

LABORATORY STUDIES ON THE APHID PARASITE,  
APHELINUS NIGRITUS HOWARD (HYMENOPTERA:  
APHELINIDAE)

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1967

Submitted to the Faculty of the Graduate College  
of the Oklahoma State University  
in partial fulfillment of the requirements  
for the Degree of  
MASTER OF SCIENCE  
May, 1970

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APHELINIDAE)

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## PREFACE

The author wishes to express sincere appreciation to his major adviser, Dr. Raymond D. Eikenbary, for his advice, guidance, encouragement, helpful suggestions, and assistance in the preparation of this manuscript.

Grateful recognition is expressed to Dr. R. R. Walton for his valuable criticisms and suggestions in this thesis. The author wishes to express his appreciation to Dr. Robert D. Morrison, Professor of Mathematics and Statistics, for his help with the statistical portion of this paper.

Special thanks are extended to Harley G. Raney, Research Assistant, for his help during this study.

Also, the author would like to thank Meredith Oliver, Technician, Oklahoma State University, for her interest and enthusiastic assistance during this study.

Indebtedness for financial support is expressed to the Oklahoma Agricultural Experiment Station, project number H-1011.

Special appreciation is expressed to the author's wife, Lou, for her encouragement, patience, and understanding during the course of this study.

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## CHAPTER I

### INTRODUCTION

The greenbug, Schizaphis graminum (Rond.), is presently considered the most destructive pest of small grains in the Central and Southwestern states. Losses of over 50 million bushels of grain annually were recorded in outbreak years (Dahms et al., 1955). In 1968 the greenbug infested large acreages of grain sorghum in Oklahoma and throughout a major portion of the sorghum-producing region of the United States. Thousands of acres of grain sorghum were killed and yields were reduced by 50 percent or more in some areas. Prior to 1968 greenbugs were not considered important pests of sorghum; however, Hays (1922) reported greenbugs damaged sorghum in 1916, and Daniels and Jackson (1968) reported greenbugs from sorghum in Texas in 1967. Greenbugs used in the present study were collected from sorghum and are considered to be 'C' biotype and are physiologically, if not morphologically, different from greenbugs collected in prior years on wheat (Harvey and Hackerott, 1969).

Due to severe losses of grain sorghum and the probability of this biotype attacking small grains at the end of the sorghum growing season, much interest has developed in improved control methods, particularly in the use of parasites and predators. Several native and imported parasites are being studied. One of the native parasites, Aphelinus nigritus Howard, was evaluated in this study.

The parasite Aphelinus nigritus Howard is of the subfamily Aphelininae of the Eulophidae (Aphelinidae) (Howard, 1908). Members of this subfamily are parasites of aphids, scales, and whiteflies; however, apparently all species of the genus Aphelinus are internal parasites of aphids (Schlinger and Hall, 1959). Howard (1908) described A. nigritus and reported it was first reared from S. graminum (Rond.) at Spartanburg and Clemson, South Carolina. Later, Webster and Phillips (1912) added information concerning the general habits of this species. A. nigritus was first found in Oklahoma on S. graminum cultures in the greenhouse (Wood, 1958). Prior to this, the species had been reported in South Carolina, New Mexico, Minnesota, Kansas, and Arizona (Wood, 1958).

Sekhar (1965) stated that in the study of a parasite species, knowledge on the influence of environment on the over-all activity of the parasite should be gained. Many biological studies have been made concerning relatives of A. nigritus. Hartley (1922) studied the native form of A. semiflavus Howard. Much later, Schlinger and Hall (1959) added some data on the imported form of this species. Earlier, Lundie (1942) studied the biology of A. mali (Hald.) which is a native parasite of the woolly apple aphid, Eriosoma lanigerum (Hausm.). More recently, the field potential of A. asychis (Walker) has been evaluated by Force and Messenger (1968). But in reference to influence of environment or biological studies, nothing was found in the literature concerning A. nigritus.



## CHAPTER II

### METHODS AND MATERIALS

#### The Host Aphids

Three aphids known to attack sorghum in this region were cultured and maintained in the laboratory. Cultures of the following species were used for experiments:

- 1) Rhopalosiphum maidis (Fitch) (corn leaf aphid)
- 2) Schizaphis graminum (Rond.) (greenbug)
- 3) Sipha flava (Forbes) (yellow sugar cane aphid)

#### The Parasites

Parasites used in this study were obtained from colonies maintained on cultures of S. graminum and R. Maidis in the laboratory. Colonies were maintained in a rearing room with temperatures between 24°C. and 27°C., at a relative humidity of 40-60 percent. These colonies originated from parasites collected on S. graminum cultures in the greenhouse. The parasite was identified by B. D. Burks of Entomology Research Division, U.S.D.A.

Studies were conducted in constant-temperature chambers as described by Force and Messenger (1964). To provide illumination approximately that of daylight, each chamber contained warm white and cool white fluorescent lamps, together with incandescent lights. The light intensity in the chamber was approximately 1100 foot candles. The

photoperiod was 12 hours of daylight in each 24-hour period.

Four constant temperatures of 24°, 27°, 29°, and 32°C. were maintained during the entire experiment. A fifth temperature was programmed at 21°C. for each 12 hours of darkness and 32°C. for each 12 hours of daylight. Temperatures were maintained with fluctuations of less than  $\pm 1^\circ\text{C}$ . at a relative humidity of  $50 \pm 10$  percent.

In each test approximately 25 2nd and 3rd instar nymphs of each aphid species were placed on a young sorghum plant grown in a 4-inch plastic pot. Ten pots of sorghum per temperature per aphid combination were used. Table VI (Appendix) shows these different aphid host combinations. The soil surface of each pot was covered with a layer of fine white sand to facilitate the recovery of any aphid mummies that might drop from the plants. The plant was caged with a clear cellulose nitrate cylinder (11 inches x 2½ inches). Ventilation of the cage was by three windows covered with fine-mesh cloth (2 on sides, 1 at top).

The parasites that were to be used during the tests were fed a solution of honey and water immediately after emergence. A few hours were allowed for mating to occur, then one mated female was placed in each pot.

Basically, this research problem was designed to give data concerning longevity, developmental period, fecundity, host preference, and sex ratio per host species per temperature of the parasite. To determine host preference, all possible combinations of the aphid species were conducted at each temperature. This includes testing the aphid species individually at each temperature. The host preference of A. nigratus was measured by comparing the number of mummies produced from the three aphid species. Three methods of caging the parasite

were used. Method I was with combinations of the three aphid species for the entire life span of the parasite; Method II was with combination of the three aphid species for 12 hours of light and 12 hours of darkness; and Method III was with individual aphid species for the entire life span of the parasite. Prior to introduction of the parasite, the aphid species were allowed 2 to 3 hours to orient themselves and become dispersed on the plants. Then the parasite would have a choice of aphid species randomly distributed over the plant.

Data concerning longevity, fecundity, sex ratio, and developmental period of the parasite were collected at the same time. During the test, mummies were removed daily, recorded, and placed in 1-oz plastic cups. Separate cups were used for each aphid species. As the adult parasites emerged, they were sexed daily and recorded as to males and females from each aphid species. Longevity was obtained when the female parasite was allowed to remain in the cage until her death. Results were obtained by recording the day of emergence and then checking daily to record the date of the parasite's death.

Developmental period was obtained in this study by recording the number of days between the date of parasitization and the date on which the adult parasites emerged from the aphid hosts.

This experiment was completely randomized design because a uniformity trial run beforehand showed insignificant variance resulted by different placement of pots in the chambers. Variation among pots within a temperature was used as the experimental error when there was no competition or sex classification. When there was competition effect between hosts or differences among sexes, or both, data was analyzed as a split-plot type of design in which the main plots were

temperatures with sub-plots becoming host and sex.

Analysis of variance of the data was performed using the data after logarithmic transformations from their original form. Transformed data were used to stabilize the variance in the experiment (Snedecor and Cochran, 1967).

## CHAPTER III

### RESULTS AND DISCUSSION

#### Host Preference

##### Method I

Figure 1 (Appendix) shows the mean number of S. graminum and R. maidis that were parasitized by A. nigrinus. These data indicated a preference of A. nigrinus for S. graminum over R. maidis when only these two species were present. Analysis of variance indicated a significant difference between hosts (Table I, Appendix). The mean number of greenbugs parasitized is higher than corn leaf aphids at all temperatures (Figure 1). Effects due to temperature were insignificant, and there was no temperature by host interaction effects (Table I). This lack of interaction is shown by the fact that the differences in the number of greenbugs and corn leaf aphids parasitized are about equal at all temperatures (Figure 1).

When all three aphids were present as hosts on the same plant (Figure 2), S. flava was not parasitized by A. nigrinus. The over-all mean number of S. graminum parasitized remained higher than that of R. maidis, although the numbers were near the same at 24°C. and the corn leaf aphid had higher parasitism at 27°C. The variation of Figure 2 from Figure 1 can perhaps best be explained by the presence of the third aphid host. This is substantiated by the analysis of

variance which showed significant differences among temperatures, hosts, and host by temperature interaction (Table II). Aphelinus nigritus attempted to oviposit in S. flava and there is the possibility of an egg being deposited and not maturing.

#### Method II

Figure 3 shows the total number of mummies produced by 10 A. nigritus females during 12 hours of light and 12 hours of darkness when caged with all three aphid species. The figure shows that the number of mummies produced varied significantly among the host aphids. The number of S. graminum mummies is higher than R. maidis and S. flava was not parasitized.

#### Method III

Figure 4 shows the measurement of host preference based upon the mean number of mummies produced by the parasite when caged with individual aphid species. Since S. flava was not parasitized, comparisons were only made between S. graminum and R. maidis. This comparison in the figure shows the over-all mean number of S. graminum mummies to be higher than R. maidis. Figure 4 is also used to estimate fecundity, which will be discussed later.

### Longevity

Analysis of variance on longevity in Table III shows significant differences among temperatures, host combinations, and temperature by combination interaction. Figure 5 shows the longevity of A. nigritus females at various temperatures. Mean longevity is the time from adult

female emergence from the cocoon until death. Male longevity was not determined in this study. Force and Messenger (1964) found various temperatures affected longevity of the parasites of the spotted alfalfa aphid, Therioaphis maculata (Buckton). This is the case with A. nigrinus (Figure 5). The range was between 16 days at 24°C. down to 9 days at 32°C. It is worthwhile to note that between 24°C. and 27°C. the difference is 5 days, while there is only 1 day difference in the next three temperatures along the scale. This would indicate longevity is affected less by the temperatures in the range between 27°C. and 32°C.

Table VI shows the mean longevity of the parasite with regard to all possible combinations of the aphid species and temperatures. When S. flava was available as the only host the longevity was greatly reduced. This may be because this aphid was not parasitized by A. nigrinus.

#### Developmental Period

The mean developmental period as used in this study refers to the time from oviposition to emergence of the adult from the cocoon. Ainslie (Webster and Phillips, 1912) found that under favorable weather conditions A. nigrinus developed from egg to adult in 12-13 days. This agrees with the data contained in Table VII. This table shows the length of the developmental period for the parasite from different hosts at various temperatures.

#### Fecundity

The fecundity of A. nigrinus as described in this study was

considered to be the mean number of mummies produced per female parasite from each host at various temperatures (Figure 4). It is considered in this manner because we are interested in the number of hosts killed and the number of emerging adult parasite offspring.

The fecundity of the parasite was tested with each species of aphid at each temperature (Figure 4). This gives an indication of the performance of the parasite with each host and whether temperature affects this performance. Analysis of variance in Table IV shows significant differences among temperatures, host combination, and temperature by host interactions. In Figure 4 the fecundity drops noticeably after the temperature rises above 27°C. When S. graminum is host 27°C. shows the greatest effect on the response, while the effect of 24°C. and 27°C. are highest and almost equal with R. maidis. In addition, the over-all mean number of S. graminum mummies produced is higher than R. maidis. These data will be useful if mass rearing and release of this parasite are considered.

Tables VIII and IX are compilations of data showing the mean number of emerging adult parasites at each temperature. The percent emerged is also calculated. From the comparison of these tables the effect of host upon the percent emergence can be seen. The two lowest percentages from R. maidis (Table VIII) are equal to the two highest percentages from S. graminum (Table IX). This indicates that the type of host has an effect on the percent emergence.

#### Sex Ratio

A. nigratus is arrhenotokus in their reproduction. That is, female progeny are produced from fertilized eggs, but unfertilized



eggs produce only male. The sex ratio of A. nigritus is interesting from the standpoint that in all cases and at all temperatures the mean number of males was larger than the mean number of females (Table X). The highest over-all ratios of males to females were produced at the higher temperatures (32°C.) with the ratio falling at the lower temperatures. Analysis of variance on sex ratio in Table V shows significant differences among temperatures, host, host by temperature interaction, and sex. This agrees with Schlinger and Hall (1959) who also found that temperature influenced the sex ratio of a parasite species.

Table X shows a comparison of the mean number of males and females from S. graminum and R. maidis mummies produced when they were on the same plant. The sex ratio of females to males averaged over-all temperatures from R. maidis is 1:1.9 and from S. graminum it is 1:1.8. However, at 32°C. the sex ratio varies significantly between the host aphids, with S. graminum female to male ratio being 1:3.1 while R. maidis is 1:1.5. This is substantiated by the analysis of variance in Table V which shows significant differences in sex by host interactions.

Figures 6 and 7 show the sex ratio of parasites emerging from S. graminum and R. maidis mummies, respectively, when caged individually. From these two hosts the sex ratio of females to males is about equal at all temperatures except 32°C. At this temperature the female to male ratio from S. graminum is 1:2.1 while R. maidis is 1:1.5. It is interesting to note that this sex ratio response of the parasite with each host individually (Figures 6 and 7) is similar to the response obtained when the hosts were in combination (Table X). This indicates that the sex ratio per host responses would be similar for each host whether the hosts are tested individually or in combination.

Since A. nigritus had more male than female offspring, the question arises whether the potential capacity to increase is too low for it to be an effective parasite. Evidence of the species having been present for the last 60 years and being recovered in several different areas would point to the probability that the species may perform differently under natural conditions.

## CHAPTER IV

### CONCLUSIONS

The primary parasite, Alphelinus nigrinus Howard, showed marked preference for certain aphids among those attacking sorghum. Wood (1958) found this to be true with other aphids as well. He found that the cabbage aphid, Brevicoryne brassicae (L.), and the spotted alfalfa aphid, Therioaphis maculata (Buckton), were not parasitized by this parasite. In this study the yellow sugar cane aphid, Sipha flava (Forbes), was not parasitized by A. nigrinus. Since S. graminum was preferred and is the most abundant aphid on sorghum, it is normal to conclude that this parasite may be helpful in reducing greenbug numbers. However, when we consider the fecundity and sex ratio it appears that it may lack the capacity to increase rapidly enough to be an effective parasite. Of course, to evaluate parasite effectiveness by means of laboratory studies, all factors affecting the individual species of parasites, the relationship between different species of parasites, and the interactions between host and parasite must be considered (Force and Messenger, 1964). Therefore, it is not feasible from data presented in this paper to attempt an explanation of the over-all effectiveness of A. nigrinus. This subject should be deferred until later when other factors, such as field surveys and field evaluations, are known.

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**APPENDIX**

TABLE I  
 ANALYSIS OF VARIANCE OF THE NUMBER OF MUMMIES  
 PRODUCED BY A. NIGRITUS FROM S. GRAMINUM  
 AND R. MAIDIS IN COMBINATION  
 AMONG TEMPERATURES

Source	df	MS
Total	99	
Temperature	4	0.5249
Error (a)	45	0.2890
Host	1	8.9729**
Host x Temperature	4	0.4178
Error (b)	45	0.5255

\*\* Significant at the 1 percent level.

TABLE II  
 ANALYSIS OF VARIANCE OF THE NUMBER OF MUMMIES  
 PRODUCED BY A. NIGRITUS FROM S. GRAMINUM,  
R. MAIDIS, AND S. FLAVA IN COMBINATION  
 AMONG TEMPERATURES

Source	df	MS
Total	99	
Temperature	4	1.0832**
Error (a)	45	0.1540
Host	1	1.5493**
Host x Temperature	4	1.1250**
Error (b)	45	0.0592

\*\* Significant at the 1 percent level.

TABLE III  
 ANALYSIS OF VARIANCE OF THE LONGEVITY OF  
A. NIGRITUS DUE TO DIFFERENCES IN  
 TEMPERATURES AND COMBINATIONS  
 OF APHID HOSTS

Source	df	MS
Total	349	
Temperature	4	992.02**
Combinations	6	228.67**
Temperature x Combination	24	83.75**
Error	315	20.40

\*\*Significant at the 1 percent level.

TABLE IV  
 ANALYSIS OF VARIANCE OF THE NUMBER OF MUMMIES  
 PRODUCED BY A. NIGRITUS FROM S. GRAMINUM  
 AND R. MAIDIS INDIVIDUALLY  
 AMONG TEMPERATURES

Source	df	MS
Total	99	
Temperature	4	1.6836**
Combinations	1	0.9535**
Combination x Temperature	4	0.2116*
Error	90	0.0608

\*Significant at the 5 percent level.

\*\*Significant at the 1 percent level.



TABLE V  
 ANALYSIS OF VARIANCE OF THE NUMBER OF OFFSPRING  
 PRODUCED BY A. NIGRITUS FROM S. GRAMINUM  
 AND R. MAIDIS IN COMBINATION AMONG  
 TEMPERATURES, HOSTS, AND SEX

Source	df	MS
Total	199	
Temperature	4	1.2533*
Error (a)	45	0.4367
Host	1	11.4527**
Host x Temperature	4	0.4767**
Error (b)	45	0.1060
Sex	1	4.5399**
Sex x Temperature	4	0.0868
Error (c)	45	0.1337
Sex x Host	1	0.4275**
Sex x Host x Temperature	4	0.0667
Error (d)	45	0.0480

\*Significant at the 5 percent level.

\*\*Significant at the 1 percent level.

TABLE VI  
 LONGEVITY<sup>a</sup> OF A. NIGRITUS AT ALL POSSIBLE  
 COMBINATIONS OF HOSTS AND  
 AT FIVE TEMPERATURES

Host Combinations <sup>b</sup>	Temperatures °C.					Over-all Mean
	24	27	29	32	21-32	
001	16.1	12.1	10.8	9.5	14.0	
002	18.0	13.0	11.5	10.1	15.1	
003	9.0	7.0	5.0	4.2	8.3	
012	17.1	12.8	11.6	10.0	14.5	
013	16.1	11.5	10.2	10.2	12.2	
023	17.8	11.6	10.5	9.2	12.0	
123	17.1	12.2	11.1	9.8	14.1	
Mean	15.9	11.5	10.1	9.0	12.8	11.4

<sup>a</sup>Recorded in mean number of days.

<sup>b</sup>001 represents R. maidis tested singly  
 002 represents S. graminum tested singly  
 003 represents S. flava tested singly  
 All others are combinations of the above

TABLE VII  
 MEAN NUMBER OF DAYS IN DEVELOPMENTAL PERIOD  
 OF A. NIGRITUS BY HOST AND TEMPERATURE

Host	Temperatures °C.					Mean
	24	27	29	32	21-32	
<u>S. graminum</u>	15.2±1.3	12.2±1.2	11.0±1.3	10.2±.5	12.0±2.9	12.2±.9
<u>R. maidis</u>	16.2±1.3	12.0±0	11.6±.2	10.5±.6	13.0±.6	12.7±1.0

TABLE VIII

MUMMIES, PARASITES, AND PERCENT OF EMERGENCE  
OF A. NIGRITUS ON R. MAIDIS AS THE HOST

Temperatures °C.	Mummies			Parasites			% Emerged <sup>b</sup>
	Low	High	Mean <sup>a</sup>	Low	High	Mean <sup>a</sup>	
24	13	84	46.1	11	73	37.6	80.4
27	16	57	43.2	11	51	33.9	76.1
29	16	48	29.8	16	34	23.4	78.5
32	4	16	11.2	4	14	8.7	77.3
21-32	20	94	66.1	20	87	56.1	88.1

<sup>a</sup> represents mean of all pots at given temperature

<sup>b</sup> represents mean number of parasites divided by mean number of mummies

TABLE IX

MUMMIES, PARASITES, AND PERCENT OF EMERGENCE  
OF A. NIGRITUS ON S. GRAMINUM AS THE HOST

Temperatures °C.	Mummies			Parasites			% Emerged <sup>b</sup>
	Low	High	Mean <sup>a</sup>	Low	High	Mean <sup>a</sup>	
24	33	71	48.6	23	59	36.6	75.3
27	42	98	68.2	26	81	49.4	72.4
29	14	56	39.8	9	49	30.1	75.6
32	8	33	19.4	5	31	15.0	77.3
21-32	20	94	60.5	10	73	46.0	76.0

<sup>a</sup> represents mean of all pots at given temperature

<sup>b</sup> represents mean number of parasites divided by mean number of mummies

TABLE X

COMPARISON OF MEAN NUMBER OF MALE AND FEMALE  
A. NIGRITUS FROM S. GRAMINUM AND  
R. MAIDIS IN COMBINATION

Temperature (°C.)	<u>S. graminum</u>		<u>R. maidis</u>	
	♂	♀	♂	♀
24	17.4	12.1	2.7	1.7
27	29.7	18.3	13.3	8.0
29	18.7	9.2	10.0	4.2
32	12.8	4.1	2.6	1.7
21-32	26.7	13.5	10.8	4.2
Mean	21.1	11.4	7.9	3.9

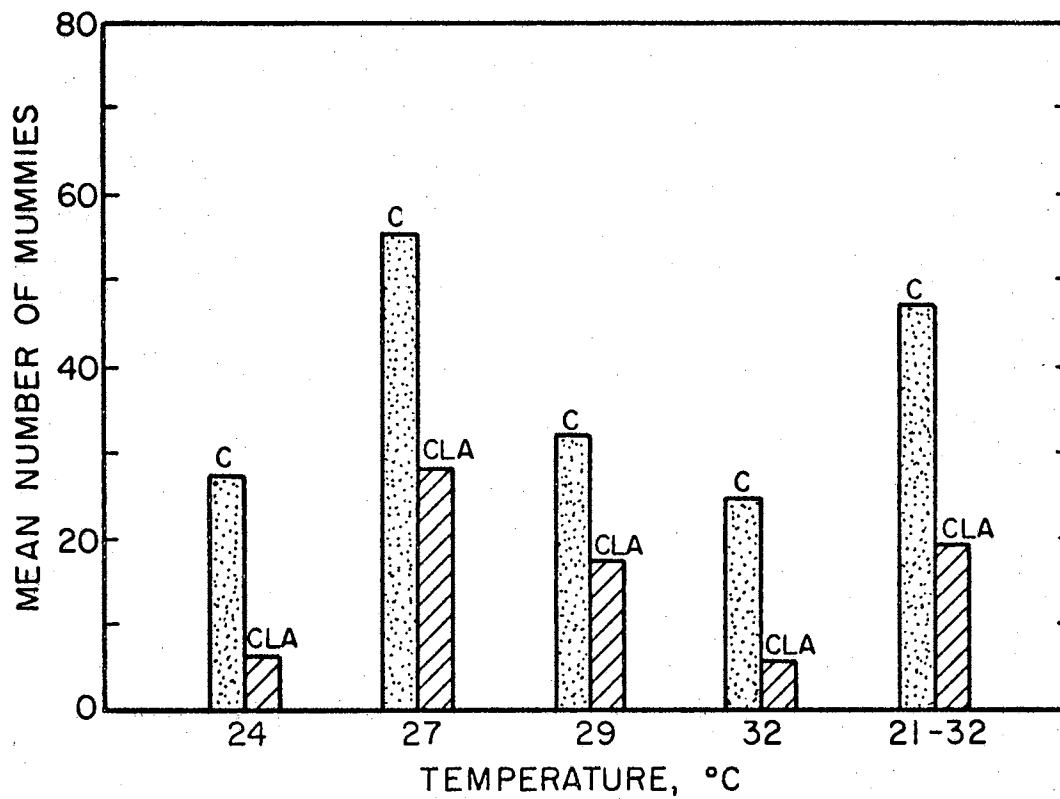


Figure 1. Mean Number of Mummies Produced by *A. nigratus* at Five Temperatures When *S. graminum* ('C') and *R. maidis* ('CLA') Were Available as Hosts

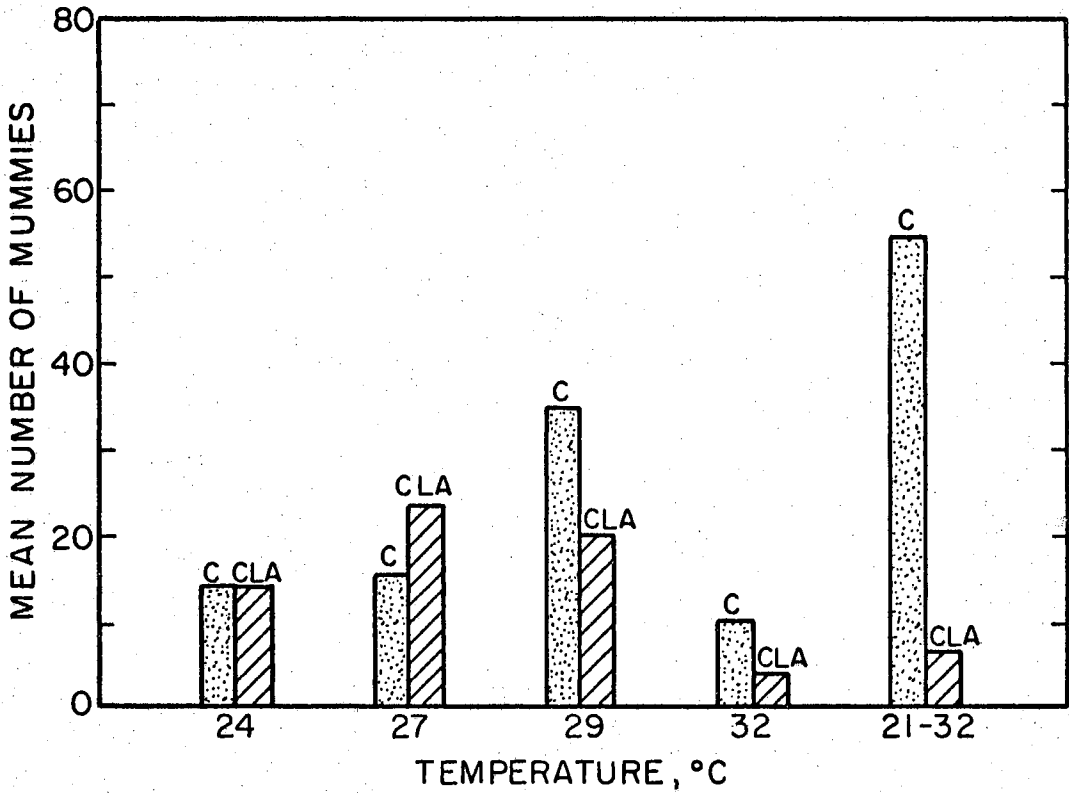


Figure 2. Mean Number of Mummies Produced by *A. nigratus* at Five Temperatures When *S. graminum* ('C'), *R. maidis* ('CLA'), and *S. flava* ('YSCA') Were Available as Hosts

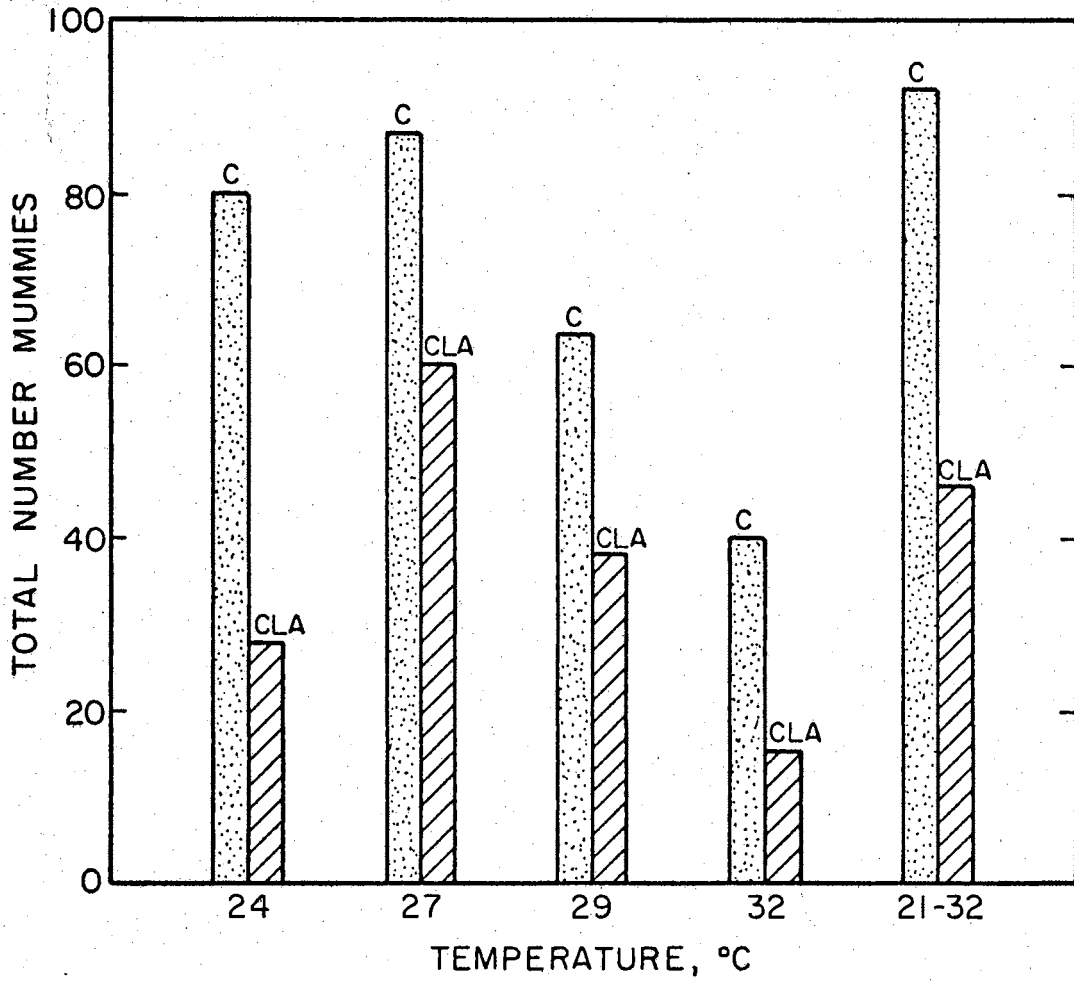


Figure 3. Total Number of Mummies Produced by Ten *A. nigritus* Females During 12 Hours of Light and 12 Hours of Darkness When Caged With *S. graminum* ('C'), *R. maidis* ('CLA'), and *S. flava* ('YSCA')

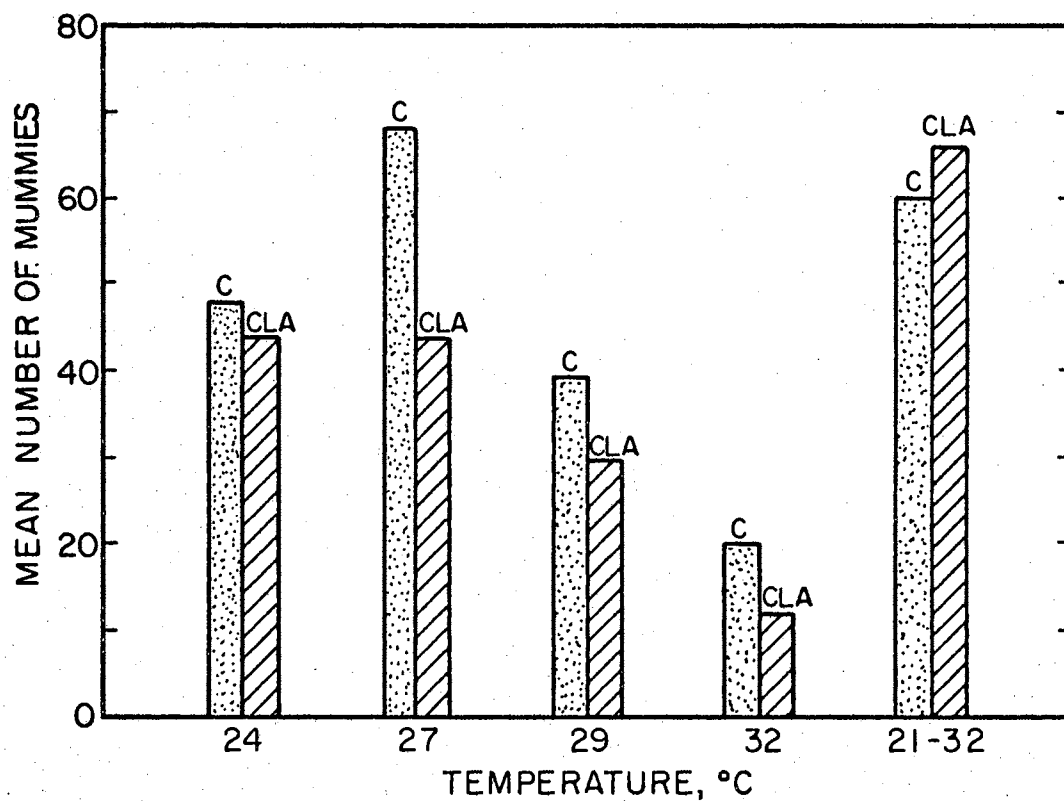


Figure 4. Mean Number of Mummies Produced by *A. nigratus* at Five Temperatures When *S. graminum* ('C') and *R. maidis* ('CLA') Were Caged Individually



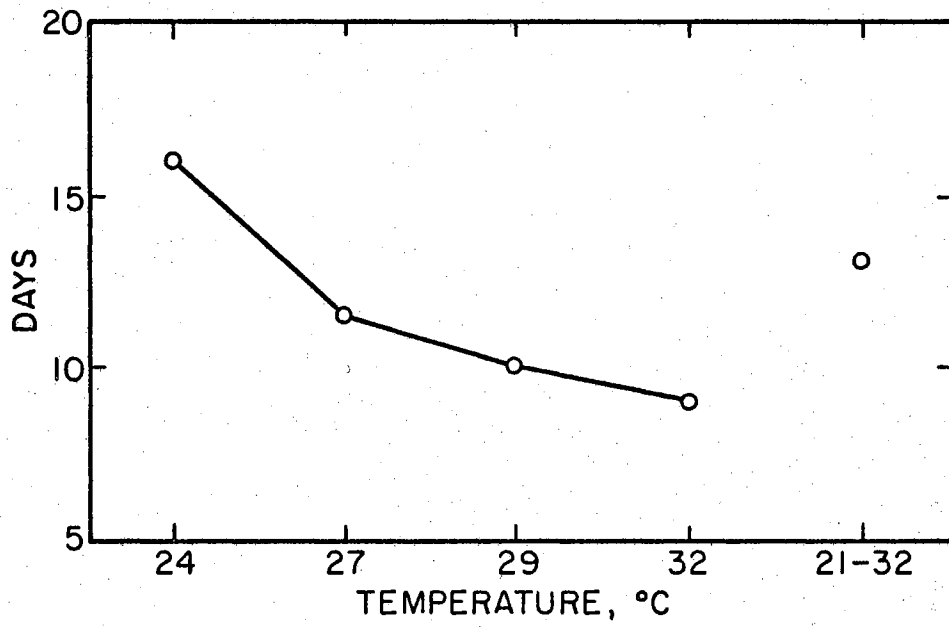


Figure 5. Mean Longevity of Female *A. nigritus* at Five Temperatures

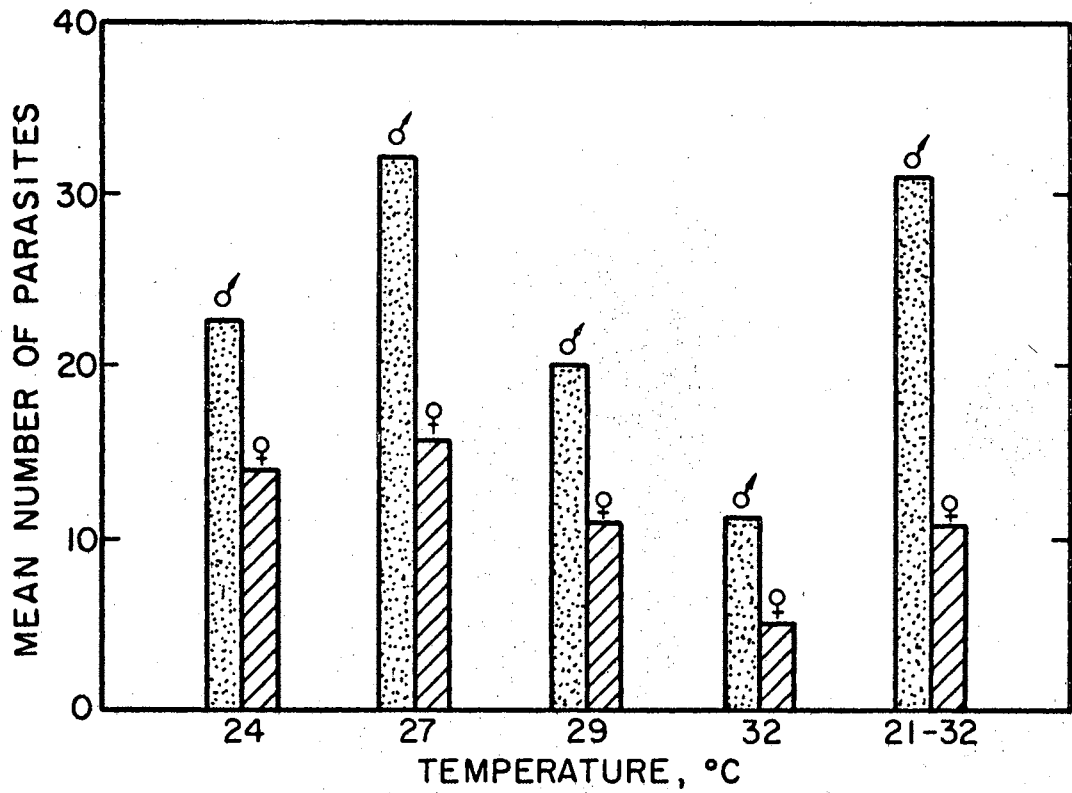


Figure 6. Mean Number of Male and Female *A. nigritus* Produced at Five Temperatures With *S. graminum* as the Host

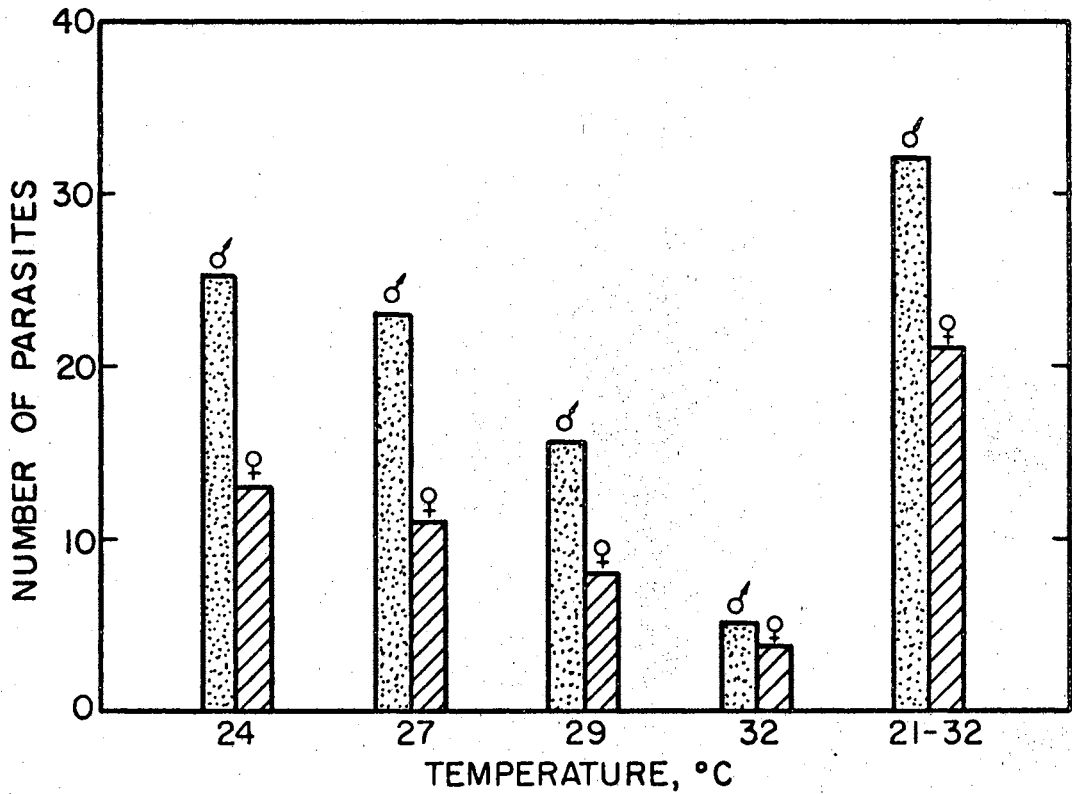


Figure 7. Mean Number of Male and Female *A. nigritus* Produced at Five Temperatures with *R. maidis* as the Host

VITA

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Dave Thomas Langston

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Master of Science

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