THE EFFECTS OF SUBSTRATE COMPACTION AND PUPAL DEPTH ON PARASITIZATION OF HOUSE FLY PUPAE BY <u>SPALANGIA</u> <u>ENDIUS</u> (WALKER)

by

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Introduction

Parasitic hymenoptera most commonly found attacking muscoid flies in accumulated animal and vegetable wastes are species in the genera <u>Spalangia</u> and <u>Muscidifurax</u>, with some being cosmopolitan in distribution (Ables and Shepard, 1974a; Kogan and Legner, 1970; Legner and Greathead, 1969; Legner and Olton, 1968, 1971; Legner et al., 1967). Most parasites are confined to top layers of the pupal substrate, however, a house fly pupa parasitized by <u>Spalangia endius</u> Walker has been recovered from a depth of 8 inches (20.3 cm) (Morgan et al., 1976). Morgan and Patterson (1975) reported that of all protelean parasites of muscoid flies, <u>S. endius</u> has the best ability to locate and parasitize its host.

Legner (1977) demonstrated that moisture, temperature, and relative humidity have an effect on the ability of <u>S. endius</u> to parasitize its host, but <u>S. endius</u> consistently penetrated to a depth of 4 cm in an experimental wheat flakes habitat. Many authors have remarked about how well <u>S. endius</u> located its host compared to other protelean parasites (Ables and Shepard, 1974b; Legner, 1969, 1977; Morgan et al., 1976; Morgan and Patterson, 1975; Weidhaas et al., 1976) but their statements are not directed to the environment of the house fly.

This study was conducted to determine the ability of <u>S. endius</u> to parasitize the house fly, <u>Musca domestica</u> L., as related to depth of pupae and compaction of the substrate within the host environment. In

addition, pupal depth and substrate compaction were studied to determine the range in which house flies could complete their life cycle.

Materials and Methods

Field Study

The field samples of pupal substrates were taken with a T-shaped probe 2.5 cm dia. in feedlots at Garden City and Hugoton in southwest Kansas and the K.S.U. Beef Research Center at Manhattan, Kansas. Each soil sample was taken to a depth of 30.5 cm when possible. The soil core was divided into 2.5 cm units and house fly pupae were extracted from each unit by floatation and recorded. A total of 65 samples were taken in high density fly pupation areas from four feedlots.

Laboratory Study

Depth and compaction studies were conducted in metal cans 20.25 cm.h. X 17.8 cm dia. A pupal substrate consisting of 1 part freeze dried bovine manure and 2 parts dried corn ensilage was used. This ratio was developed in preliminary experiments. The pupal substrate was mixed in a 208 liter (55 gal.) barrel and aliquots placed in the metal cans. Enough substrate was placed in each container to allow 2 cm of substrate below the lowest pupal depth and 4 cm above the substrate for flight area of the parasites. One hundred house fly pupae (<u>Musca domestica</u> L.) were then placed at one of the following depths: 0, 2, 4, 6, 8, 10, 12, and 14 cm. House flies were reared using the C.S.M.A. procedure. When pupation began, 12-24 hr. old pupae were selected from the population and placed in the substrate at specified depths. The substrate was then compacted at levels of 0, 20.3 X 10⁻³, 40.6 X 10⁻³,

60.9 X 10^{-3} , and 81.2 X 10^{-3} nt/m² by suspending lead bricks of 0, 12, 24, 36, and 48 Kg., respectively, on a compaction device that consisted of a lever press. The lead weights were suspended on the compaction device for 2 minutes for consistency of treatment.

Twenty adult female <u>S. endius</u> were placed on top of the substrate and honey provided ad libitum. Each can was covered with muslin cloth and held in place with 2 strong rubber bands to prevent parasites from escaping. The parasites were supplied by Dr. Philip Morgan, U.S.D.A., Gainsville, Florida.

A Factorial design using 3 replications blocked through time was utilized, with each replication consisting of a combination of 8 depths and 5 compactions. A total of 80 treatments were used, 40 which contained parasites and 40 without parasites. All cans were placed randomly on 3 shelves in a rearing room maintained at 24° C, 50% R.H. The cans were held until all adult flies emerged and the number of adult flies recorded. Pupae were then extracted from the substrate by flotation and those that did not eclose were dissected to determine if parasitization occurred.

Natural mortality was defined as death at 0 Kg compaction and 0 cm depth with no factors influencing adult emergence. Mortality induced by compaction and depth was considered to be pupae that did not eclose combined with adults which eclosed from the pupal case but died before reaching the surface.

The flow charts in fig. 1 illustrate the possible effects compaction and depth exert on house fly pupae with and without the introduction

of parasites. Analysis of data was conducted by Analysis of Variance and Duncan's Multiple Range Test. Fig. I. Flow chart showing effects of substrate compaction and depth of pupae on house fly emergence.

Fig. II. Flow chart showing effects of substrate compaction and depth of pupae on house fly emergence. <u>S. endius</u> introduced.



Figure 1





Results and Discussion

Field Study

Depth of pupae in the substrate and compaction of the substrate are two factors which can affect the success of house fly development. A survey of representative fly developing areas in Kansas feedlots showed 91.7% of the pupae sampled were found between depths of 2 and 7.5 cm with the optimum being 5 cm (Fig.III). Determination of the degree of compaction of field samples was not feasible. The apparent reasons for flies pupating below the surface are to reduce desication and/or to reduce the possibility of attack by predators and parasites. In feedlots fly pupae are found along feed bunks, fence rows, and in pens where cattle do not step. These areas are characteristically undisturbed and moist.

Depth and Compaction without Parasites

The effects of compaction and depth without parasites on house fly pupae are shown in tables 1 and 2. As compaction and depth increase the % induced fly mortality increases and the % adult fly emergence decreases. In table 1 the upper levels of compaction and depth (48 kg and 14 cm, respectively) produced a mortality of 86.67% and the lower level, 0 compaction and 2 cm depth, produced a mortality of 19.67% which indicates the treatment range is adequate for this study.

Depth and Compaction with Parasites

The effects of compaction and depth on the ability of <u>S.</u> endius to parasitize its host are shown in tables 3, 4, and 5. As compaction Fig. III. House fly pupation in feedlots at various depths.



Figure III

		Com	paction	(kg)			
Depth (cm)	0	12 24 36 % Induced Mortality		36 ality	48	Depth $\frac{11}{X}$	
0	9.7	-	-	-	-		
2	19.7	19.8	40.5	45.5	57	36.5	a
4	20.0	51.3	44.1	62.3	60.3	47.6	ab
6	44.7	59.7	64.0	59.7	68.7	58.7	ь
8	51.1	57.4	58.7	60.5	66.0	59.3	bc
10	29.3	53.9	65.3	65.6	83.2	62.1	bc
12	41.6	67.3	67.3	76.1	93.3	66.5	cd
14	50.1	75.7	82.2	83.3	86.7	75.6	d
Compaction $\frac{11}{X}$	36.63	54.99	60.32	64.72	73.61		
	а						
		ь	ь	b c	с		

Table 1. Average percent house fly mortality at different levels of substrate compaction and depth of pupae.

Percents followed by different letters are different at 5% level of significance.

Depth (cm)	0	12 % Adult	24 Fly Em	36 ergence	48	Depth $\frac{1}{X}$
0	90.3	-	-	-	-	
2	80.3	80.2	59.8	54.4	43.0	63.6 a
4	80.0	48.7	55.9	37.7	39.7	52.4 ab
6	55.3	40.3	36.6	40.3	31.3	41.3 bc
8	48.9	42.6	41.3	39.5	34.0	40.7 bc
10	70.7	32.7	34.7	34.4	16.8	37.9 c
12	58.4	56.1	32.7	20.5	6.7	32.9 cd
14	49.9	24.3	17.7	16.7	13.3	24.4 d
$\frac{11}{X}$	63.37	45.0	39.73	34.8	26.4	
	а	b	b			
			с	c d	d	

Table 2. Average percent adult house fly emergence at different levels of substrate compaction and depth of pupae.

¹Percents followed by different letters are different at 5% level of significance.

		Compaction (kg)						
Depth (cm)	0	12 % Ind	12 24 36 % Induced Mortality		48	Depth $\frac{11}{X}$		
0	1.7	-	-	-	-			
2	14.3	26.8	29.0	41.5	40.8	30.5 a		
4	28.1	61.3	62.5	65.0	65.4	56.5 b		
6	35.8	45.5	61.7	54.9	66.5	52.9 b		
8	30.3	48.0	60.4	74.6	63.4	55.4 b		
10	38.9	61.0	68.6	71.9	76.5	63.4 bc		
12	42.3	70.4	74.0	82.0	95.3	72.8 c		
14	48.6	71.0	73.0	81.7	92.3	73.3 c		
Compaction $\frac{1}{X}$	34.04	52.38	61.33	67.38	71.48			
	а	ь	ь					
			c	с	с			

Table 3. Average percent house fly mortality at different levels of substrate compaction and depth of pupae. <u>S. endius</u> introduced.

¹Percents followed by different letters are different at 5% level of significance.

		Com	paction	(kg)			
Depth (cm)	0	12 % Par	24 asitized	36 Pupae	48	Depth	$\frac{11}{x}$
0	93.7	-	-	-	-		
2	70.3	23.4	34.4	13.7	3.7	29.1	a
4	38.8	5.3	•7	1.3	1.3	9.5	ь
6	6.1	0.0	.7	0.0	.7	3.1	bc
8	13.5	1.0	.7	• 3	0.0	1.5	с
10	0.0	0.0	.3	0.0	0.0	.4	с
12	0.0	.4	.3	• 3	0.0	.1	с
14	.7	0.0	0.0	0.0	1.0	.1	с
Compaction $\frac{11}{X}$	18.49	8.64	5.3	2.24	.96		
	а	b	Ь	_			

Table 4.	Average percent house fly pupae parasitized by S. endius at	
	different levels of substrate compaction and depth of pupae	

Percents followed by different letters are different at 5% level of significance.

Depth (cm)	0	12 24 36 % Adult Fly Emergence		48	Depth $\frac{11}{X}$	
0	4.6	-	-	-	-	
2	15.3	50.2	36.6	44.8	55.5	40.5 a
4	33.1	38.1	36.8	33.7	33.2	35.0 ab
6	58.1	54.5	37.6	45.1	32.8	45.6 a
8	56.2	51.0	38.9	25.0	36.6	41.5 a
10	61.1	39.0	31.0	28.5	23.5	36.6 ab
12	57.6	28.5	25.7	17.7	3.7	26.6 ь
14	50.8	29.0	27.0	18.3	7.7	26.5 Ь
$\frac{11}{\text{Compaction X}}$	47.47	39.57	33.37	30.43	27.57	
	а	ь	b			
			с	с	с	

Table 5. Average percent house fly emergence at different levels of substrate compaction and depth of pupae. <u>S. endius</u> Introduced.

 $^{1}\mbox{Percents}$ followed by different letters are different at 5% level of significance.

and depth increase the ability of <u>S. endius</u> to parasitize house fly pupae decreases. The analysis for percent induced mortality showed depth and compaction to be significant factors. Greatest differences were between means of 2 to 4 cm depth and 0 to 12 kg compaction. The differences beyond these levels were not as great and this is shown with Duncan's test (table 3). The introduction of <u>S. endius</u> did not have a significant impact on induced mortality.

The analysis of percent parasitized pupae showed an interaction between depth and compaction. Parasitism was best achieved with zero compaction not deeper than 4 cm. These results coincide with Legner (1977) as he discovered <u>S. endius</u> consistently penetrated a wheat flake substrate to a depth of 4 cm. Morgan et al. (1976) found a pupa which was parasitized by <u>S. endius</u> at a depth of 8 in (20.32 cm) which also coincides with this study because parasitized pupae were found at depths of 14 cm (5.5 in) with a compaction of 48 kg, however, no significant results were recorded at these levels. Compactions greatly reduced the effectiveness of the parasite and significant parasitism occurred only to a compaction level of 12 kg and not deeper than 2 cm. The interaction occurred between depth and compaction because increases of both compaction and depth compounded the problem <u>S. endius</u> encountered in penetrating the substrate.

The analysis of % adult fly emergence indicated depth and compaction were significant variables and also showed an interaction. Duncan's test could not detect significantly different ranges of fly emergence because of the impact <u>S. endius</u> had on emerging flies at low

compaction and depth levels. At low levels of compaction and depth a low level of emergence was achieved and as the compaction at low depths increased adult fly emergence also increased (table 5). These results are caused by the penetration and parasitization ability of <u>S. endius</u>. <u>S. endius</u> had no effect on adult fly emergence at depths of 6 cm and beyond and adult fly emergence in this range was comparable to the unparasitized study (table 2).

In feedlot situations a 435 kg steer would produce approximately 3 times more compaction on a dry substrate than was produced in this study. This is one reason house fly pupae are not found in the middle of feedlot pens. Compaction of the pupal substrate could be achieved in several ways: by a highway roller, by a sheeps-foot roller, by using portable pens and moving them to allow cattle to walk on the substrate which is along fence rows, or by a combination of these. Adult fly emergence can be reduced with compaction of the pupal substrate.

The release of <u>S. endius</u> in areas where compaction is not feasible will also reduce adult fly emergence. Areas refered to are around feed bunks, drainage ditches, and permanent fence rows. Further studies of compaction and the release of <u>S. endius</u> need to be continued in feedlots to determine their feasibility in controlling house flies.

Literature Cited

- Ables, J. R. and M. Shepard. 1974a. Hymenopterous parasitoids associated with poultry manure. Env. Entomol. 3(5):884-886.
- Ables, J. R. and M. Shepard. 1974b. Responses and competition of the parasitoids <u>Spalangia endius</u> and <u>Muscidifurax</u> raptor (Hymenoptera: Pteromalidae) at different densities of house fly pupae. Canadian Entomol. 106:825-830.
- Kogan, M. and E. F. Legner. 1970. A biosystematic revision of the genus Muscidifurax (Hymenoptera: Pteromalidae) with descriptions of four new species. Canadian Entomol. 102:1268-1290.
- Legner, E. F. 1969. Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. Ann. Entomol. Soc. Am. 62:220-226.
- Legner, E. F. 1977. Temperature, humidity and depth of habitat influencing host destruction and fecundity of muscoid fly parasites. Entomophaga 22 (2):199-206.
- Legner, E. F. and D. J. Greathead. 1969. Parasitism of pupae of East African populations of Musca domestica and <u>Stomoxys</u> calcitrans. Ann. Entomol. Soc. Am. 62:128-133.
- Legner, E. F. and G. S. Olton. 1971. Distribution and relative abundance of dipterous pupae and their parasitoids in accumulations of domestic animal manure in the Southwestern United States. Hilgardia 40:505-535.
- Legner, E. F. and G. S. Olton. 1968. Activity from parasites from Diptera <u>Musca domestica</u>, <u>Stomoxys calcitrans</u> and species of Fannia, Muscina, and Ophypa. 11. At sites in eastern hemisphere and Pacific area. Ann. Entomol. Soc. Am. 61:1306-1314.
- Legner, E. F., E. D. Bay, and E. B. White. 1967. Activity of parasites from Diptera: <u>Musca domestica</u>, <u>Stomoxys</u> <u>calcitrans</u>, <u>Fannia conicularis</u>, and <u>Fannia femoralis</u>, at sites in the western hemisphere. <u>Ann. Entomol. Amer.</u> 60:462-468.
- Morgan, Philip B., A. Benton, and R. S. Patterson. 1976. The potential use of parasites to control flies in the Caribbean area. Virgin Islands Agric. and Food Fair, Feb. 14-16. 43 pp.

- Morgan, Philip B. and R. S. Patterson. 1975. Possibilities of controlling stable flies and house flies with protelean parasitoids. 46th Ann. Meeting, Florida Anti-Mosquito Association, April 13-16, pp. 29-35.
- Weidhaas, D. E., D. G. Hale, P. B. Morgan, and G. C. LaBrecque. 1976. A model to simulate control of house files with a pupal parasite, <u>Spalangia endius</u>. Environ. Entomol. 6(4):489-500.

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Abstract

Comparisons of various levels of compaction and depth of a simulated substrate were studied to determine the ability of <u>Spalangia</u> <u>endius</u> (Walker) to parasitize house fly pupae (<u>Musca domestica</u> L.). The optimum adult house fly emergence occurred from depths of 2-4 cm at all compaction levels. As compaction and depth increased, mortality induced by these factors also increased but adult fly emergence decreased. The introduction of <u>S. endius</u> to the treatment showed a significant reduction of emerging adult flies at lower levels of compaction and depth, however, there was no significant reduction in fly mortality. Compaction influenced the penetration ability of <u>S. endius</u> and no significant parasitism occurred in compaction and depth levels greater than 12 kg and 2 cm, respectively. As compaction and depth increased, the penetration and parasitization ability of <u>S. endius</u> decreased. Optimum penetration and parasitism occurred at a compaction level of 0 kg and depth of 4 cm. APPENDICES

APPENDIX A

REVIEW OF LITERATURE

Review of Literature

Control of disease-bearing or annoying insects has depended on a variety of methods including sanitation, source reduction, other physical or mechanical measures, insecticides, quarantines, and naturallyoccurring biotic agents (LaBrecque, et al., 1975). When insecticides were developed they rapidly reduced dense populations but resulted in other problems such as resistance of insects to insecticides and environmental residues. The over-use of insecticides has led researchers to an integrated approach of control and along with this more emphasis is being placed on biological control.

<u>Spalangia endius</u> (Walker) is a hymenopterous protelean parasitoid of muscoid flies. An insect can be termed protelean and parasitoid only if the immature stages are parasitic and only if the parasitism results in death of the host (Askew, 1971). Other members of this group are <u>S.</u> <u>cameronia</u> (Perkins), <u>S. nigra</u> (Latreilla), <u>S. nigroaenes</u> (Curtis), <u>Muscidifurax raptor</u> (Girault and Sanders), <u>Mormonella vitripennis</u> (Walker), <u>Pachyrepcideus vindemiae</u> (Rondani), <u>Nasonia vitripennis</u> (Walker), and <u>Tachinaephagus zealandieus</u> (Ashmead). There are predominantly four species in the western hemisphere which use <u>M. domesticus</u> (L.), <u>Stomoxys</u> <u>calcitrans</u> (L.), <u>Fannia canicularis</u> (L.), and <u>Fannia femoralis</u> (Stein) as hosts. These parasites are <u>M. raptor</u>, <u>S. cameroni</u>, <u>S. endius</u>, and <u>S.</u> <u>nigroaenea</u> (Legner, et al., 1967). Of these four parasites <u>S. endius</u> is seriously being considered for control of the stable fly (<u>Stomoxys calcitrans</u> L.) and the house fly (<u>Musca domestica</u> L.) because of its host

specificity, ease of rearing, low cost of production, and safety to the environment when compared to chemical control. A million parasites can now be produced for \$500.00 with the major cost due to labor and materials required to rear the host (Morgan, et al., 1976b). According to Morgan and Patterson (1975a) <u>Spalangia endius</u> (Walker) has the best ability to find and parasitize its host when compared to all other protelean parasites of muscoid flies.

Life History

The female <u>S. endius</u> is ready to mate and oviposit immediately upon emergence of the host pupal case. When parasitizing pupa the female proceeds through 4 distinct phases: finding the host area, finding the fly pupae, drumming and drilling, and ovipositing and feeding. Once the pupa is located, she examines the surface by drumming and tapping it with the tips of her antennae (Morgan, et al., 1976a). If she is satisfied, she taps the puparium with her abdomen which places the tip of the ovipositor into position for drilling. However, Legner and Gerling (1967) report there may be detection of a previously attacked host by some external stimuli and therefore rejection may occur.

Oviposition will take from 10 min. to 1 hr. and when she pierces the puparium, one egg is deposited between the pupal case and fly pupa. After oviposition the female obtains proteins necessary for optimum egg production by ingesting exuviants of the ovipositional wound (Gerling and Legner, 1968). Within 33-35 days the parasite will develop from egg to adult and completely destroy the developing fly (Morgan and Patterson, 1975a; Edwards, 1955).

Larval Development

Development of the embryo requires 2-3 days, after which the fully formed 1st-instar larva is clearly visible through the chorion. Eclosion is through the anterior of the egg. Once eclosed, the larva stands erect on its hind segments on the host and moves its head and thorax to and fro before crawling.

The first-instar larva has 13 body segments and 9 pairs of spiracles; its body is about 1 mm long and tapers from the third thoracic segment to the pointed last abdominal segment. The cuticle is translucent and the digestive tract, particularly the light-brown mandibles and gut, is clearly visible.

Feeding sites of the first and second instar larvae are under the wing pads of the fly pupa, in the cleft between the thorax and head, or near the legs rather than on the dorsal abdominal and thoracic regions. The second-instar larva develops from the 7th to 10th day after the egg is deposited. Like the first-instar it has 13 body segments and 9 pairs of spiracles. The mouth appendages include a pair of mandibles and a ring-like sclerite which provides apodemes for mandibular articulation.

The third-instar larva is easily recognized by 2 longitudinal rows of 11 tubercles, a row latero-dorsally on each side of the body. Large mandibles and other oral sclerites are clearly visible and can be studied in detail after being shed. Most third instar larvae feed on the dorsum of the fly pupa and often more than 100 brown feeding marks on the house fly pupa were observed by Gerling and Legner (1968). The third instar larva terminates its wanderings on the dorsal service of the thoracic and cephalic regions where it transforms into a pupae. The pupal stage averages 15-18 days depending on whether male or female. Future adult appendages are recognizable in young pupa and melanization occurs gradually. Pupae are usually white until the 11th day and then melanization occurs. Male pupae are usually melanized by the 10th day but females are usually still white until the 11th day.

Morgan and Patterson (1975a) concluded that the average progeny of <u>S. endius</u> is 9.4-9.6 per day on 1-2 day old house fly pupae and has the potential of parasitizing 36.7 hosts in its life span. The total number of pupae parasitized by a female <u>S. endius</u> is directly correlated with the density and size of the host (Wylie, 1967; Legner, 1969). Each female when mated produces a sex ratio of 2 females to 1 male. Unmated females only produce male offspring and the average life-span of a female is 3.88 days (Morgan, et al., 1976a).

<u>S. endius</u> is most abundant and active during the hottest periods of the year (Legner and Brydon, 1966). During this period (June to September), it predominates because of high survival rates and good searching capacity (Legner, 1967; Ables and Shepard, 1974; Ables, et al., 1976). However, <u>S. endius</u> is not effective during the early spring and fall because of its low tolerance of cold (Legner and Brydon, 1966).

Many studies have been conducted with the release of <u>S. endius</u> in areas where high populations of house flies exist (Morgan, et al., 1976b; Morgan and Patterson, 1975b; Morgan, et al., 1975; Mourier, 1972). Monty (1972) concluded that even though <u>S. endius</u> was well established within a population of house flies, it still could not maintain itself at densities high enough to effectively control fly populations. Morgan

et al. (1976b) solved this problem by weekly releases of laboratory reared parasites and was able to completely suppress a house fly population in a poultry house within 35 days. Many other reports have been published to substantiate the effectiveness of <u>S. endius</u> in controlling house fly populations (Ables and Shepard, 1976; Legner and Brydon, 1966; Legner and Detrick, 1972; Legner and Greathead, 1969; Legner, et al., 1965; Morgan, et al., 1975a; Weidhaas, et al., 1976).

Past failures in the release of <u>S. endius</u> occurred when inadequate numbers were released because of poor estimation of the natural fly population (Knipling, 1972). Population sampling and estimation of populations are areas which need attention before effective control of the house fly can be obtained with the release of parasitoids.

Bibliography

- Ables, J. R. and M. Shepard. 1976. Seasonal abundance and activity of indigenous hymenopterous parasitoids attacking the house fly (Diptera: Muscidae). Can. Entomol. 108(8):841-844.
- Ables, J. R. and M. Shepard. 1974. Responses and competition of the parasitoids <u>Spalangia endius</u> and <u>Muscidifurax raptor</u> (Hymenoptera: Pteromalidae) at different densities of house fly pupae. Can. Entomol. 106:825-830.
- Ables, J. R., M. Shepard and J. R. Holman. 1976. Developments of the parasitoids <u>Spalangia</u> <u>endius</u> and <u>Muscidifurax</u> <u>raptor</u>, in relation to constant and variable temperature simulation and validation. Environ. Entomol. 5(2):329-332.
- Askew, R. R. 1971. <u>Parasitic Insects</u>. American Elsevier Publication Company, Inc., New York. p. 316.
- Edwards, R. L. 1955. The host finding and oviposition behavior of <u>Mormoniella vitripennis</u> (Walker) (Hymenoptera: Pteromalidae), a parasite of muscoid flies. Behavior 7:8-112.
- Gerling, D. and E. F. Legner. 1968. Developmental history and reproduction of <u>Spalangia cameroni</u>, parasite of synanthropic flies. Ann. Entomol. Soc. Am. 61:1436-1443.
- Knipling, E. F. 1972. Entomology and the management of man's environment. Aust. Entomol. Soc. 11:153-167.

- LaBrecque, G. C., D. L. Bailey, D. W. Meifert, and D. W. Weidhaas. 1975. Density and mortality rate evaluations of stable fly (<u>Stomoxys calcitrans</u> L.) populations in field cages. (Diptera: Muscidae). Can. Entomol. 107:597-600.
- Legner, E. F. 1969. Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. Ann. Entomol. Soc. Am. 62:220-226.
- Legner, E. F. 1967. Behavior changes in the reproduction of <u>Spalangia</u> <u>cameroni</u>, <u>S. endius</u>, <u>Muscidifurax</u> <u>raptor</u>, and <u>Nasonia vitripennis</u> (Hymenoptera, Pteromalidae) at increasing fly host densities. Ann. Entomol. Soc. Am. 6:819-826.
- Legner, E. F. and H. W. Brydon. 1966. Suppression of dung-inhabiting fly populations by pupal parasites. Ann. Ent. Soc. Am. 59:638-651.
- insects to control filth breeding flies in California. Proc. 40th Annu. Conf. Calif. Mosquito Control Assoc., Inc. pp. 129-130.

Legner, E. F. and E. J. Detrick. 1972. Inundation with parasitic

- Legner, E. F. and D. Gerling. 1967. Host feeding and oviposition on <u>Musca domestica by Spalangia cameroni, Nasonia vitripennis</u>, and <u>Muscidifurax raptor</u> (Hymenoptera: Pteromalidae) influences their longevity and fecundity. Ann. Entomol. Soc. Am. 60:678-691.
- Legner, E. F. and D. J. Greathead. 1969. Parasitism of pupae of East African populations of <u>Musca domestica</u> and <u>Stomoxys</u> <u>calcitrans</u>. Ann. Entomol. Soc. Am. 62:128-133.
- Legner, E. F. and E. D. Bay, and C. W. McCoy. 1965. Parasitic natural regulatory agents attacking <u>Musca</u> <u>domestica</u> L. in Puerto Rico. J. Agric. Univ. P. R. 49:368-376.

- Morgan, Philip B. and R. S. Patterson. 1975a. Possibilities of controlling stable flies and house flies with protelean parasitoids. 46th Ann. Meeting, Florida Anti-Mosquito Association, April 13-16. pp. 29-35.
- Morgan, Philip B. and R. S. Patterson. 1975b. Field parasitization of house flies by natural populations of <u>Pachycrepoideus vindemiae</u> (Rondani), <u>Muscidifurax raptor</u> (Girault and Sanders), and <u>Spalangia</u> <u>nigroaenea</u> (Curtis). The Fla. Entomol. 58(3):202.
- Morgan, Philip B., R. S. Patterson, and G. C. LaBrecque. 1976a. Hostparasitoid relationship of the house fly, <u>Musca domestica</u> L., and the protelean parasitoid, <u>Spalangia endius</u> (Walker). J. Kans. Entomol. Soc. 94:483-488.
- Morgan, Philip B., R. S. Patterson and G. C. LaBrecque. 1976b. Controlling house flies and stable flies at a dairy installation by releasing a protelean parasitoid, <u>Spalangia endius</u> (Hymenoptera: Pteromalidae). J. Georgia Entomol. Soc. 11(1):39-43.
- Morgan, Philip B., G. C. LaBrecque, D. E. Weidhaas, and A. Benton. 1975. Suppression of field populations of house flies with <u>Spal</u>angia endius. Science 189:388-389.
- Mourier, H. 1972. Release of house flies on Danish farms. Vidensk. Medd. Dan. Naturhist. Foren. 135:129-137.
- Weidhaas, D. E., D. G. Hale, P. B. Morgan, and G. C. LaBrecque. 1976. A model to simulate control of house flies with a pupal parasite, <u>Spalangia endius</u>. Environ. Entomol. 6(4):489-500.

Wylie, H. G. 1967. Some effects of host size on <u>Nasonia vitripennis</u> and <u>Muscidifurax raptor</u> (Hymenoptera: Pteromalidae). Can. Entomol. 99:742-748. APPENDIX B

PRELIMINARY STUDIES

Optimum Moisture Content

Materials and Methods

A preference test was utilized to determine the optimum moisture content for house fly pupation. Ten grams of freeze dried bovine manure was reconstituted to one of the following moisture contents: 80, 60, 40, 20 and 0%, and all moisture contents placed in a plastic container 15.24 cm dia X 3.81 cm.h. The different moisture contents were separated by aluminum foll boundaries which did not extend high enough to limit migration of the larvae. A 35 mm. dia. X 10 mm.h. petri dish, containing saturated C.S.M.A. and 25 third-instar house fly larvae, was placed in the center of the reconstituted manure. The C.S.M.A. was saturated with distilled water to force the larvae from the petri dish into the reconstituted manure.

A plastic lid with a 2.5 cm dia. hole in the middle was used to cover each container and regulate the humidity within the container. The treatments were placed in a growth chamber and held at 26° C and 60% R.H. until all larvae had pupated for 24 hrs. The house fly pupae were removed from each substrate by flotation and recorded. Six replications, blocked by treatments, were utilized and analysis was conducted by Analysis of Variance and Duncan's Multiple Range Test.

Results

The analysis showed a significant difference at the .05 level between moisture contents; however, the Duncan's test could not distinctly separate moisture contents. This happened because some of the

larvae would pupate on top of the substrate and not within it at high moisture contents (table 5).

Another study was conducted in the same manner as the previous test, using 9 replications and reconstituted manure with 0, 20, and 40% moisture contents. The analysis of this data showed significant difference between 0% and 20% moisture contents but not between 20% and 40% moisture contents. This study showed that house flies will pupate in any moisture content of the substrate, however, a dry substrate is most preferred (table 6).

		%	Moisture Co	ontent	
Replication #	0	20 N	40 o. Pupae/sub	60 strate	80
1	9	0	2	1	1
2	12	4	1	1	3
3	2	4	4	2	0
4	6	3	6	7	0
5	5	2	12 *	3	0
6	8	3	7	0	1
\overline{x}^{1}	7.0	5.3 e	2.7	2.3	.8
	а	b c	a b	b c	с

Table 1. Number of pupae found in each different moisture content.

¹Percents followed by different letters are different at 5% level of significance.

	% Moisture Content						
Replication #	0	20 No. Pupae/substrate	40				
1	6	2	15				
2	14	1	4				
3	14	2	6				
4	20	0	1				
5	15	8	0				
6	18	4	2				
7	14	8	3				
8	11	- 14	0				
9	12	11	2				
$\frac{11}{x}$	13.8	5.6	3.				
	а	Ь	b				

Table 2. Number of pupae found in each different moisture content.

 $^{1}\mathrm{Percents}$ followed by different letters are different at 5% level of significance.

Optimum Pupal Substrate for Parasitism

Materials and Methods

Four types of pupal substrate, soil and manure, soil and ensilage, manure and ensilage, and manure and ensilage and soil, were utilized in this study. The substrates were made by mixing equal parts, by volume, of each material. Each substrate was placed in metal cans 17.8 cm dia. X 20.5 cm.h. to a depth of 3 cm and 100 house fly pupae, 12-24 hr. old, were placed at a depth of 1 cm within the substrate. House flies were reared using the standard C.S.M.A. technique.

Twenty female <u>S. endius</u> were placed on the surface of the substrate along with a droplet of honey to supply nurishment for the parasites while searching for house fly pupae. Each metal can was covered with muslin cloth and held in place with 2 strong rubber bands to keep parasites from escaping.

Four replications were utilized and each replication was blocked by treatments. A total of 32 cans, 16 with parasites and 16 without parasites, were placed randomly on 3 shelves in a rearing room and held at 23° C with 50% R.H. The treatments were held until all adult flies emerged and died and adult fly emergence was recorded. Pupae which did not eclose were extracted from the substrate by floatation and dissected to determine if parasitism occurred. Analysis of the data was conducted by Analysis of Variance and Duncan's Multiple Range Test.

Results

The analysis showed parasitism was significantly different between the pupal substrates. Duncan's test showed the best parasitism was achieved with a substrate composed of ensilage and freeze dried manure (table 7).

Substrate	1 2 3 4 % Pupae Parasitized				$\frac{1}{x}$	
Ensilage & Manure	97	95.9	97	92.1	95.5	a
Ensilage & Soil & Manure	82.1	86	85.1	80	83.3	Ь
Ensilage & Soil	76.8	70	75.5	49	67.8	с
Soil & Manure	5	4	3.2	7	4.8	d

Table 3.	Percentage	of pupae	parasitized	by s	S. endius	in	various	com-
	binations of	of substra	ate.					

 $^1\mathrm{Percents}$ followed by different letters are different at 5% level of significance.

				Dept	h of P	upae (cm)		
Sample #	2.5	5.0	7.5	10 Numbe	12.5 er of P	15.0 upae Fou	17.5 nd	20.0	22.5
1	2	2	1	0	0	0	0	0	0
2	2	0	1	0	0	0	0	0	0
3	5	5	0	1	0	° 0	0	0	0
4	2	17	1	0	0	0	0	0	0
5	4	6	1	0	0	0	0	1	0
6	0	7	0	0	0	0	0	0	0
7	3	15	6	1	0	0	3	0	0
8	15	16	1	0	0	1	0	0	0
9	29	3	1	1	0	0	1	0	0
10	0	7	1	0	1	0	0	0	0
11	13	12	0	0	0	0	0	0	0
12	6	6	4	0	1	1	0	0	0
13	1	18	0	0	0	0	0	0	0
14	11	4	8	2	0	0	0	0	0
15	1	11	0	0	0	0	0	0	0
Total	94	131	25	5	1	2	4	1	0
x	6.2	8.73	1.6	- 33	.067	. 133	.267	.067	0

Table 4. Number of pupae found in 2.5 cm units at Redd Beef Feeders in Hugoton, Kansas.

				Dep	th of P	upae (cm)		
Sample #	2.5	5.0	7.5	10 Numb	12.5 er of Pi	15.0 upae Fou	17.5 nd	20.0	22.5
1	5	6	5	1	2	1	0	0	0
2	0	6	1	2	0	0	0	0	0
3	16	8	6	0	0	0	0	0	0
4	11	6	6	2	1	0	0	0	0
5	4	1	6	16	1	0	-	-	-
6	2	12	9	1	-	-	-	-	-
7	2	1	0	1	-	- ,	-	-	-
8	2	4	0	0	-	-	- 1	-	-
9	1	3	1	1	-	-	-	-	-
10	1	14	2	2	0	0	0	0	0
11	0	8	1	1	-	-	-	-	-
12	2	5	1	3	0	0	0	0	0
13	22	8	1	0	0	0	0	0	0
14	4	2	2	3	0	0	0	0	0
15	2	2	2	1	3	2	1	0	0
16	3	6	3	0	-	-	-	-	-
17	5	2	0	0	0	0	-	-	-
18	3	7	3	2	1	1	0	1	0
19	4	11	7	2	0	0	0	0	0
20	2	6	4	1	-	-	-	-	-
21	6	12	4	3	0	0	0	0	0
22	9	11	3	3	2	1	0	0	0
23	2	2	-	1.1	-	+ -	-	-	-
24	3	2	0	0	-	-	-	-	-
25	7	9	4	0	-	-	-	-	-
26	2	2	0	0			-	_	-

Table 5. Number of pupae found in 2.5 cm units at the Beef Research Center at K.S.U., Manhattan, Kansas.

				Dep	th of Pu	upae (cr	n)		
Sample #	2.5	5.0	7.5	10 Numbe	12.5 er of Pu	15.0 upae Foi	17.5 und	20.0	22.5
27	0	4	3	0	-	-	-	-	-
28	3	6	3	0	-	-	-	-	-
29	5	2	0	0	0	0	0	0	0
30	7	10	4	2	0	1	0	0	0
Total	135	178	81	47	13	6	1	1	0
x	4.5	5.9	2.79	1.6	.62	• 35	.067	.067	0

		_							
				Dept	th of Pup	ae (cm)		
Sample #	2.5	5.0	7.5	10 Numbe	12.5 er of Pup	15.0 ae Fou	17.5 nd	20.0	22.5
1	5	4	0	0	-	- 1	-	-	-
2	9	11	2	3	0	0	-	-	-
3	3	4	0	0	0	-	-	-	-
4	1	0	2	0	0	0	-	-	-
5	1	9	2	2	0	0	-	-	-
6	0	5	2	0	0	-	-	-	-
7	0	1	3	0	0	0	0	0	0
8	7	5	1	0	0	0	-	-	-
9	4	8	3	1	0	0	0	0	0
10	6	9	7	3	1	0	0	0	0
Total	36	56	22	9	1	0	0	0	0
x	3.6	5.6	2.2	.9	.11	0	0	0	0

Table 6. Number of pupae found in 2.5 cm units at Finney Co. Feedyard, Garden City, Kansas.

				Dep	th of Pu	pae (cm)		
Sample #	2.5	5.0	7.5	10 Numb	12.5 er of Pu	15.0 pae Fou	17.5 nd	20.0	22.5
1	3	1	0	-	-		-	-	-
2	0	2	3	1	0	0	0	0	0
3	8	27	17	6	1	0	0	0	0
4	3	4	2	3	3	0	0	0	0
5	2	0	1	1	2	0	-	-	-
6	1	5	0	0	-	-	-	-	-
7	4	9	3	1	0	0	-	-	-
8	2	1	4	1	0	0	0	0	0
9	6	11	4	3	1	0	0	0	0
10	1	3	3	2	0	1	0	0	0
Total	30	63	37	18	7	1	0	0	0
x	3.0	6.3	3.7	1.8	• 7	.1	0	0	0

Table 7. Number of pupae found in 2.5 cm units at Bonita Beef, Garden City, Kansas.

Data for study of substrate compaction and pupal depth effects on the ability of \underline{S} . <u>endius</u> to parasitize house fly pupae. Table 8.

					Paras	sitized			Control	
ompaction	(kg)	Depth	(cm)	Pupae Recovered	F1y Emergence	Nat. Mortality	Number Parasitized	Pupae Recovered	F1y Emergence	Nat. Mortallty
0		0		100 100	0 0 41	- 52	98 88 88	99 100	91 86 93	8 14 7
o		2	•	100	39	3 29 11	97 64 50	100	79 74 88	21 26 12
0		4 .		99 100	34 41 24	37 40 7	28 19 69	100 100	66 85 89	34 15 11
0		9		97 98 100	43 46 83	42 52 11	12 6 0 2	100 104 100	29 52 87	71 52 13
0		¢	•	96 001 99	19 61 87	40 38 11	37	97 100 99	28 37 80	69 63 19
0		10		101	29 84	- 30 71 16	000	98 100	57 73 81	41 27 19
•		12		94 100	47 41 82	47 59 18	000	97 99 99	49 36 87	48 62 12
0		11		96 100	55 22 73	41 78 25	007	94 99 100	23 37 88	71 62 12

				Par	rasitized			Control	
Compaction (kg)	Depth ((cm)	Pupae Recovered	F1y Emergence	Nat. Mortality	Number Parasitized	Pupae Recovered	Fly Emergence	Nat. Mortality
12	•		96 100	0 21	0=1	96 98 72	100 100 100	68 68 416	119
12	2		99 100 100	45 45 66	36 34 10	19 27 24	100 100 98	81 77 81	19 23 17
12	4		. 95 100	4 26 84	95 62 22	0 12 4	98 100	10 46 90	88 54 10
12	ę		95 98 99	0 78 83	95 20 16	000	93 100 100	. 39 82	93 61 18
12 .	ω		95 100 100	0 78 78	95 25	00m	98 001 98	0 36 90	98 49 8
12	10		93 100	34 83 83	93 66 17	000	97 99 100	0 11 87	97 88 13
12	12		95 100	0 23 62	99 77 34	001	96 101	31 31 76	65 70 25
12	14		46 66 66	0 14 72	94 85 27	000	96 100	0 11 62	96 89 38

			Par	asîtîzed			Control	
Compaction (kg)	Depth (cm)	Pupae Recovered	F1y Emergence	Nat. Mortality	Number Parasitized	Pupae Recovered	F1y Emergence	Nat. Mortality
36	0	99 100 100	-00	0 m 10	98 86	100 100	77 93 79	23 7 21
36	2	99 100	33	64 40 20	2 27 12	99 100	30 48 86	69 52 15
36	4	100 100	0 31 70	100 65 30	0 -7 0	100 100	1 29 83	99 71
36	9	98 98 100	0 59 75	98 39 25	000	98 100 100	0 49 72	98 28 28
36	80	94 100	0 16 59	46 40 40	00-	95 99 100	0 54 64	95 45 36
36	10	95 98	0 14 70	95 86 29	000	99 102	0 37 67	99 65 33
36		99 100	0 11 42	99 89 57	00-	108 100 98	0 38 23	108 62 65
36	14	100 100	0 14	100 86 59	000	98 100	0 20 30	98 07 70

				Par	asitized			Control	
Compaction (kg) Der	pth (cm	Pupae Recovere	Fly d Emergence	Nat. Mortality	Number Parasitized	Pupae Recovered	Fly Emergence	Nat. Mortality
48	•	0	97 100 100	0 27 27		96 89 69	100 100	84 93	11 16 7
48		2	98 100	27 55 84	69 43 9	785	100 100	43 80 80	94 57 20
48		4	99 100 99	0 28 71	99 72 24	004	96 100 100	31 0 88	96 69 12
48		9	97 102 100	0 26 73	97 75 26	0	96 99 99 99 99	. 25 68	96 74 31
48		80	94 100 99	0 40 69	94 60 30	000	93 100	310	93 69 29
48		10	100	0 28 42	100 72 57	000	96 100 100	0 31	96 81 68
48		12	94 100	000	94 92 94	000	94 100	17 3	94 83 97
48		14	106 100	0 6 17	106 94 83	000	94 100	0 1 0 30	94 90 70

THE EFFECTS OF SUBSTRATE COMPACTION AND PUPAL DEPTH ON PARASITIZATION OF HOUSE FLY PUPAE BY SPALANGIA ENDIUS (WALKER)

bу

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MASTER OF SCIENCE

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Abstract

Comparisons of various levels of compaction and depth of a simulated substrate were studied to determine the ability of <u>Spalangia</u> <u>endius</u> (Walker) to parasitize house fly pupae (<u>Musca domestica</u> L.). The optimum adult house fly emergence occurred from depths of 2-4 cm at all compaction levels. As compaction and depth increased, mortality induced by these factors also increased but adult fly emergence decreased. The introduction of <u>S. endius</u> to the treatment showed a significant reduction of emerging adult flies at lower levels of compaction and depth, however, there was no significant reduction in fly mortality. Compaction influenced the penetration ability of <u>S. endius</u> and no significant parasitism occurred in compaction and depth levels greater than 12 kg and 2 cm, respectively. As compaction and depth increased, the penetration and parasitization ability of <u>S. endius</u> decreased. Optimum penetration and parasitism occurred at a compaction level of 0 kg and depth of 4 cm.