Olfactometer Studies of Host Seeking by the Parasite *Spalangia endius* Walker (Hymenoptera: Pteromalidae)

by

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Abstract

Behavioral studies using a Y-tube olfactometer demonstrated that larval house fly media such as bovine and poultry manure were attractive to the parasite Spalangia endius Walker. Fermenting ensilage and C.S.M.A., a fly rearing medium, were also attractive by the chi-square test for significant differences. The presence of a host seeking stimulant was suggested. Manure containing or having contained house fly larvae was favored over fresh manure. Sterile cultures of third instar larvae were found attractive, indicating the attractant was a larval metabolite. Only a larvae and larval medium combination approximately four days old appeared to be consistantly attractive to the parasite as compared to the medium alone. This was probably dependent upon the fly larval density and the concentration of a kairomone substance. A minimum of 25 larvae per 100 grams of manure were found necessary for attraction by the parasite. A kairomone released by the house fly could reduce the host habitat searched by the parasite to that containing potential pupal hosts once the larval medium had been located.

Introduction

Experimental releases of the pupal parasite Spalangia endius Walker in poultry and dairy installations demonstrated the potential use of these wasps in the control of the house fly, Musca domestica L. (Morgan et al. 1975; Morgan et al. 1976). Sustained releases of these wasps suppressed the fly population. For successful fly control, the parasite must locate and parasitize the host. This process of parasitization was divided into four phases by Doutt (1959); host habitat location, host finding, host acceptance and host suitability.

Chemical stimuli or cues involved in the host habitat and host finding phases may be derived from the host's food, the host or a combination of food and host factors (Vinson 1976). The cue sequence has been worked out for Cardiochiles nigriceps, a parasite of Heliothis virescens, the tobacco budworm (Vinson 1975). Other parasite species have been attracted to odors from the food plants or larvae of their hosts (McKinney and Pass 1977; Vinson 1976). The parasite Nasonia vitripennis is attracted to meat. Meat which contains or had contained host pupae appears to be most attractive (Edwards 1954; Wylie 1958). The objective of this study was to determine if Spalangia endius Walker was attracted to house fly contaminated medium, uncontaminated house fly larval medium and determine if S. endius

was attracted to the developmental stages of the house fly. The response of the parasite to individual larval migration tracks was also tested.

Materials and Methods

I. Parasite Attraction to House Fly Rearing Media and the House Fly Developmental Stages.

A Y-tube olfactometer was utilized to study the response of S. endius to house fly larval medium that had no prior exposure to the developmental stages of the house fly and medium that contained developmental stages of the house fly. The olfactometer modified after Chaudhury et al. (1972) (Figure 1) consisted of a glass Y-tube (Kimax 29/42) with each arm a straight glass tube (Kimax 29/42). A glass chamber, 6.5 cm in diameter, formed the parasite introduction chamber attached to the base of the Y-tube. The chamber was closed at its base by a large rubber stopper containing #60 brass mesh screened holes for the passage of air. Each arm of the Y-tube contained a glass funnel. Any parasite passing through the funnel was considered to have made a choice. A vacuum-compression pump was used to force air through the olfactometer. The air was first passed through three chambers of activated charcoal and glass wool for cleaning then split into two streams, each passing through a calibrated Rogar Gilmont flowmeter. The air then passes through four 250 ml Erlenmyer filtration flasks and into the arms of the olfactometer. The first flasks contain the water for establishing a uniform humidity and the second two contain the test

materials. PVC tubing was utilized for connecting the pump, flowmeter and flasks to each arm of the olfactometer.

The parasites utilized in all studies were reared in Florida by the USDA and mailed to Kansas State University at weekly intervals. The parasites were in the pupal stage upon arrival. The parasites were then held in a dark room at 25.5°C and 70% relative humidity. The house fly pupae containing the parasites were subdivided into groups of 50 pupae and held in 4.5 cm x 4.5 cm x 2 cm plastic boxes for emergence. After emergence, the parasites were retained for 12 to 24 hours to obtain a uniformly aged sample for the studies. The parasites were fed honey ad libitum. To minimize any moisture response the parasites were then preconditioned to water. The parasites were placed in a 100% relative humidity chamber (Figure 2) over water for 12 to 24 hours before test trials in the olfactometer. Humidifying the air in the olfactometer also minimized any moisture response (Table 1).

The house flies utilized in this study were reared by standard C.S.M.A. procedures or on a sterile synthetic medium modified from Monroe (1962) (Appendices D and E). C.S.M.A. is a fly rearing medium (from Chemical Specialties Manufacturing Association) manufactured by the Purina Company. The flies in the bovine and poultry manure samples were field collected samples from the Kansas State University Beef Research Center.

A minimum of two trials with 40 to 50 parasites each were made for each test combination. The trials were conducted

in total darkness due to the strong phototrophic response of the parasite. The trials were conducted for one hour at an airflow of 1000 ml per minute. After each trial, the olfactometer was washed, rinsed with acetone and the position of the airflow to the olfactometer switched to eliminate any left-right bias. The PVC tubing was replaced with each test combination.

The fly larval and pupal substrates tested; fresh manure, soil, corn ensilage and poultry feed, were obtained from the Kansas State University Beef Research Center and the Kansas State University Poultry Research Center. The fresh bovine and poultry manure was collected at defication and never contained any flies. Dry manure was obtained by air drying the fresh bovine manure samples. Corn ensilage and poultry feed were taken from the feeding bunkers of the cattle and chickens, respectively. The Tully silty clay loam soil samples were taken at the Beef Research Center perifary. The substances were selected from conditions naturally encountered by the house fly and by the parasite in a feedlot or poultry farm situation. C.S.M.A. and the synthetic medium were also utilized in the tests.

Since house flies are attracted to ammonia in fermenting substances (Richardson and Richardson 1922), ammonium carbonate was used as an adult female house fly attractant for egging the laboratory colonies. Ammonium carbonate was tested (1.75 g / 100 ml H_2O) to determine if the parasites were also attracted to ammonia.

The olfactometer results were analyzed by the chi-square test for heterogeneity and for significant differences in choice between the odors presented. If the test was not significant for heterogeneity, a pooled chi-square was used (Appendix B).

II. Parasite Tracking of House Fly Larvae

House fly larvae prefer to migrate from the larval medium to a drier site to pupate. To evaluate the tracking of individual house fly larvae to the pupation site by female parasites, a single chambered test arena without airflow was used (Figure 3). The test arena consisted of a transparent plastic box measuring 38.1 cm x 27.9 cm x 10.2 cm uniformly illuminated from below by a diffused light source. The bottom of the arena was covered with a sheet of Whatman Number 3 filter paper. One photonegative third instar house fly larva was then guided from the arena center along a straight line using a clean glass rod and a beam of light. The larva was removed to prevent a visual response and a single female parasite was introduced into the chamber. Her movements were traced from above on plastic sheets. The procedure was conducted for six minutes. After each trial, the chamber was washed with hot soap and water and a complete new set up was used. Twelve replications were conducted. The larva was not rinsed of rearing media because any chemical attractant present could have been lost.

Figure 1. Y-tube Olfactometer. A, Parasite release chamber; B, Y-tube; C, Funnel; D, Arm of olfactometer; E, Test flask; F, Water flask; G, Flowmeter; H, Activated charcoal and glass wool (only one chamber shown); I, Direction of air flow.

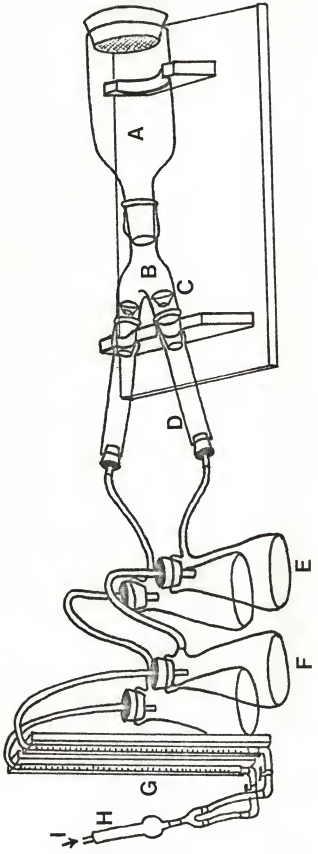

A
B
C
D
E
F
G
H

Figure 2. Parasite Preconditioning Chamber. A, Plastic lid; B, Muslin retaining ring; C, Muslin cloth upon which the parasites are placed; D, Metal ring with nylon mesh for support; E, Plastic base of container which contains the water.

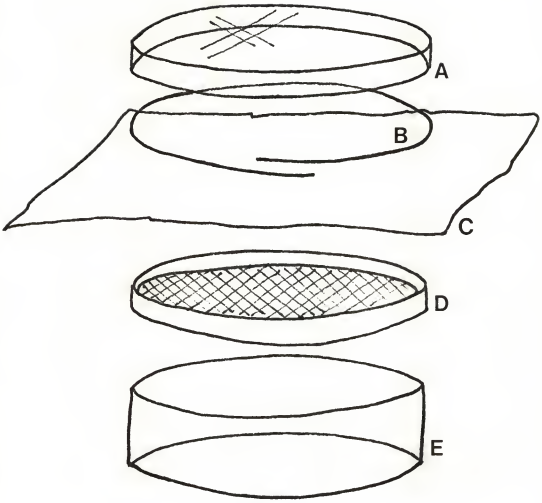
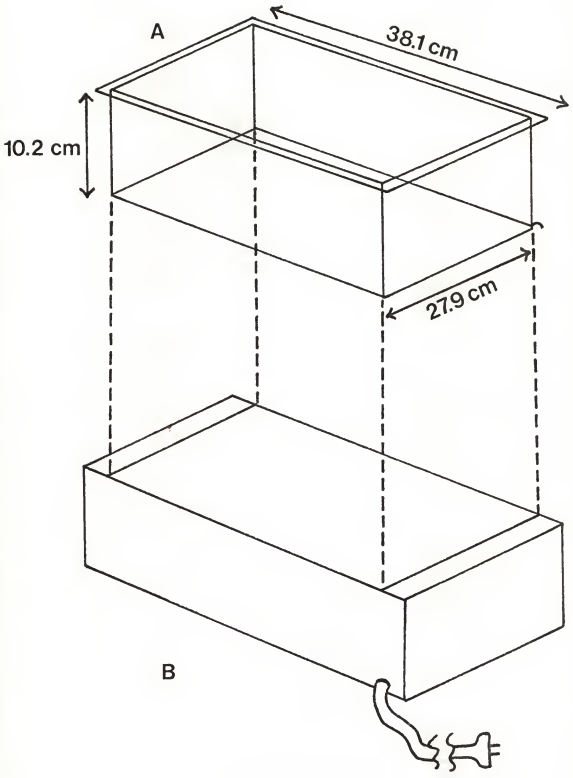

A
B
C
D
E

Figure 3. Test Arena for Parasite Tracking of House Fly Larvae. A, Illumination source; B, Tracking arena.


A
38.1 cm
10.2 cm
27.9 cm
B

Results and Discussion

Odors from cattle and poultry manure, corn ensilage and fermenting C.S.M.A. were significantly attractive to Spalangia endius (Tables 2 and 4). Dry bovine manure, uncontaminated by the house fly, was also attractive to the parasite. Ammonia, a fermentation product attractive to ovipositing flies, was also attractive to the parasite. Soil taken from near the Beef Research Center was not attractive. The manure substates were concentrated by man in feedlots and poultry houses providing a habitat for the house fly and S. endius. In many cases, the substrate may be scattered and may change over the parasite's month developmental period (66 days at 18°C and 17 days at 35°C, Ables et al. 1976) due to dessication or removal of the substrate in a sanitation program. As a result, the parasite could be removed from fresh substrate at emergence necessitating a search for these potential host habitats.

Once the female adult parasite has located a potential host habitat, she must find the house fly pupae which were not uniformly distributed through the habitat and may not even be present. The female parasite may search randomly for host pupae or she may be directed through cues from the host. There are two basic approaches. The parasite could be attracted directly to the fly pupae. However, the pupae were not found attractive and can be eliminated (Table 3). The other cue source could be the fly larvae.

Table 1. Choices made by Spalangia endius in the Y-tube olfactometer to moisture and moisture conditioning.

Test	A[1]	B	No Response	χ^2	P
Blank vs Blank	84	70	16	1.27p[2]	n. s.[3]
Moisture vs Dry 12 Hrs.	43	37	4	0.45p	n. s.
Moisture vs Dry 36 Hrs.	59	19	3	20.51p	P<.0001
H_2O vs Drierite[4] Unconditioned Parasites	37	55	8	10.07	P<.01
H_2O vs H_2O Unconditioned Parasites	35	44	5	1.02p	n. s.
H_2O vs Drierite Preconditioned Parasites	39	41	9	0.05	n. s.
H_2O vs H_2O Preconditioned Parasites	48	37	8	1.42	n. s.

[1]Material named first is A; material named second is B.
[2]p represents pooled chi-square values.
[3]n. s. = P>.05
[4]Drierite brand desiccant is manufactured by the W. A. Hammond Drierite Company.

Table 2. Choices made by Spalangia endius in the Y-tube olfactometer to host free materials.

Test	A[1]	B	No Response	χ^2	P
Blank vs Fresh Bovine Manure	19	66	7	32.92	P<.0001
Blank vs Dry Bovine Manure	57	122	17	29.46	P<.0001
Blank vs Fresh Poultry Manure	42	108	9	29.04p[2]	P<.0001
Fresh vs Dry Bovine Manure	133	48	18	45.75	P<.0001
Blank vs Corn Ensilage	58	132	14	32.58	P<.0001
Blank vs Poultry Feed	42	57	2	2.27p	n. s.[3]
Blank vs Soil	70	79	19	0.54p	n. s.
Blank vs Ammonium Carbonate	28	56	8	9.33p	P<.005
Blank vs Parasites	27	25	40	8.02	P<.025

[1]Material named first is A; material named second is B.
[2]p represents pooled chi-square values.
[3]n. s. = P>.05

Table 3. Choices made by Spalangia endius in the Y-tube olfactometer to host contaminated materials.

Test	A[1]	B	No Response	χ^2	P
Fresh Bovine Manure vs Larvae in Dry Manure[2]	50	135	13	47.01	P<.0001
Fresh Bovine Manure vs Dry Manure with All Larvae Removed	37	160	3	80.59	P<.0001
Blank vs Pupae	36	34	16	0.05p[3]	n. s.[4]
Blank vs Poultry Feed with Pupae	50	112	12	23.72p	P<.0001
C.S.M.A. vs Larvae with C.S.M.A.[2]	53	113	12	32.91	P<.0001
Blank vs Larvae with C.S.M.A. Removed[5]	38	60	1	4.93p	P<.05

[1]Material named first is A; material named second is B.
[2]Contains approximately one fly larva per cubic centimeter.
[3]p represents pooled chi-square values.
[4]n. s. = P>.05
[5]Contains approximately 5 fly larvae per cubic centimeter, a total of 200 larvae.

Table 4. Choices made by Spalangia endius in the Y-tube olfactometer to C.S.M.A. (CSMA) over the period of larval development.

Test	A[1]	B	No Response	χ^2	P
Blank vs CSMA Dry	35	45	10	1.25p[2]	n. s.[3]
Blank vs CSMA Fermented	20	58	4	21.54p	P<.0001
CSMA vs CSMA with Larvae, Day 1	46	37	10	3.04	n. s.
CSMA vs CSMA with Larvae, Day 2	93	76	17	18.78	P<.0001
CSMA vs CSMA with Larvae, Day 3	80	94	15	7.37	n. s.
CSMA vs CSMA with Larvae, Day 4	75	121	5	20.65	P<.001
CSMA vs CSMA with Larvae, Day 5	74	107	6	6.01p	P<.025
CSMA vs CSMA with Larvae, Day 6	71	99	15	4.61p	P<.05

[1]Material named first is A; material named second is B.
[2]p represents pooled chi-square values.
[3]n. s. = P>.05

Table 5. Choices made by Spalangia endius in the Y-tube olfactometer to differing densities of third instar house fly larvae.

Test	A[1]	B	No Response	χ^2	P
Manure vs Manure, 0 Larvae	45	50	3	0.26p[2]	n. s.[3]
Manure vs Manure, 5 Larvae	42	49	6	0.54p	n. s.
Manure vs Manure, 25 Larvae	23	65	12	20.05p	P<.0001
Manure vs Manure, 50 Larvae	35	62	1	7.56p	P<.01
Manure vs Manure, 75 Larvae	26	73	1	22.31p	P<.0001
Manure vs Manure, 150 Larvae	37	58	6	4.64p	P<.05

[1]Material named first is A; material named second is B.
[2]p represents pooled chi-square values.
[3]n. s. = P>.05

Table 6. Choices made by Spalangia endius in the Y-tube olfactometer to third instar house fly larvae reared in aseptic culture.

Test	A[1]	B	No Response	χ^2	P
Blank vs Synthetic Diet	48	86	18	10.78p[2]	P<.001
Synthetic Diet vs Synthetic Diet with Larvae	45	101	12	21.48p	P<.0001

[1]Material named first is A; material named second is B.
[2]p represents pooled chi-square values.

The female parasite must locate a house fly pupa within 48 hours of the fly's pupation or the house fly can still emerge after parasitization (personal communication from Dr. P. B. Morgan, USDA). The presence of larvae would indicate the potential presence of one or two day old pupae. Dry manure containing a high density of mature, third instar house fly larvae (approximately one fly larva per cubic centimeter as collected at the Beef Research Center) was significantly more attractive to the parasite than fresh manure (Table 3). Even after all the larvae had been removed, the dry manure was still more attractive than the fresh manure, indicating the presence of an attractive substance left in the manure by the larvae. Perhaps a house fly larval metabolite acted as a parasite attracting stimulant or kairomone and was used in the detection of larvae to reduce the area searched by the parasite.

Bryant and Hall (1975) demonstrated the effects of larval medium conditioning on the reduction of oviposition by the female house fly adult and as an attractant to other house fly larvae whose excretion products stimulate the growth of the microbiota upon which fly larvae feed. "Collectively, these processes create local pockets of intermediate larval densities which reap the benefits of enhanced food availability, while avoiding overcrowding through regulation of ovipositional behavior (Bryant and Hall 1975)." The chemical agent(s) involved in conditioning (or some other metabolite) are possible canidates for the attractive substance left in

the manure. Since the mutual attraction of larvae would counteract a normal random diffusion from the oviposition site, the larval conditioning agent(s) might restrict the parasite's searching to habitats where higher density fly populations exist.

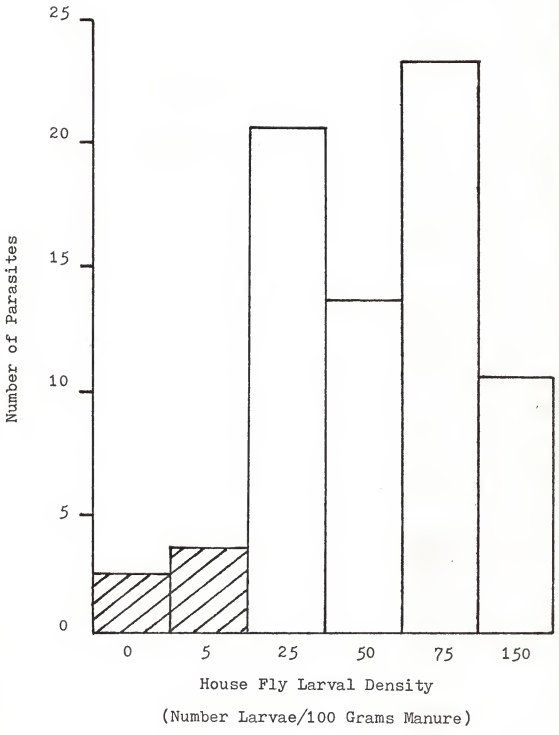
Based on this possibility, three experiments were performed utilizing the Y-tube olfactometer. These were to determine if the attractant appeared as the larvae developed, to determine if the attractant elicited a response at various house fly larval densities and to determine if the house fly larvae alone were the source of the attractant.

C.S.M.A. with larvae were compared to identical C.S.M.A. lacking larvae over the developmental period of the larvae (six days at 25.5°C) to test if the attractant appeared as the larvae developed (Table 4). Days one and three showed no preferences by the parasite. Day two was significantly different, but the results were heterogeneous with the parasites favoring the C.S.M.A. in half the trials and favoring the C.S.M.A. with larvae in the other trials. The heterogeneity in these trials may be due to variations in the larval densities resulting in differing amounts of the chemical attractant. By day four, the parasites were more consistantly attracted to C.S.M.A. containing the mature, third instar larvae which were concentrated in pockets at the bottom of the rearing containers. The results are probably dependent upon the fly larval density and the resulting concentration of the attractant since the larvae begin conditioning the medium within 24 hours after hatching (Bryant and Hall 1975).

In order to determine if the parasite's response to the attractant was larval density dependent, thirty-six, 100 gram aliquots of manure were placed in petri dishes into which newly hatched house fly larvae were placed. Eighteen of the manure aliquots received 0, 5, 25, 50, 75, and 100 larvae per aliquot in three replications. The control was the other eighteen aliquots of manure without larvae. The developing larvae were held until the third instar and compared with the manure controls. Two trials for each level of larvae were performed in the olfactometer. The parasites did not respond to the lower density of 5 larvae per aliquot of manure, but were significantly attracted by 25 or more house fly larvae per aliquot of manure (Table 5; Figure 4). Therefore, approximately 25 larvae per 100 grams of bovine manure were required to elicit a response from the parasites.

The attractant associated with the house fly larvae could be a metabolite of the larvae as previously suggested, a metabolite of the microbiota present or could be a product of a larval, microbiota interaction. House fly larvae were reared on a sterile medium and compared with sterile medium without larvae to determine if the attractant was actually a larval metabolite. The preparation of the aseptic medium (modified from Monroe 1962) and the sterile innoculation of the medium with house fly eggs are discussed in Appendix E. The third instar larvae in the aseptic cultures were significantly attractive to the parasites (Table 6) indicating a larval metabolite alone is acting as a host attractant for the parasite.

Figure 4. Parasite Response to Varying Densities of House Fly Larvae. The plot represents the observed number of parasites attracted to the larvae minus the expected number of parasites if there were no differences.

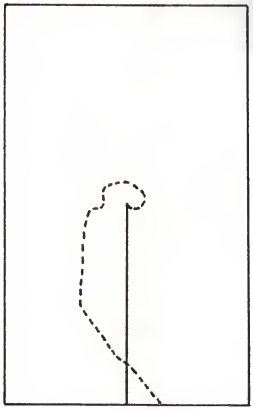

25
20
15
10
5
0
Number of Parasites
0
5
25
50
75
150
House Fly Larval Density
(Number Larvae/100 Grams Manure)

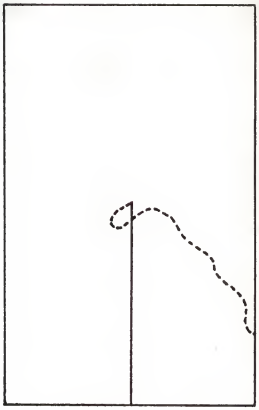
Once the parasite locates the house fly larvae, the pupae must be found. When the larvae migrate to a pupation site, the attractant appears to be carried over to the pupation site, at least at high fly densities. A high density of pupae (approximately 1 pupa per 2 cm^2) in spilled chicken feed in a poultry house was attractive although fly pupae and chicken feed uncontaminated by fly developmental stages were not. The fly larvae had left the nearby poultry manure to pupate in the dry feed. However, in the tracking tests, the parasites did not respond to the individual tracks of a larva (Figure 5). The attractant on one larva was not sufficient to elicit a response although a high density of larve (200 larvae) cleaned of C.S.M.A. substrate were attractive in the Y-tube olfactometer (Table 3).

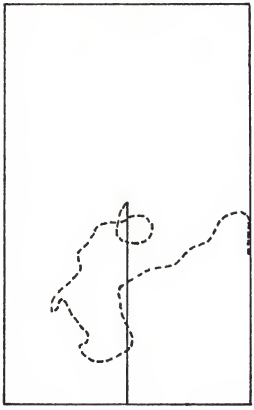
The results of many of the olfactory tests were significant for heterogeneity. If there was no variation in the parasite's response, an equal number would be expected at each arm of the olfactometer every trial. A constant number of parasites should also respond to an attractant at each trial. Heterogeneity among the trials resulted mostly from varying degrees of response rather than from a preference reversal. These response variations may be due to moisture, a parasite interaction or from variations in the materials tested.

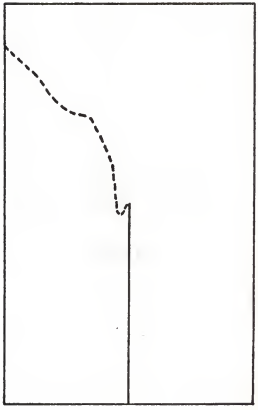
The parasite's response to moisture became significant by 24 hours and extremely significant by 36 hours post emergence (Table 1). A reversal of the behavioral response

Figure 5. Four replications of parasite tracking of house fly larvae. The dotted line represents the female parasite. The solid line is the fly larval track.









to water was obtained by preconditioning the parasites at 100% relative humidity. There was no significant difference in response between the moisture or the Drierite brand desiccant, $CaSO_4$, using preconditioned parasites. Therefore, a moisture response did not introduce heterogeneity with preconditioned parasites.

An interaction between female parasites or test material variations could also introduce response variations. A parasite interaction was tested by the response of the parasites to other female parasites and by their response in the blank trials. The response to the other female parasites were divided between the female parasites and the blank in different trials giving a heterogeneous test although the overall response was about the same (Table 2). Many of the parasites failed to respond to the presence of other female parasites. In addition, the parasite's responses in the blank versus blank trails were homogeneous meaning a parasite interaction did not introduce heterogeneity. The heterogeneity can perhaps be attributed to variations in the amount of attractive chemical in the materials tested, resulting in odor variations in the airflow.

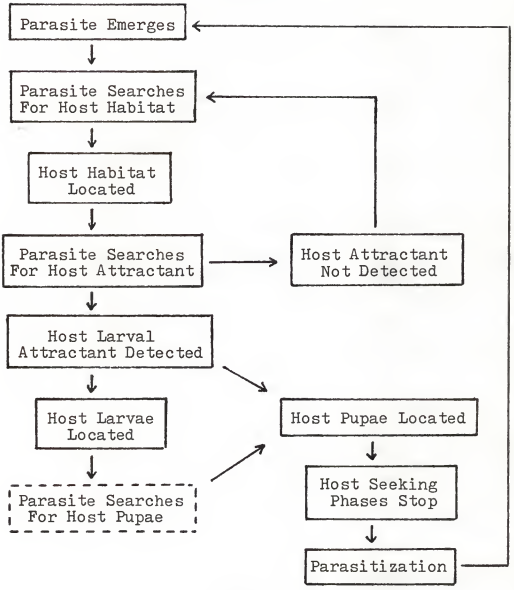
In summary, the study has determined Spalangia endius was attracted to uncontaminated house fly larval medium, to house fly contaminated medium, both septic and aseptic, and to the larval stages of the house fly at densities greater to or equal than 25 larvae per 100 grams of manure. The pupal stage of the house fly was not attractive to the

parasite. A possible cue sequence for the host habitat and host finding phases of parasitization can be formulated based upon the olfactory responses (Figure 6). The parasite was first attracted to the host habitat (house fly substrates such as fresh manure). If the substrate contained no flies, the parasite proceeds searching. However, S. endius will be attracted to the host through the presence of a chemical attractant released by the fly larvae (possibly a kairomone) if the larval density was high enough and the larvae are mature. The attractant may be present only in the larval substrate or it may carry over to the pupation site. If the attractant was present with the pupae, the host searching by the parasite stops and the host acceptance phase begins. If the parasite located a concentration of larvae, the parasite must still search for the fly pupae. Since the parasite does not track individual larvae to their pupation site, perhaps the parasite begins a random search, a directed search pattern or utilizes some other chemical or physical cue to locate dispersed pupal populations.

If S. endius was more effectively attracted to higher host densities, biological control efficiency would be limited at low fly population densities which require more searching by the parasite and better at higher fly populations. Additional olfactory studies with behaviorally discriminating assays to determine the mechanisms of response (taxis or kinesis) and extractions to isolate the attractant would allow better delineation of the host seeking process.

An understanding of these processes could lead to a better utilization of the parasite in controlling house flies.

Figure 6. The Host Seeking Sequence for Spalangia endius.


Parasite Emerges
Parasite Searches For Host Habitat
Host Habitat Located
Parasite Searches For Host Attractant
Host Attractant Not Detected
Host Larval Attractant Detected
Host Larvae Located
Host Pupae Located
Parasite Searches For Host Pupae
Host Seeking Phases Stop
Parasitization

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Appendix A

A Review of the Literature

Experimental field releases of the parasitic wasp Spalangia endius Walker (Hymenoptera: Pteromalidae) in poultry and dairy installations demonstrated the potential use of these wasps in the control of the house fly, Musca domestica L. (Morgan et al. 1975; Morgan et al. 1976b; Morgan and Patterson 1977). These wasps suppressed the fly population and maintained it at a low level. The biological control potential of S. endius has been discussed by Legner and Brydon (1966); Morgan, Benton and Patterson (1976); and Morgan and Patterson (1975).

As defined by Askew (1971), these parasitic wasps are properly termed protolean parasitoids. Protolean insects are those in which only the immature stages are parasitic and parasitoid if the parasitism results in the death of the host. Other members of this group include S. cameroni Perkins, S. nigra Latreille, S. nigroaenes Curtis, Muscidifurax raptor Girault and Sanders, Pachyrepoides vindemiae (Rondani), Nasonia vitripennis (Walker) and Tachinaephagus zelandieus Ashmead. In the western hemisphere, four species predominate on the hosts M. domestica, Stomoxys calcitrans (L.), Fannia canicularis (L.) and F. femoralis Stein. These are M. raptor, S. cameroni, S. endius and S. nigroaenea (Legner, Bay and White 1967). Spalangia spp. have been shown to possess a greater searching capacity than M. raptor or N. vitripennis (Legner 1967). Spalangia spp. were found at greater depths in the manure deposits while M. raptor parasitized more hosts

near the surface. Spalangia spp. also actively searches for higher host densities and would appear to be the preferred biological control agent.

The life history for Spalangia spp. is described by Morgan, Benton and Patterson (1976). "The female is ready to mate and oviposit immediately upon emergence from the host puparium and proceeds through four distinct phases when parasitizing the house fly pupae; namely, finding the host area, finding the fly pupae, drumming and drilling, and ovipositing and feeding. Once she has found the pupa, she systematically examines the surface while drumming with the tips of the antennae. Then she begins tapping with the tips of the antennae, followed by tapping with the tip of the abdomen on the surface of the puparium. This activity apparently places the tip of the ovipositor in place for drilling, a procedure that requires from 10 minutes to 1 hour." For S. cameroni, Gerling and Legner (1968) reported a duration for drumming of up to 10 minutes; for tapping, 1 to 60 seconds; and drilling, 10 to 120 minutes. "When the wall of the puparium is pierced, the entire length of the ovipositor is inserted; and 1 egg is deposited on the developing fly. After depositing the egg, the female withdraws the ovipositor and then obtains nourishment by ingesting the blood of the fly flowing from the oviposition wound. During the next 33-35 days, the wasp develops from an egg to a mature adult and in the process completely destroys the house fly host which had served as a source of food for the developing wasp." While

one larvae on a house fly pupa is normal for S. endius, S. cameroni and M. raptor, Wylie (1971) observed as many as 25 larvae of N. vitripennis maturing on a house fly pupa and Olton and Legner (1974) observed 18 larvae of T. zealandicus matured on one pupa. In the case of S. cameroni and M. raptor, supernumerary individuals in superparasitized hosts are eliminated by cannibalism (Wylie 1971).

The developmental life history of S. cameroni was studied by Gerling and Legner (1968). The egg required 2 days; the first instar larva, 5 days; the second instar larva, 3 days; the third instar larva, 5 days; the prepupa, 2 days and the pupa, 15 days. These are the minimum development times for the male. The female pupation period is three days longer. The larva feeds upon the surface of the host, puncturing the integument with the mandibles and imbibing fluids. The first and early instars prefer to feed under the wing pads of the fly pupa, between the thorax and head or near the legs. The third instar feeding site is usually the dorsum of the fly pupa. The development of S. endius appears to be similar.

In laboratory studies, numerical host-parasite relationships have been worked out. Morgan et al. (1976a) established the mean mortality of the female S. endius to be 3.88 days upon emergence. Each female possesses 24 fully developed eggs. With an average 2.6 progeny per day, a mean total of 10 progeny per female was produced. Legner and Gerling (1967) had similar results with S. cameroni. However, Ables and Shepard (1974) obtained 15-40 progeny with S. endius. Mated

females produce males and females at a ratio of 1:2 while unmated females produce males only. In another study, the relationship of developmental rate of S. endius to temperature was described, simulated and validated (Ables, Shepard and Holman 1976). Ables and Shepard (1974) were able to describe the functional response of S. endius by a linear relationship between attack rate and density of house fly pupae. A model to simulate the control of house flies with S. endius has been developed based on such numerical relationships (Weidhaas et al. 1977).

For successful fly control, the parasite must locate and parasitize the host. Doutt (1959) divided the process of parasitism into the following four phases; host-habitat location, host finding, host acceptance and host suitability. Vinson (1975) proposes a fifth step, host regulation. The host may be found through random searching, but many appear to be directed to the host through a series of physical and chemical cues. The cues reduce the area searched. As long range factors, the cues involved in habitat location would be volatile chemicals (odors) derived from the host's food, the host or a combination of food and host factors. Short range cues from the host would be important in host location. Contact chemicals, those of low volatility or perceived in high concentrations, and non-chemical factors could identify a host.

Host seeking stimulants or kairomones are chemicals which are released and/or produced by one organism that induces a

response by an individual of another species, the response being adaptively advantagous to the receiving organism (Brown, Eisner and Whittaker 1970). The kairomone usually serves the host producing it in some manner. Bryant and Hall (1975) demonstrated theeffects of larval media conditioning on the oviposition of the female house fly and as a density regulator on the microbiota upon which fly larvae feed. The chemical agent(s) involved could be utilized by the parasite in the detection of larvae and narrow the area searched for potential pupal hosts.

The attraction or repulsion to an odor source is divided into its components by Kennedy (1977a). His first division of the responses is between those operating at a distance and those operating at close range, the long range and short range factors already mentioned. His second division is between directed locomotor responses (taxis) and undirected locomotor responses (kineses) relative to the odor source. Frankel and Gunn (1961) discuss the taxic and kinetic response while Dethier et al. (1960) summarizes the types of locomotor responses with which an organism can respond.

Kennedy (1977b) points out the mechanisms involved in distant and close range responses to cues are fairly distinct. For example, concentrated odor gradients permitting a chemotactic response would only be found close to an odor source. The type of response can help discriminate between a distant or close range response which would indicate the stimulus role in host habitat location or host finding. However, the

classical Y-tube olfactometer is a non-behaviorally discriminating assay. The concentrated odor gradients in the olfactometer may involve responses unlikely at a distance in the field and distant responses con not be identified. The olfactometer actually assays the odor for a response. Another drawback is the parasite may not respond to the same stimulus in the same way walking as it does when flying. Despite these drawbacks, the information provided by the olfactometer may give insight into the parasite's host seeking without answering the question of the mechanism.

The basic cue sequence has been demonstrated and the kairomones identified for Cardiochiles nigriceps, a parasite of Heliothis virescens, the tobacco budworm (Vinson 1975). After approaching the plant, the parasite is attracted to damaged plant tissue. If the damage was not caused by the host, scanning of the plant is resumed. But, if the plant was damaged by H. virescens, the female parasite becomes excited and examines the area with her antennae. The female tracks the trail to the host. The host seeking stimulant is from the mandibular gland of the host.

Other parasite species have been attracted to odors from the food plants, larvae of their hosts or host products in an olfactometer (Vinson 1976; McKinney and Pass 1977). The only parasite of filth breeding flies upon which any host seeking work has been done is N. vitripennis. Edwards (1954) and Wylie (1958) found only meat containing or that contained host pupae attractive, but Laing (1937) found just meat

attractive. Nothing definite is known of how S. endius or similar protolean parasitoids find their host's habitat and the host.

Vinson (1975) constructed a model of the factors involved in successful parasitism by C. nigriceps. A similar cue sequence for Spalangia endius together with numerical developmental data will allow the formulation of a population model of the host parasitoid relationship. The model will help evaluate the potential for biological control of the house fly using S. endius.

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Appendix B

Chi-Square Test for Heterogeneity

1. Determine E_{ij}

$$E = \frac{R_i C_j}{N}$$ where R_i = Row totals, i = 1, 2,..., r

C_j = Column totals, j = 1, 2,..., c

2. Determine χ^2

$$\chi^2 = \sum_{ij} \frac{O_{ij}^2}{E_{ij}} - N$$ where O_{ij} = Observed values

3. Compare computed χ^2 to $\chi^2_{(r-1)\,(c-1)}$ in a standard table for the P value.

4. If χ^2 is not significant, then the data for all trials can be pooled and tested for differences in preference with one degree of freedom. The pooled test is more powerful than the cumulative chi-square.

Appendix C

Table 1. Parasite response in the Y-tube olfactometer under different times and air-flow conditions.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Blank	2	4	4	2.024	n. s.	0.61	n. s.
½ Hour	0	2	8				
100 ml/ minute	4	2	4				
	1	1	8				
	4	6	0				
	11	15	24				
Blank vs Blank	3	0	7	2.64	n. s.	3.26	n. s.
½ Hour	1	0	9				
1900 ml/ minute	3	1	6				
	2	1	7				
	2	2	6				
	11	4	35				
Blank vs Blank	7	1	2	4.79	P<.05	5.15	n. s.
½ Hour	2	2	5				
1000 ml/ minute	4	5	3				
	4	6	2				
	4	3	3				
	21	17	15				

Table 1 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Blank	11	7	0	34.56	P<.0001	35.62	P<.0001
1 Hour	9	10	1				
100 ml/ minute	12	7	2				
	13	3	3				
	0	20	0				
	3	14	3				
	48	61	9				
Blank vs Blank	7	6	7	3.93	P<.05	4.34	n. s.
1 Hour	10	4	6				
1900 ml/ minute	9	11	2				
	7	6	7				
	4	7	9				
	9	6	4				
	46	40	35				
Blank vs Blank	8	10	4	4.29	P<.05	4.37	n. s.
1 Hour	7	11	1				
1000 ml/ minute	9	8	1				
	9	7	3				
	12	5	3				
	6	7	7				
	51	48	19				

Table 2. Parasite response in the Y-tube olfactometer to moisture.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Blank	19	18	2	3.99	P<.05	4.54	n. s.
1 Hour	27	16	1				
1000 ml/ minute	21	15	1				
	16	19	2				
	16	20	3				
	15	15	2				
	114	103	11				
Moisture vs Dry	22	20	2	0.07	n. s.	0.45	n. s.
Parasites 12 Hrs.	21	17	2				
Post Emergence	43	37	4				
Moisture vs Dry	25	12	3	0.83	n. s.	4.50	P<.05
Parasites 24 Hrs.	20	15	4				
Post Emergence	45	27	7				
Moisture vs Dry	31	8	1	0.63	n. s.	20.51	P<.0001
Parasites 36 Hrs.	28	11	2				
Post Emergence	59	19	3				

Table 2 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Moisture vs Dry	35	3	3	10.48	P<.005	28.63	P<.0001
Parasites 48 Hrs.	23	15	2				
Post Emergence	58	18	5				
Moisture vs Dry	29	9	3	0.12	n. s.	17.25	P<.0001
Parasites on Honey	24	9	3				
Diet, 36 Hrs.	53	18	6				

Table 3. Parasite response in the Y-tube olfactometer after addition of moisture flasks and parasite conditioning.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Blank	20	20	7	0.96	n. s.	1.27	n. s.
1 Hour	24	17	6				
1000 ml/ minute	19	18	2				
	21	15	1				
	84	70	16				
Blank vs Blank	16	20	3	0.23	n. s.	0.21	n. s.
After minor	20	20	2				
adjustments	36	40	5				
Blank vs Blank	11	22	5	0.03	n. s.	6.25	P<.025
1 Hour	11	20	7				
1900 ml/ minute	22	42	12				
Blank vs Drierite	18	17	3	3.25	n. s.	0.77	n. s.
	17	29	2				
	17	21	6				
	21	17	5				
	73	84	16				

Table 3 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
H_2O vs Drierite	27	25	5	6.82	P<.01	10.07	P<.01
Unconditioned	10	30	3				
Parasites	37	55	8				
H_2O vs H_2O	19	20	3	0.61	n. s.	1.02	n. s.
Unconditioned	16	24	2				
Parasites	35	44	5				
H_2O vs Drierite	16	23	5	1.81	n. s.	0.05	n. s.
Preconditioned	23	18	4				
Parasites	39	41	9				
H_2O vs H_2O	25	15	4	1.11	n. s.	1.42	n. s.
Preconditioned	23	22	4				
Parasites	48	37	8				

Table 4. Parasite response in the Y-tube olfactometer to natural materials.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Reconstituted Freeze Dried Bovine Manure	11 16 27	31 31 62	2 2 4	0.65	n. s.	13.76	P<.001
Blank vs Fresh Bovine Manure	4 15 19	41 25 66	2 5 7	9.99	P<.005	32.92	P<.0001
Blank vs Dry Bovine Manure	14 7 18 18 57	31 35 27 29 122	5 4 5 3 17	6.74	P<.01	29.46	P<.0001
Fresh vs Dry Bovine Manure	30 36 31 36 133	17 8 16 7 48	2 6 3 7 18				

Table 4 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Soil near feedlot	21	17	4	2.14	n. s.	0.54	n. s.
	12	19	9				
	17	22	4				
	20	21	2				
	70	79	19				
Blank vs Corn Ensilage	10	38	4	4.43	P<.05	32.58	P<.0001
	17	30	5				
	8	29	3				
	13	35	2				
	58	132	14				
Blank vs Poultry Feed	24	29	0	0.40	n. s.	2.27	n. s.
	18	28	2				
	42	57	2				
Blank vs Poultry Feed with Numerous House Fly Pupae	12	35	2	3.00	n. s.	23.72	P<.0001
	12	35	2				
	12	20	1				
	14	22	2				
	50	112	7				

Table 4 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Fresh Poultry Manure	13	24	3	1.68	n. s.	29.04	P<.0001
	10	24	3				
	10	29	1				
	9	31	2				
	42	108	9				
Fresh Bovine Manure vs Larvae in Dry Manure	8	40	2	10.09	P<.001	47.01	P<.0001
	9	36	4				
	13	33	3				
	20	26	4				
	50	135	13				
Fresh Bovine Manure vs Dry Manure with Larvae Removed	4	44	2	6.25	P<.025	80.59	P<.0001
	13	37	0				
	8	41	1				
	12	38	0				
	37	160	3				

Table 5. Parasite response in the Y-tube olfactometer to C.S.M.A., house fly larval development and the immature stages of the house fly.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Dry CSMA	18 17 35	25 20 45	3 7 10	0.13	n. s.	1.25	n. s.
Blank vs CSMA Fermented	7 13 20	31 27 58	2 2 4	2.02	n. s.	21.54	P<.0001
Fermented CSMA vs Larvae with CSMA	14 17 17 5 53	34 23 20 36 113	0 7 5 0 12	15.47	P<.0001	32.91	P<.0001
Blank vs Larvae without CSMA	21 17 38	28 32 60	0 1 1	0.69	n. s.	4.93	P<.05
Blank vs Pupae	17 19 36	15 19 34	11 5 16	0.07	n. s.	0.05	n. s.

Table 5 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Pupae Washed as Mature Larvae, Clean Pupation	20 13 33	20 21 41	3 8 11	1.03	n. s.	0.86	n. s.
Blank vs Ammonium Carbonate	16 12 28	33 23 56	1 7 8	0.02	n. s.	9.33	P<.005
Blank vs Parasites	3 22 27	13 14 25	30 10 40	7.95	P<.005	8.02	P<.025
Fermented CSMA vs CSMA with Larvae, Day 1	20 26 46	22 15 37	5 5 10	24.99	P<.0001	3.04	n. s.
Fermented CSMA vs CSMA with Larvae, Day 2	16 16 29 32 93	23 27 15 11 76	6 7 2 2 17	17.246	P<.0001	18.78	P<.001

Table 5 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Fermented CSMA vs CSMA with Larvae, Day 3	16 22 19 23 80	33 25 20 16 94	5 2 1 7 15	6.29	P<.025	7.37	n. s.
Fermented CSMA vs CSMA with Larvae, Day 4	15 17 28 15 75	33 30 21 37 121	1 3 0 1 5	10.43	P<.005	20.65	P<.001
Fermented CSMA vs CSMA with Larvae, Day 5	19 19 21 15 74	24 26 27 30 107	1 1 1 3 6	1.45	n. s.	6.01	P<.025
Fermented CSMA vs CSMA with Larvae, Day 6	16 17 17 21 71	24 20 32 23 99	3 7 0 5 15	1.96	n. s.	4.61	P<.05

Table 6. Parasite response in the Y-tube olfatometer to varying densities of house fly larvae in 100 gram aliquots of manure.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Manure vs Manure, 0 Larvae	24 21 45	26 24 50	0 3 3	0.03	n. s.	0.26	n. s.
Manure vs Manure, 5 Larvae	20 22 42	26 23 49	2 4 6	0.27	n. s.	0.54	n. s.
Manure vs Manure, 25 Larvae	14 9 23	37 28 65	0 12 12	0.11	n. s.	20.05	P<.0001
Manure vs Manure, 50 Larvae	15 20 35	33 29 62	1 0 1	0.96	n. s.	7.56	P<.01
Manure vs Manure, 75 Larvae	15 11 26	35 38 73	0 1 1	0.73	n. s.	22.31	P<.0001

Table 6 Continued.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Manure vs Manure, 150 Larvae	20 17 37	28 30 58	3 2 5	0.32	n. s.	4.64	P<.05

Table 7. Parasite response in the Y-tube olfactometer to house fly larvae reared in aseptic culture.

Test	A	B	No Response	χ^2 Heterogeneity	P	χ^2	P
Blank vs Synthetic Diet	18	32	3	0.219	n. s.	10.78	P<.001
	15	24	11				
	15	30	4				
	48	86	18				
Synthetic Diet vs Synthetic Diet with Larvae	13	33	4	0.294	n. s.	21.48	P<.0001
	15	34	6				
	17	34	2				
	45	101	12				

Appendix D

C.S.M.A. House Fly Rearing Medium

Ingredients	Amount
Dry C.S.M.A.	600 grams
Baker's Yeast	20 grams
Water	500 cc
Diamalt	50 cc

The ingredients are mixed in any suitable container, clay crocks were used by the author. Freshly oviposited house fly eggs (approximately 200-300) are added to the C.S.M.A. formula and held at 25.5°C and 70% relative humidity until pupation. The pupae are removed and caged for fly emergence.

The Fleischmann's Diamalt used is composed of corn syrups, vinegar and fungal enzymes. Instead of diamalt, 15 g malt and 400 cc of evaporated milk may be used.

Appendix E

Aseptic Synthetic Medium for Rearing House Fly Larvae
(Modified from Monroe 1962)

I. Preparation of the Dry Synthetic Medium

Nutrients	Parts by Wt.	Grams
Micropulverized Casein	70.0	140
Alphace (powdered cellulose)	3.0	6
Sodium Oleate	2.0	4
Ribose Nucleic Acid	1.0	2
Wesson's Salts	4.0	8
Agar	20.0	40

The dry ingredients are mixed together in a ball mill for at least one hour.

II. Cholesterol Addition

Two hundred mg of purified cholesterol were dissolved in 100 ml of ether to make a 0.1% cholesterol medium. The medium and another 50 ml ether are added to the cholesterol, stirring in a fume hood, until the solvent is completely evaporated.

III. B-Vitamin Addition

The vitamins are weighed and dissolved in 100 ml of distilled water with the aid of 2-3 drops of concentrated NH_4OH.

B-vitamin	mg
Thiamine hydrochloride	100
Riboflavin	50
Nictoinic Acid	200
Pantothenic Acid	100
Pyridoxine hydrochloride	50
Choline chloride	2000
Inositol	1000
Folic Acid	10
Biotin	10

The B-vitamin solution was added to the medium in 1400 ml of distilled water. The suspension was stirred and heated to near boiling. The medium was then poured into 16 erlenmyer filter flasks (approx. 90 ml each) and stoppered with foam dispo plugs. The stems are plugged with cotton. By using the filtration flasks, the medium can be directly incorporated into the Y-tube olfactometer. The flasks were autoclaved at 15 lbs. pressure (121°C) for 20 minutes. The flasks were swirled to suspend soluble material and immersed in ice water to solidify upon removal from the autoclave. The medium can be stored in a refrigerator until ready for innoculation with house fly eggs.

IV. Innoculation of the Medium with House Fly Eggs

Freshly oviposited house fly eggs were washed several times in distilled water, then surface sterilized in 0.1% sodium hypochlorite for 20 minutes (Dilute 1 ml Clorox, 5.25% sodium hypochlorite, in 50 ml of distilled water for sterilization).

With a sterilized, calibrated pipette approximately 200 eggs were added to seven of the flasks observing proper sterile technique. Seven of the other flasks were the controls for comparison with the flasks containing house fly larvae. The remaining two flasks were for determining if the parasite was attracted to the sterile medium. Sufficient cultures were made to insure three replications, sterile, were obtained.

The cultures were held at 26-28°C for 4-5 days until the third instar larvae had developed before tests in the olfactometer were performed.

Olfactometer Studies of Host Seeking by the Parasite *Spalangia endius* Walker (Hymenoptera : Pteromalidae)

by

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Abstract

Behavioral studies using a Y-tube olfactometer demonstrated that larval house fly media such as bovine and poultry manure were attractive to the parasite Spalangia endius Walker. Fermenting ensilage and C.S.M.A., a fly rearing medium, were also attractive by the chi-square test for significant differences. The presence of a host seeking stimulant was suggested. Manure containing or having contained house fly larvae was favored over fresh manure. Sterile cultures of third instar larvae were found attractive, indicating the attractant was a larval metabolite. Only a larvae and larval medium combination approximately four days old appeared to be consistantly attractive to the parasite as compared to the medium alone. This was probably dependent upon the fly larval density and the concentration of a kairomone substance. A minimum of 25 larvae per 100 grams of manure were found necessary for attraction by the parasite. A kairomone released by the house fly could reduce the host habitat searched by the parasite to that containing potential pupal hosts once the larval medium had been located.