.

It is interesting that the relative efficiency rankings of the various models does not change as the duration of the arrestment period changes, at least for the two arrestment durations tested here. This finding indicates that resource quantity per stimulus source has little bearing on the relative foraging rankings of the movement algorithms tested here. This is surprising since one might expect algorithms that are highly accurate but require appreciable time to find targets, such as transverse klinotaxis, to be a disadvantage when resources are small, since proportionally more time would be spent

finding resources than consuming them. Is is possible that the range of resource sizes (i.e., arrestment durations) tested here is not broad enough to include resources small enough for the potential disadvantage of transverse klinotaxis to be detected. The foraging efficiency of klinotaxis can be influenced by resource size as illustrated by the results of one simulation using a variant of klinotaxis (results not shown). The mover sampled four points in the foward direction instead of two, thus consuming four time units at each step inside the active space. In this case, klinotaxis was less efficient than klinokinesis without arrestment (model 4) when the arrestment duration (for klinotaxis) was five time units. At an arrestment duration of fifty time units, however, klinotaxis was now more efficient than klinokinesis. This result can be explained by the high path directionality but large time investment required for klinotaxis. Once within the active space, a mover responding via klinotaxis, unlike a klinokinetically-responding mover, never moves in directions away from the stimulus source, but the cost for this high path directionality is the time required to sample potential directions before each step. When resource reward is large, this increased path efficiency is rewarded with a large consumable resource, but when resource size is small, the reward is not commensurate with the time investment required to find the resource.

The distance over which stimulus gradients extend may influence the foraging-efficiency rankings of the movement algorithms. For instance, klinotaxis might have a foraging-efficiency advantage over klinokinesis plus arrestment when the stimulus gradient extends over fairly short distances, but not necessarily when the gradient extends

over longer distances. Thus, the foraging efficiencies of the movement algorithms may depend on the nature of the stimulus gradient as well as on the reward at the stimulus source.

The choice of model parameters influences the ability of a simulation model to mirror reality. To reduce bias in parameterization of movement algorithms, parameters influencing movement were chosen to maximize foraging efficiency within the constraints of each algorithm. The precision in stimulus-discrimination ability of movers and spatial distribution of stimulus intensities in the model is probably higher than that in the real world, but any attempts to limit precision could potentially introduce artifacts which are even less justifiable. It seems unlikely that the qualitative nature of the results would differ appreciably even if precision were more accurately represented.

One important contribution of simulation modelling to understanding any system is the elucidation of where gaps exist in our knowledge of the system. The lack of information on how chemical gradients are distributed and how well they maintain their integrity in nature became painfully obvious as I designed the movement arena. Hopefully, simulation studies such as this will point the way to where our limits of knowledge are most severe, and, thus, guide future research.

GENERAL DISCUSSION

The results of these simulations have important implications for locomotory ecology, not the least of which are implications for the evolution of resource finding mechanisms. It is not difficult to imagine a situation where resources were at sufficiently high

densities that an organism utilizing random movement alone could encounter resources often enough that basic nutritional needs were met. As resources became depleted, however, organisms with sophisticated movement algorithms would have a competitive advantage over less efficient foragers. Thus, sophisticated finding mechanisms should be selected for.

Selection for movement algorithm sophistication might not necessarily be direct, since algorithm sophistication requires appropriate morphological structure for stimulus perception in addition to the apppropriate information capabilities. It is not unlikely that accurate finding algorithms might arise opportunistically after the requisite morphological structures had evolved, these structures being favored for reasons other than superior foraging ability. For example, it is difficult to imagine the appendages bearing the receptors required for tropotaxis being selected for merely for the function of housing receptors. It seems far more likely that such appendages would have been selected for because of their their utility in, perhaps, movement, later being "colonized" opportunistically by chemoreceptors used to detect stimulus gradients.

In addition to becoming more competitive at foraging than less-sophisticated contemporaries, organisms with the ability to move more accurately toward resources could successfully colonize areas where resources were more sparsely distributed. It is tempting to speculate that increases in resource-finding ability may have been an important in the diversification of primeval life forms owing to the resultant expansion of foraging ranges. Foraging-range expansion could foster

diversification by exposing organisms to new habitats and food sources. Given fortuitous mutations in genes regulating food or habitat selection, or geographical events separating subpopulations of a species, gene flow would be reduced, enhancing the probability of species formation.

The observation that a mover equipped with the klinotaxis algorithm foraged as efficiently as one capable of klinokinesis plus arrestment has an interesting implication for the evolution of search behaviors. If indeed these models are performing similarly to their real-world analogues, one might question why a mechanism such as klinotaxis, which is more complicated than klinokinesis, would ever evolve if it did not result in increased foraging efficiency. Aside from the neutral explanation that klinotaxis will not be selected against if it happens to arise (since its foraging efficiency was no different from klinokinesis plus arrestment), klinotaxis might have a selective advantage in situations not tested in this study. For example, I did not consider foraging for resources that were not at fixed locations in the arena. It seems likely that the tracking of a mobile resource might be accomplished more efficiently with an accurate algorithm such as klinotaxis, rather than with a mechanism that relies on random direction changes (klinokinesis). Alternatively, structural constraints might prohibit some organisms from utilizing klinokinesis efficiently. This might be the case for fly larvae, which are the best-known examples of organisms that utilize transverse klinotaxis. These animals are legless and move by crawling. Their locomotory abilities might preclude klinokinesis since the temporal comparisons of stimulus intensities required for klinokinesis might occur over too broad a time scale relative to the maggot's rate of

movement. Also, since maggots are very susceptible to desiccation, they might not be able to afford excursions that divert them from the stimulus source, which is often the major source of moisture in the maggot's vicinity.

I hope this study will stimulate further investigation into the impact of sensory and behavioral response capabilities on the foraging characteristics of animals. Most models of foraging behavior will undoubtedly benefit from the inclusion of the response characteristics of organisms to stimuli associated with the resources to which they respond. This conclusion is in accord with Pyke's (1978) finding that predictions of bumblebee foraging movements were accurate only when the sensory abilities of the bees were considered. The added level of complexity required to incorporate sensory capabilities of foragers should result in greater yields in our efforts to understand the locomotory ecology of animals, particularly when resource finding is at a premium.

SUMMARY

How has our understanding of insect chemical ecology been increased as a result of the information presented in this dissertation? I believe the contents of this dissertation have broadened our perception of insect responses to natural products, and, perhaps more importantly, the results presented herein may guide future research in new directions.

First, the malleability of host-plant ovipositional acceptance in the face of host deprivation is a phenomenon that has major implications for potential crop damage by insect herbivores, yet has received little attention in the past. The impact of host-plant chemostimuli on ovipositional behaviors has been widely recognized, but their impact on insect reproductive processes is barely appreciated. It is clear that much more research needs to be done in the area of host acceptance by insects. In particular, attention must be paid to the changes in the physiological and behavioral states of the insect as a result of age, experience, and nutritional status. Sorely needed is more information regarding the decision-making process of insect herbivores: What inputs are relevant, what are the decision-making rules, how these rules are modified by changes in physiological and behavioral states, to name a few. In addition, further exploration of the dynamics of the host-acceptance process is needed; we know too little about this process which impacts not only host-plant acceptance, but also areas as diverse as natural control by parasites and medical/veterinary entomology. I hope the first section

of this dissertation sheds light on the host-acceptance process, and makes clear the need for further researh in this area.

Secondly, the need for an increased understanding of insect movement behavior is becoming critical. Movement (spatial displacement) is a phenomenon fundamental to many insect processes: host finding, migration, and population dynamics, to name a few. The coordination of efforts for regional pest management programs depends on sound understanding of insect movement, as do programs exploring "alternative" agricultural practices, such as intercropping and no-tillage farming. In particular, we need to know how insects alter their movments in response to stimuli in their environments, and how resource distribution affects the ability to locate resources. While the second section of this dissertation does not involve investigation of movement behavior of real animals, it does shed light on factors of importance to insects as they maneuver in their environments. It points out the necessity of understanding in greater depth the nature of the physical distribution of stimuli, and emphasizes the need for carefully-considered methods for analyzing insect movement.

Perhaps the most exciting product of this dissertation is the possibility offered for integrating knowledge of host accptance behavior and movement behavior. I envision a simulation model that mimicks the movement of an insect in predefined arenas as it travels from resource to resource, allowing the responsiveness to resources to change depending on what the "insect" encounters. For instance, reproductive status would be updated as eggs were matured, laid, or resorbed, and response thresholds would change depending on the resources encountered, the unlaid-egg load, and, perhaps, age of the

"insect." It would be very easy to validate the model by constructing real-life arenas similar to the simulated ones and provisioning the arenas with resources of known acceptability. Such a simulation experiment has the potential for richly enhancing our knowledge of insect ovipositional acceptance behavior, and providing insights into the meaning of the results of choice tests. As widely as choice tests are used in entomolgical research, the fundamental processes generating the results of choice tests are largely mysterious. It seems highly likely that a combination of movement modelling and experimental reproductive physiology are a potent team for attacking a problem of such fundamental importance to entomological research.

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APPENDICES

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):

Experimental and Theoretical Studies in Insect Chemical Ecology: Ovipositional Biology of Delia flies and Simulation Modelling of Insect Movement

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)

Paul Adam Weston

Date 2-21-86

*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. P. Weston 1986		28		10	10	10		MSU
Delia platura Rondani	MI: Grant Newaygo Co. P. Weston 1986		14		10	10	10		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Paul Adam Weston

Date 2-21-86

Voucher No. 1986-2
Received the above listed specimens for deposit in the Michigan State University Entomology Museum

Curator 21 February 1986
Date

APPENDIX 2

INFLUENCE OF CAGE DESIGN ON PRECISION OF TUBE-TRAP BIOASSAY FOR ATTRACTANTS OF THE ONION FLY, *Delia antiqua*

Journal of Chemical Ecology, Vol. 11, No. 4, 1985

INFLUENCE OF CAGE DESIGN ON PRECISION OF TUBE-TRAP BIOASSAY FOR ATTRACTANTS OF THE ONION FLY, *Delia antiqua*[1,2]

PAUL A. WESTON and J. R. MILLER

Department of Entomology and
Pesticide Research Center
Michigan State University
East Lansing, Michigan 48824

(Received June 8, 1984; accepted August 1, 1984)

Abstract—Responses of onion flies, *Delia antiqua*, to known attractants were measured in the laboratory with a novel tube-trap bioassay. The relative numbers of flies caught in tube traps baited with enzymatic yeast hydrolysate, brewer's yeast, and *n*-dipropyl disulfide were similar to those obtained previously with cone traps in the field. Changing the shape of the bioassay cage from a cuboid to a cylinder decreased the experimental error obtained from analysis of variance, as did rotating the floor of the circular cage. This bioassay should be useful in evaluating attractants for other insects that orient along the substrate.

Key Words—*Delia (Hylemya) antiqua*, Diptera, Anthomyiidae, onion fly, onion maggot, tube trap, insect attractants.

INTRODUCTION

For more than a decade, vegetable entomologists have been interested in developing attractants for monitoring the onion fly, [*Delia antiqua* (Meigen)], or for use in poisoned baits. Since decomposing onions have been found to be one of the most potent attractants of onion flies (Dindonis and Miller, 1980a; Ishikawa et al., 1981), we sought to generate decomposing onions and extract the attractants therefrom. Since not all rots developed by onion tissue are equally attractive to onion flies (Miller et al., 1984), it became necessary to assay rotting

[1] Diptera: Anthomyiidae.
[2] Paper No. 11327 of the Michigan State University Agricultural Experiment Station. Received for publication June 8, 1984.

0098-0331/85/0400-0435$04.50/0

onions for attractancy prior to extraction to ensure that the starting material was indeed attractive. Assaying attractancy in the field had several drawbacks, however.

First, the attractancy of microbe-infested onion tissue changes dramatically over time (Miller et al., 1984). By the time sufficient flies were caught in cone traps (Dindonis and Miller, 1980a) to permit statistical analysis of results, the once attractive material was often beyond its prime. Complicating this problem are fluctuations in fly activity in the field due to meteorological conditions and fluctuations in fly populations due to natural phenology. Here we report a rapid laboratory bioassay for *D. antiqua* attractants as well as modifications that can maximize its precision.

METHODS AND MATERIALS

The bioassay traps (tube traps, Figure 1), were 400-ml glass beakers with three equally spaced holes (1 cm diam) around the basal circumference. The holes were placed at the bottom of the traps since earlier work (Dindonis and Miller, 1980b) showed that onion flies approach sources of volatiles via short, hopping flights along the substrate. Inserted through the holes were 4-cm lengths of glass tubing (0.8 cm ID), which projected ca. 1 cm outside the beaker. The entrance tubes decreased the random entry of flies into the beaker. Sitting on

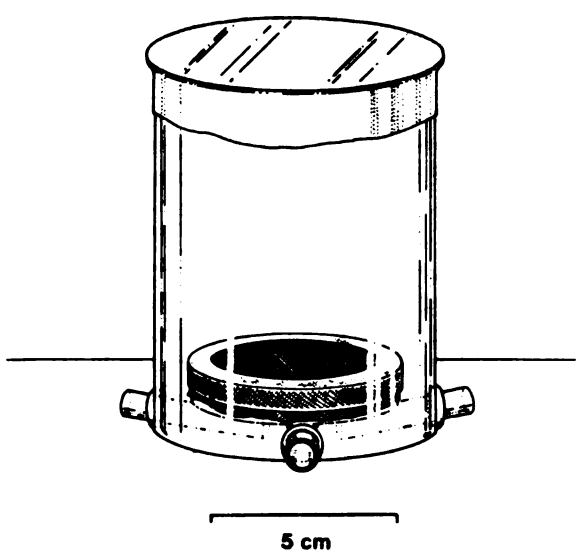

FIG. 1. Tube trap for assaying onion fly attractants.

top of these tubes was a 5.5-cm Petri dish containing the test material covered with plastic screening to prevent contact of the flies with the bait. Assembly of a tube trap was completed by covering the beaker with Parafilm.

Three cage designs were compared in this study. The first was a cuboid (80 × 60 × 60 cm) with a screen ceiling and plastic sides. The bottom 10 cm of the back and side walls were screen, and the floor was asbestos board. The other two cages were acetate cylinders (55 cm diam × 60 cm) with Plexiglas lids. In the center of each lid was a 10-cm hole covered with plastic screen for ventilation. One of these cages (designated "circular cage") had a stationary floor of screen and hardware cloth, while the other (designated "rotating cage") rested over a screen disk that rotated at 4 rph when powered by a small electric motor. The gap between the disk and the sides of the cylinder was blocked with a length of foam weatherstripping. Each cage had a small (ca. 200 cm^2) plastic door that permitted access to the inside.

The cages were placed in a controlled-environment chamber maintained at 21 ± 1°C and 35 ± 5% relative humidity under a 16:8 light-dark regime. Flies were provided with water continuously and the diet of Ticheler (1971) between replicates. Food was removed during bioassays to increase the responsiveness of the flies to baits. The flies were drawn from a population that had been in laboratory culture for two years. To ensure uniformity of age structure, only those flies eclosing during a 4-day span were included in a common stock cage. Experimental cages were stocked by first aspirating 600 flies of each sex, in groups of 10, from the stock cage. Groups were then chosen at random and assigned to the experimental cages in rotation, until each cage contained 200 flies of each sex. As flies died during the experiment, they were removed and replaced with new ones from the stock cage.

We elected to test only four baits simultaneously since the traps could be spaced uniformly in the rectangular cage (i.e., one in each corner). Treatments chosen covered a range of attractancy to adult females, based on trap catch data from the field (Miller and Haarer, 1981). Enzymatic yeast hydrolysate (EYH; ICN, Cleveland, Ohio) was the most attractive treatment, while brewer's yeast (BY; Bio-Serv, Frenchtown, New Jersey) and *n*-dipropyl disulfide (Pr_2S_2; Eastman Kodak, Rochester, New York) were intermediate; an empty trap served as a negative control. The yeast baits were presented as 5 g powder, while Pr_2S_2 was presented as 100 μl of 0.7 mole fraction in peanut oil in a size 3 BEEM polyethylene enclosure (Dindonis and Miller, 1981).

The experimental design was randomized complete block, with a total of six blocks conducted (over time) per cage. The three cages were tested simultaneously, with 1–3 days between blocks. Traps were placed at the corners of an imaginary square, 45 cm on a side, centered in each cage. Treatments were assigned to positions at random for each block. After 24 hr, flies caught in the traps were sexed, counted, and released back into their respective cages. Data

for each cage were analyzed separately with analysis of variance (ANOVA), and the mean square errors (MSEs) from the analysis of each cage were compared using Bartlett's test for homogeneity of variances (Steel and Torrie, 1980).

RESULTS

The numbers of male flies caught were very low, averaging less than 15% of the total flies caught. Therefore, only numbers of females were analyzed. The relative numbers of females caught by the treatments (Table 1) were similar to field-trapping results, except that EYH and BY caught nearly the same numbers of flies in the laboratory, while EYH caught several-fold more flies than BY in the field (Miller and Haarer, 1981). However, the same trends in trap catch were observed in all three cages, indicating uniformity of response of the

TABLE 1. FEMALE ONION FLY CATCHES IN TUBE TRAPS AS AFFECTED BY CAGE DESIGN

	Mean trap catch[a] (±SD)		
Treatment	Rectangular	Circular	Rotating
EYH	18.7 ± 11.7a	12.8 ± 4.6a	16.8 ± 6.3a
BY	11.0 ± 11.4ab	14.8 ± 3.5a	15.1 ± 6.3ab
Pr_2S_2	7.0 ± 8.9bc	10.7 ± 6.5a	9.8 ± 6.0b
Control	0.8 ± 0.8c	2.0 ± 1.3b	2.2 ± 1.2c
Total	37.5 ± 20.9	40.3 ± 7.7	43.9 ± 13.2

[a] Means followed by the same letters within columns are not significantly different at the 5% level as determined by the LSD test on data transformed to $(x + 0.5)^{1/2}$.

TABLE 2. COMPARISON OF MSES AND TREATMENT *F* VALUES FROM SEPARATE ANOVAS OF TRAP CATCHES (SQUARE ROOT-TRANSFORMED) IN EXPERIMENTAL CAGES

Cage type	MSE[a]	Treatment F^b
Rectangular	1.76a	5.69**
Circular	0.71ab	9.47***
Rotating	0.48b	16.25***

[a] Mean square errors followed by the same letter are not statistically different at the 5% level as determined by pairwise Bartlett's tests for homogeneity of variances with 15 degrees of freedom.

[b] **, significant at the 0.01 level; ***, significant at the 0.001 level.

three fly populations and similar performance of traps in each cage type. The total numbers of flies caught per cage per replicate were not statistically different ($F_{2,10} = 0.54$, square root-transformed data).

A measurement of the ability to detect treatment differences is the MSE from analysis of variance. A comparison of the MSEs and treatment F values among cage types (Table 2) shows that the rectangular cage had the highest MSE and lowest treatment F, while the rotating cage had the lowest MSE and the highest treatment F. In addition, the rotating cage had the highest degree of homogeneity of treatment variances according to Bartlett's test ($\chi^2 = 4.76$, ns), while the rectangular cage had the lowest ($\chi^2 = 9.39$, $0.01 < P < 0.05$).

DISCUSSION

A desirable feature of any bioassay is that it be able to detect differences between treatments as quickly as possible. Since responses of caged *D. antiqua* to attractants have large variances associated with them, it is often necessary to replicate such bioassays many times. Increasing the precision (i.e., the ability to detect treatment differences by decreasing experimental error) of the bioassay decreases the number of replicates needed to detect differences at the same confidence level and can therefore facilitate the isolation of biologically active materials.

Modifying the shape of the bioassay cage from a cuboid to a cylinder was prompted by the preliminary observation that traps in some positions in the rectangular cage caught more flies than others regardless of the treatments placed there. This modification resulted in a large decrease in the experimental error and a corresponding increase in the treatment F value for the same number of treatments and replicates in each cage (Table 2). This improvement was likely due to the removal of corners from the cage. If flies favor certain corners of the cage more than others, this would increase their chances of randomly entering a trap in that location. Obviously, a circular cage has no corners and is therefore less susceptible to such effects. Refining the circular cage by rotating the floor further improved the precision of this bioassay as judged by the decrease in MSE and the increase in treatment F value. This increased precision can most likely be attributed to allowing each treatment to spend equal time in all positions within the cage. This same result can be achieved by manually rotating treatments on a regular schedule, but having the floor rotate by itself reduces labor and allows the assay to run unattended for considerable lengths of time. Although the percentage decrease in MSE of the circular vs. the rotating circular cage was smaller than the rectangular vs. circular cage (32% as opposed to 60%), the additional gain in precision may be justified since the best separation of treatment means was obtained in the rotating cage.

The rotation of treatments to reduce experimental error is not a new idea.

For example, DeVaney et al. (1971) used a rotating cage when measuring the response of screwworm flies to potential attractants, and Ellis and Hardman (1975) placed test plants on turntables inside their bioassay cages when measuring the responses of cabbage root flies. The intuitive advantage of using rotation to even out exposure of treatments to locations in the cage is substantiated by the significant decrease in MSE in the experiments reported here.

We believe the tube traps used in these experiments measure attraction, albeit indirectly. Since the flies must pass through narrow tubes to enter the trap, it is unlikely that arrestment is the mechanism responsible for flies accumulating in the trap. Rather, it is much more likely that some chemotactic mechanism is responsible for guiding the flies into a trap, and thus the traps can be said to measure attractancy of the test materials.

This laboratory bioassay should prove to be useful for measuring the attractancy of test materials to a variety of other insects. Such insects might include other anthomyiid flies, beetles, and most other insects that approach the source of an attractant along the substrate.

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