LABORATORY EVALUATION OF *APHELINUS ASYCHIS*, AN IMPORTED PARASITOID OF THE GREENBUG, *SCHIZAPHIS GRAMINUM*

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PREFACE

Due to an increased concern over possible detrimental effects insecticides might have on the environment, biological control has been placed in the forefront as a possible alternative method of insect pest regulation. The research presented herein deals with one facet of biological control of the greenbug: use of an imported parasitoid.

I wish to express my gratitude to Dr. Raymond D. Eikenbary, Professor, Department of Entomology, for his guidance in this research and preparing the manuscript; to Dr. Thomas Archer, Research Associate, Department of Entomology, for his assistance in the research; to Dr. R.D. Morrison, Professor, Department of Statistics, for his invaluable assistance in designing the experiments, analyzing the data, and critical reading of the manuscript; to Dr. W.A. Drew, Professor, Department of Entomology, Dr. K.J. Starks, Associate Professor, Department of Entomology and Investigations Leader, Entomology Research Division, United States Department of Agriculture, and Dr. M.R. Curd, Associate Professor, Department of Zoology, for their helpful suggestions and critical reading of the manuscript; and to Dr. J.R. Sauer, Associate Professor, Department of Entomology, for the use of his laboratory facilities and aid in preparation of manuscripts. Appreciation is also extended to R.L. Burton, Research Entomologist, for the use of his laboratory facilities and to D.R. Molnar, Research Assistant, Department of Entomology, for his assistance with some of the photography.

Finally, I would like to express my deepest gratitude to my wife, Lee, for her understanding and assistance, and to my children, James and Laura. All have sacrificed much throughout the preparation of this manuscript. May their devotion be fully repaid in the future.

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CHAPTER I

INTRODUCTION

The greenbug, Schizpahis graminum (Rondani), first described in Italy in 1852 (Hunter and Glenn 1909), and first recorded in the United States in 1882 (Webster and Phillips 1912), has since been a serious pest of small grains in the midwestern states. Oklahoma has recorded several major outbreaks on small grains (Rogers et al. 1972) but in 1968 a new biotype, designated as the C biotype, caused severe damage to sorghum crops in Oklahoma, as well as other southwestern and western states (Wood et al. 1969, Harvey and Hackerott 1969 a, b).

The impact of the greenbug on sorghums and small grains, the cost of chemical control, and the increased concern over insecticides in the environment has stimulated research dealing with possible biological control agents as regulators of greenbug populations. One phase of research has been the importation of predators and parasitoids. Doutt (1964) defines parasitoid as a parasitic insect that: (1) is large in size compared to its host; (2) kills its host during its development; (3) is of the same taxonomic class as its host; (4) is parasitic as a larvae only; (5) does not exhibit heterocism; and (6) resembles a predator more than a parasite.

The Insect Identification and Parasite Introduction Research Branch, USDA, initiated a search in Asia which led to the importation of Aphelinus asychis (Walker) a hymenopteran, family Eulophidae, from Iran (Raney et al. 1971). The research presented herein was undertaken to implement and broaden previous studies concerning the potential of *A. asychis* as a regulator of greenbug populations.

CHAPTER II

PARASITIZATION OF THE GREENBUG BY APHELINUS ASYCHIS AND THE EFFECT OF FEEDING BY THE PARASITOID ON APHID MORTALITY

Interest in methods of control that might decrease the use of insecticides has stimulated research on biological control agents as a means of controlling the greenbug, Schizaphis graminum (Rondani), an important pest of sorghum in Oklahoma. One potential agent, Aphelinus asychis (Walker), imported from Iran is being cultured in our laboratory (Jackson et al. 1971). Results thus far indicate that this parasitoid can survive during the summer months in Oklahoma (Jackson et al. 1971). Esmaili and Wilde (1972) suggested that A. asychis would not overwinter in this region because the pupal stage cannot withstand low temperatures. However, more extensive research is needed to demonstrate if any of the insect's other stages can overwinter in the Midwest. Results of cold storage studies conducted thus far suggest that adults may endure long periods of cold with few apparent ill effects (Archer and Eikenbary 1973).

The corn leaf aphid, Rhopalosiphum maidis (Fitch), the yellow sugarcane aphid, Sipha flava (Forbes), the sunflower aphid, Aphis helianthi Monell, the oat-bird cherry aphid, Rhopalosiphum padi (L.) and the biotype C greenbug may be parasitized by A. asychis

(Jackson and Eikenbary 1971, Raney et al. 1971, Rogers et al. 1972). This range of hosts may allow populations of A. asychis to build up in the field before the greenbug becomes an economic problem.

Little research has been conducted concerning the daily ovipositional pattern of A. asychis. Most of the available data indicate only the total number of mummies formed and the percent emergence. Jackson et al. (1971) encountered some difficulty in locating mummies because the parasitized aphid often migrated into a leaf axil or crawled into crevices in the soil. The number of mummies formed off the plant could be important in field studies in which total numbers of parasitized aphids are desired. If substantial numbers of parasitized aphids migrate from the plant and emerge, A. asychis could be a much better parasitoid than previously thought because of the greater number of aphids parasitized but never observed in the field.

Esmaili and Wilde (1972) fould that A. asychis fed on some of the stung greenbugs, but no data were presented on the number of greenbugs killed by parasitoid feeding. Hartley (1922) noted that A. semiflavus fed on its host, Myzus persicae (Sulzer) and Marchal (1909) observed Tetrastichus xanthomelana Rondani feeding on the eggs of Galerucella luteola (Müller). Rockwood (1917) published observations on the feeding habits of Aphelinus lapisligni Howard on the host Aphis baker Gowen. DeBach (1943) noted a similar phenomenon with the scale parasite, Metaphycus helvolus (Compere). He stated that those studying biological control agents should ascertain if a parasite species exhibits host feeding, and if so, steps should be taken to measure this mortality factor, which may be relatively more important than parasitization.

Continued studies of A. asychis and the parasitization of the greenbug were conducted with objectives to: (1) determine quantitatively the importance of the host-feeding mortality factor, (2) establish daily ovipositional patterns, (3) quantitatively determine mummification sites, and (4) determine success of emergence and sex ratio of the emergent parasitoids.

Materials and Methods

This study consisted of 4 consecutive trials. A greenbug susceptible sorghum hybrid, RS-610, was reared in a greenhouse in 3 and 4-inch pots and brought into the laboratory when 14 days old. Only the healthiest in appearance of several possible plants in each pot was used in the experiments. Preliminary studies indicated that a plant this age could host a beginning population of ca. 20 aphids of random age, with the plant living for a sufficient length of time for the parasitized aphids to mummify (ca. 7 days). Stock cultures of greenbugs were maintained on sorghum plants in the laboratory. Twenty greenbugs of random age were transferred with a camel's-hair brush from the culture onto the stem and lower leaves of each test plant ca. 1 h before the parasitoids were introduced. One control container with one plant and 20 greenbugs, but without parasitoids, was set up each day during all 4 trials. Sexing, transferring, and caging procedures for the parasitoids were similar to those used by Raney et al. (1971).

A. asychis mummies produced on greenbug cultures were placed into 1-oz. cups daily. Adult parasitoids less than 12 h old having no previous contact with aphids were collected from the cups with a mouth aspirator (Childs and Gillespie 1932). A total of 31 female A. asychis was utilized in the 4 trials. Mating was presumed to have occurred in the cups because both males and females were present. Esmaili and Wilde (1972) reported that A. asychis mate immediately after emergence. However, as a safeguard in our experiments, a male was placed into the container with the plant at the same time the female was transferred. The plants with the parasitoids were randomly arranged in trays under growth lights in the laboratory.

Paired parasitoids were transferred at 24 h intervals to new plants having at least 20 aphids. At the time of transferal all dead aphids were counted and their ages approximated by size. The container was then stored in the laboratory. Seven days after removal of the parasitoids, the plants were checked for mummies. The mummy sites were recorded from the leaf, stem, whorl of the plant or on the sand or container. The mummies were cut from the plants and placed into 1-oz. cups which were stored in the laboratory until emergence. Each emergent parasitoid was then sexed.

Of the 31♀ parasitoids used, 20 lived a minimum of 15 days. For statistical purposes, most of the data presented here represent the 20♀ parasitoids which lived 15 days or longer. Ten of the female parasitoids died within the first 9 days. Most of this mortality probably was caused by daily transferal of the parasitoids to aphid cultures.

Results and Discussion

Longevity

The mean longevity of the 31♀ parasitoids was 17.3 days. If the 10 which died in the first 9 days were removed from this data, the mean longevity would be 23.4 days. Both figures are similar to those reported by other authors. Raney et al. (1971) found a mean longevity of 18 days for *A. asychis* when parasitizing greenbugs. However, in their experiments the parasitoids were not transferred daily. A mean longevity of ca. 19 days would seem acceptable. Longevity in this study ranged from a minimum of 3 to a maximum of 33 days. Raney et al. (1971) reported a maximum longevity of 28 days for *A. asychis* reared at 23.9° C. Force and Messenger (1964) found that *A. semiflavus* lived ca. 29 days maximum at 26.7° C, and Hartley (1922) found that *A. semiflavus* lived for a maximum of 12 days on aphids compared to 39 days on honey. From this study it appears that the natural maximum longevity for *A. asychis* is 30-36 days.

Parasitoid Feeding

Although secondary to aphid mortality caused by oviposition, considerable aphid death appears to have been caused by parasitoid feeding in that during all tests only 4 control aphids died. During feeding a progressive color change occurred in the greenbugs from the typical green to a greenish-yellow to a brown. The yellow to brown color change usually occurred after the parasitoid left the aphid, but in cases in which they fed for longer than 45 minutes, the aphid became brown while the parasitoid was feeding.

In practically every case, the first aphid stung after emergence was fed upon by the female parasitoids. Since the parasitoids had emerged several hours earlier, they may have been in a "starved" condition, accounting for this feeding on its first available host. The largest number of aphids killed by feeding of A. asychis was on the fourth day of parasitization with a mean of 2.45/female parasitoid (Figure 1). A total of 454 aphids was killed by the feeding of the 20♀ parasitoids in the first 15 days of their lifespan, representing a daily mean of 1.5 aphids fed upon per female parasitoid. The 20♀ parasitoids killed, by feeding, a total of 608 aphids in their entire lifespan.

Ovipositional insertion may last from 0.5 to 120 minutes. Interruptions of less than 5 minutes due to no apparent reason occurred among most parasitoids in which oviposition lasted longer than 20 minutes. The parasitoids usually fed only from the aphids into which the ovipositor was inserted for more than 5 minutes. The act of oviposition varied from aphid to aphid. The ovipositor was placed into either the thoracic or abdominal regions from the side, anterior, or posterior of the aphid. The ovipositor was withdrawn and reinserted several times or left in the aphid until the completion of the ovipositional thrust. Many times the parasitoid would slowly move its abdomen up and down causing the ovipositor to go deeper into the aphid.

After the wound was opened the parasitoid would turn, position its body over the aphid (Figure 2) and feed for varying amounts of

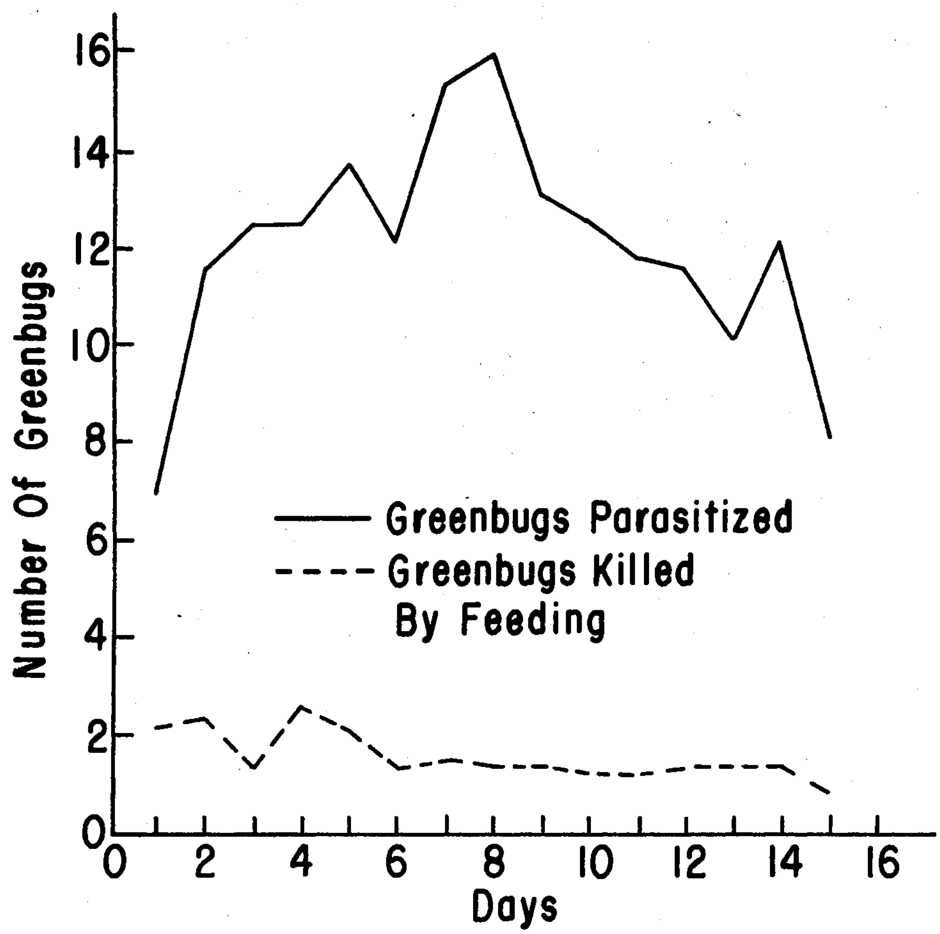

Figure 1. The mean number of greenbugs killed daily by feeding and oviposition by 20♀ *A. asychis* during the first 15 days of the parasitoids' life in the laboratory

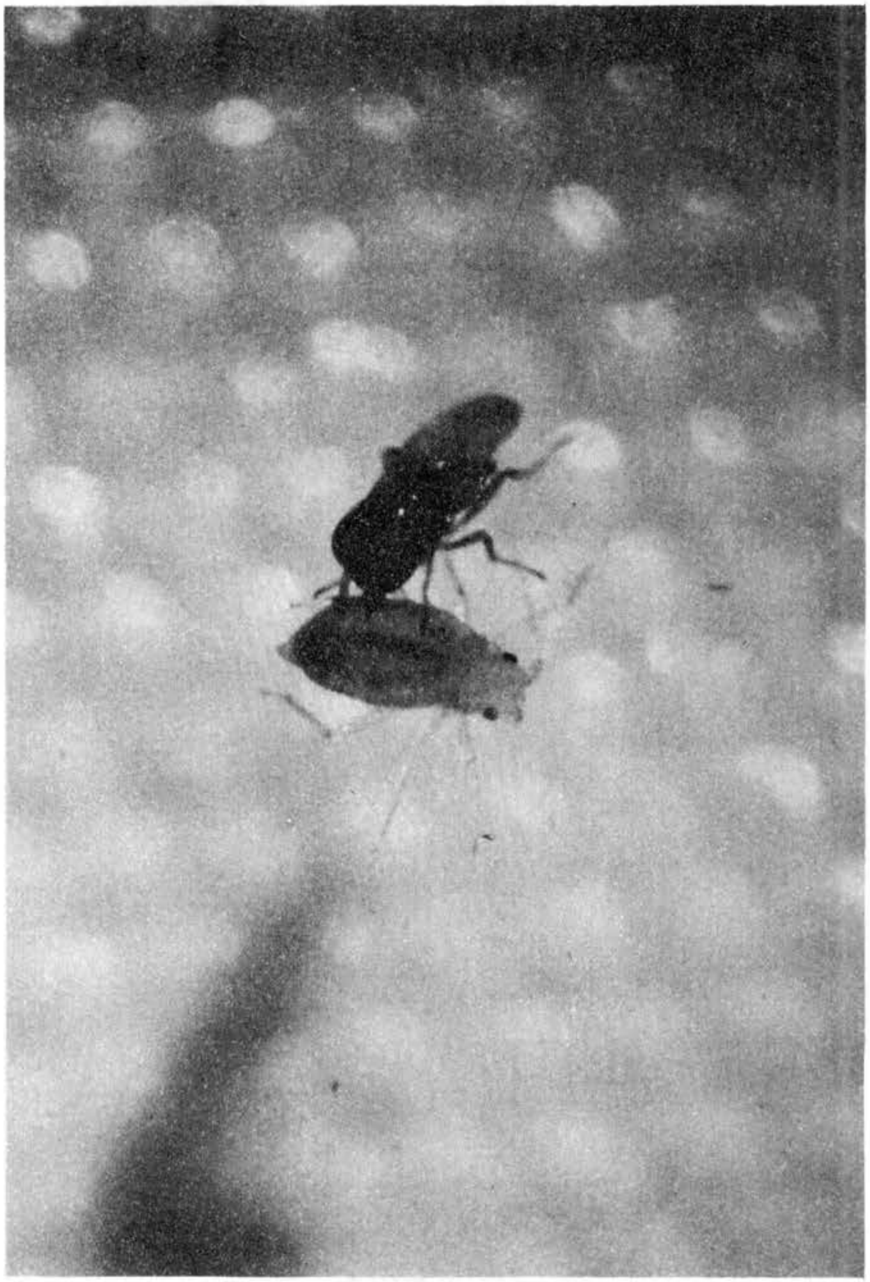

Figure 2. A. asychis feeding from the ovipositional wound in a greenbug

time. On various occasions the parasitoid stopped eating, turned around, and again inserted its ovipositor. After withdrawing the ovipositor, the parasitoid would return to its former position and resume feeding; this was repeated several times when the aphid and parasitoid were in contact. It was not possible to determine conclusively if the ovipositor was inserted into the same wound, but from the speed and number (usually 1) of ovipositional thrusts, it seemed unlikely.

Interesting phenomena were noted in several preliminary experiments in which more than 1♀ _A. asychis_ were placed in cultures of aphids. After a parasitoid had finished feeding on an aphid, a second parasitoid approached the aphid, made no ovipositional attempt, but immediately began feeding. This could be common in nature. Another noteworthy event demonstrating a slightly different behavior of the parasitoid was ovipositional probes by a second parasitoid on an aphid that had previously been stung and fed upon. The second parasitoid did not feed upon the aphid but left immediately after oviposition.

Mummies

A temperature of $26.7 \pm 4^{\circ}$ C was maintained throughout the experiments because Raney et al. (1971) found the highest number of greenbugs parasitized by _A. asychis_ and the highest percent emergence of _A. asychis_ at this temperature. The data concerning mummies were taken in such a way as to determine if a particular site on or off the plant was preferred by greenbugs becoming mummies. During the first 15 days a total of 1967 mummies was found on the leaves and 1200 on the stems. There was little difference in the counts on the whorl, sand, and container with 121, 172, and 114 mummies respectively.

From observations made in the laboratory and from the results of the data it seems unlikely that the parasitized aphid normally moves to any extent after it starts mummifying. Mummies found on or in the soil probably are physically knocked from the plant. Wind, rain, and animals could account for this occurrence in the field. Some of these could have accounted for those mummies found off the plant in these laboratory studies. If, however, the plant becomes overloaded with aphids, or for some reason begins to die, the aphids will migrate from that plant. This was sitnessed on several occasions during these experiments and accordingly, larger numbers of mummies were found on the soil and container. In the field these aphids could move to a neighboring plant. However, some of the aphids would probably form mummies on or in the soil. Factors such as excessive moisture or desiccation and heat from the high soil temperatures would probably limit and possibly terminate parasitoid emergence from mummies on or in the soil. However, more research needs to be conducted to determine if emergence does occur on, or in the soil, and its importance.

The considerable number of mummies found in the whorl was interesting because the greenbug is not primarily an inhabitor of the whorl. The ability of this parasitoid to find and oviposit in aphids in the whorl is an important attribute of this parasitoid, and indeed it is not surprising for one of its major native hosts, the corn leaf aphid, inhabits the whorl.

Figure 1 shows the mean number of mummies produced daily by 20 parasitoids during the first 15 days of their life. Maximum oviposition occurred on the eighth day when an average of 16 mummies/parasitoid was formed. Total number of mummies formed for the 15 days was 3574, with a mean of 179 for each parasitoid. The minimum number of mummies produced by one parasitoid for the 15 days was 93, whereas the maximum number produced was 296. The minimum number produced by 1 parasitoid over the entire lifespan was 115, and the maximum was 417. The total number of mummies produced over the entire lifespan of the 20 parasitoids was 4655.

Emergence

Of the total 4655 mummies formed, 3792 (81%) emerged. This is in agreement with Raney et al. (1971). Emergence occurred in 4-7 days after mummification with the males generally the first to emerge. Of the total emergent parasitoids, 56% were females.

Figure 3 presents the percent emergence of the parasitoids from aphids the first 15 days of the parasitoids' lives. Total percent emergence and percent female emergence remained reasonably constant throughout the 15-day period. Since mating occurred early in the lifespan and all the males died during the first week of the experiments, female progeny representing approximately 56% of the total emergent group up through the 15th day exemplifies that sperm of *A. asychis* can remain viable for at least two weeks under laboratory conditions.

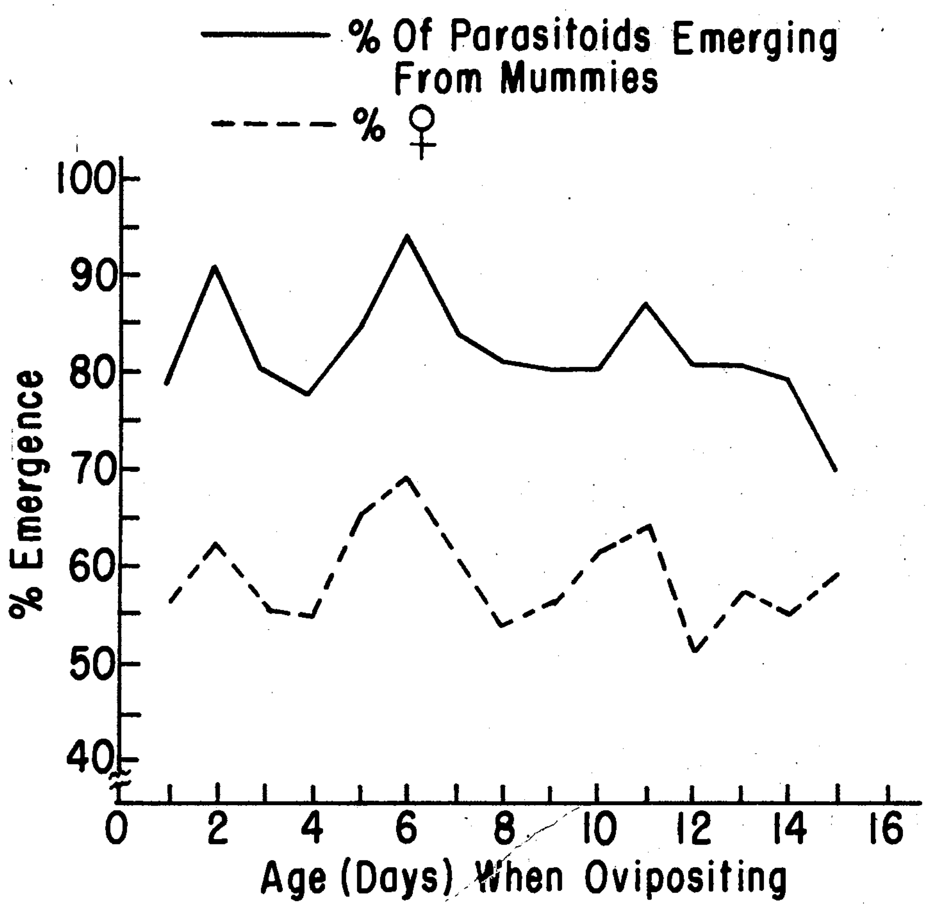

Figure 3. Emergence and sex of _A. asychis_ resulting from daily exposure to greenbugs [a/]

$$\text{[a/] Percent emergence} = \frac{\text{Number of parasitoids emerged on a particular day}}{\text{Total number of mummies available for parasitoid emergence on that day}} \times 100$$

$$\text{Percent females} = \frac{\text{Number of females emerged on a particular day}}{\text{Total number of parasitoids emerged on that day}} \times 100$$

A bias due to handling the parasitoids daily must be considered in interpreting all the data. Regardless of the care taken, the technique of "sucking" a parasitoid into a glass cylinder and expelling it into a container where it might hit plant, sand, or container wall could have injured the insects. The first 3-5 times the parasitoids were transferred in this manner they attempted to avoid the aspirator. The greater the attempt at avoidance, the more likely injury would result. Several times the insect appeared stunned after transferal and remained motionless on the sand. In most cases they recovered. Overall, A. asychis is a good laboratory animal to work with because of its tendency to remain on the plant and its hesitancy to take flight.

In summary (Figure 4), it appears that host feeding of A. asychis plays a role in the reduction of greenbug populations. The ratio of killed by eating to killed by mummification was ca. 1:7.66 over the entire lifespan. If the number of aphids eaten (608) is added to the number of mummies formed (4655), the total number of 5263 aphids killed for the 20 parasitoids becomes not only a more impressive number but a better indicator of the potential of A. asychis as a biological control agent of the greenbug.

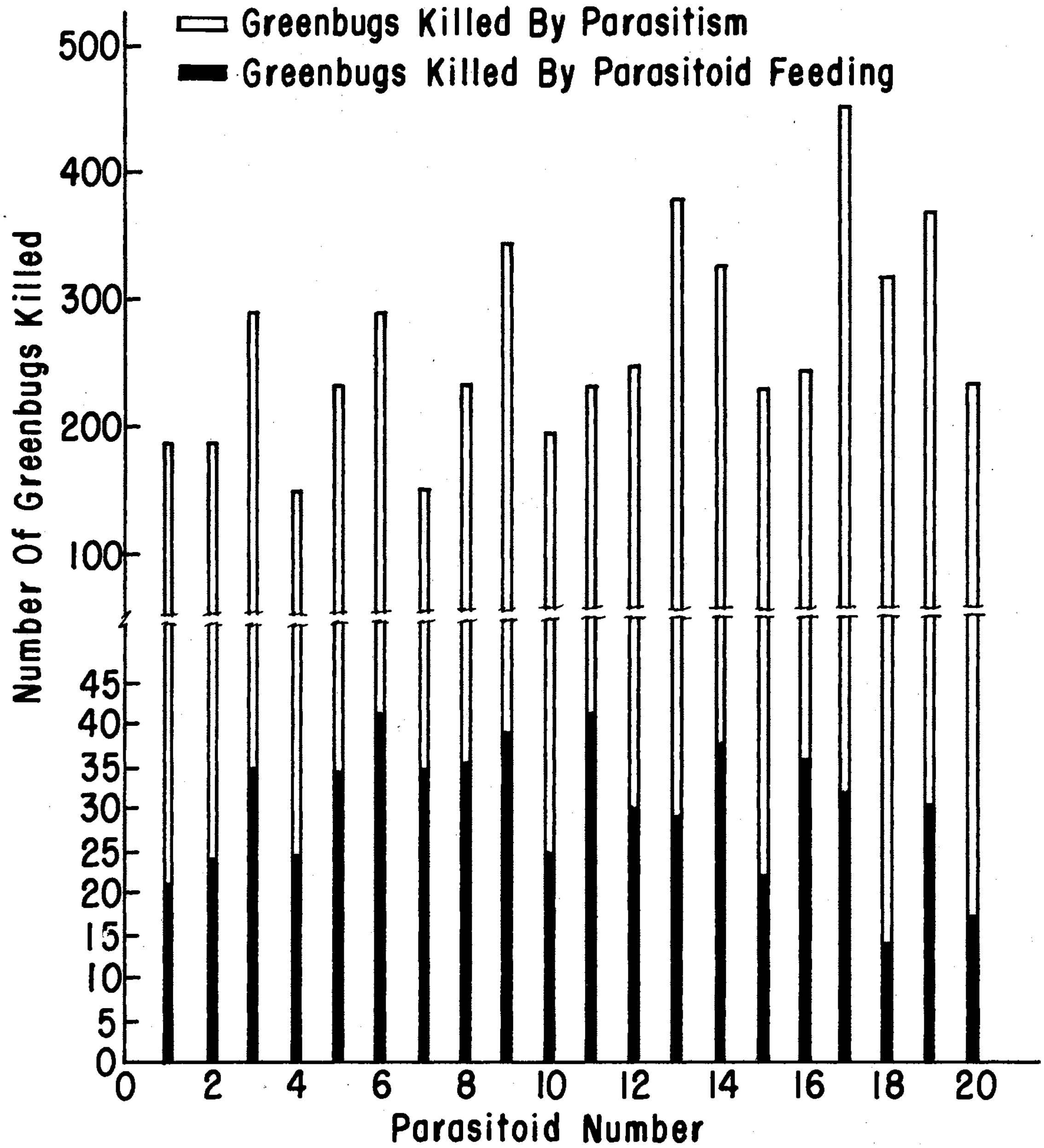

Figure 4. Mortality of greenbugs by feeding and parasitization by each of 20♀ _A. asychis_ in the laboratory

CHAPTER III

AGE OF GREENBUGS PREFERRED FOR OVIPOSITION AND FEEDING BY THE PARASITOID *APHELINUS ASYCHIS* AND THE EFFECT OF PARASITIZATION ON GREENBUG FECUNDITY

Following importation of the parasitoid *Aphelinus asychis* (Walker) numerous studies have been conducted to determine the potential of this parasitoid as a biological control agent of aphids, particularly the greenbug, *Schizaphis graminum* (Rondani), (Jackson et at. 1971, Jackson and Eikenbary 1971, Raney et al. 1971, Rogers et al. 1972, Esmaili and Wilde 1972, and Cate et al. 1973). *A. asychis* may regulate greenbug populations in two ways: (1) feeding on hosts by adults, and (2) by parasitism (Jackson et al. 1971, Jackson and Eikenbary 1971, Raney et al. 1971, Rogers et at. 1972, Esmaili and Wilde 1972, and Cate et al. 1973). Although Cate et al. (1973) found aphid mortality caused by parasitoid feeding to be of lesser importance than that by parasitism, both means of causing mortality should be considered in assessing the impact of *A. asychis* on aphid populations.

Esmaili and Wilde (1972) stated that a majority of aphids killed by parasitoid feeding were young nymphs. Hartley (1922) commented that *A. semiflavus* (Howard) (often synonymized with *A. asychis*) preferred the younger nymphs of Myzus persicae (Sulzer) for feeding and oviposition. However, neither of these studies included data to support the claim of a preferred host age.

Studies were conducted to determine if oviposition by A. asychis might reduce greenbug fecundity. Hight et al. (1972) found that parasitization of the greenbug by a native parasitoid Lysiphlebus testaceipes (Cresson) decreased reproduction in all ages and completely eliminated reproduction in aphids parasitized when less than 3 days old.

Materials and Methods

All studies were conducted under laboratory conditions of $26 \pm 4^{o}C$ and 12-14 h photoperiod. To obtain progeny of known age, ca. 400 reproductive greenbugs were placed on RS-610 sorghum plants in 6" pots covered with a ventillated cellulose nitrate cage. The progeny were removed 12 h later and maintained on sorghum in the absence of parasitoids until they reached the desired age. All aphids and parasitoids were transferred in the manner described by Cate et al. (1973). The experiments were conducted in a randomized block design.

In all studies 5-7 dm vials 3/4 filled with white coarse grain sand were used. A freshly cut 1/2" section of sorghum was placed vertically in the sand, allowing the top 1/4" to be exposed. The sand was then moistened, but not saturated, with tap water. The aphids were then transferred to the leaf section in numbers reported below.

Feeding Preference

In the feeding study 3 age groups of aphids were used: (1) less than 12 h old, (2) 60-66 h old, and (3) 100-106 h old (used as the mature group because aphids of this age are not capable of reproduction, thus allowing equal numbers to be maintained in all age groups). Four aphids from each of the 3 groups, producing a total of 12 aphids, were placed on the leaf section in each of the 10 vials in all 6 replicates. This gave a total of 120 aphids per replicate. One female parasitoid was then introduced into each vial, allowed 6 h for stinging and feeding, and then removed. The studies were conducted daily from 11 a.m. to 7 p.m. during the spring of 1973. The live aphids from each vial were separated according to size and each group placed on a separate plant. When these replaced aphids mummified they were cut from the plant and placed into 1-oz. cups and the parasitoids sexed upon emergence.

Ovipositional Preference

Ovipositional preference was determined in a manner similar to the technique used in the feeding preference study. Unstarved female parasitoids less than 10 days old were used and the number of aphids/age group in each vial was increased from 4 to 12. This increased the number of aphids in each vial from 12 to 36. Only 3 replicates were conducted in this study. The remaining part of the study was conducted as previously described.

Greenbug Fecundity

To determine the effect of parasitization on fecundity of 1, 2, 3, 4, 5, 6, and 7 day old greenbugs, 3 replicates, each consisting of 10 stung and 10 unstung aphids/age group, were conducted. Aphids of a particular age were placed on leaf sections without aphids in glass vials as previously described. A single female parasitoid which had been exposed to males was placed into each vial. A control was conducted without female parasitoids. The parasitoids and aphids were observed. As soon as stinging ceased the stung aphid was placed onto a leaf section in a vial similar to those previously described. An aphid from the control was then placed on another leaf section in another vial. The stung and unstung (control) aphids in the vials were checked daily for 6 days, the number of progeny recorded, and then removed. The mummies were checked daily and the emergent parasitoids sexed.

Results and Discussion

Feeding Preference

In addition to the data reported by Cate et al. (1973) a preference for age of aphids utilized by *A. asychis* for feeding was found to be young > old > intermediate aged aphids (Figure 5). Analysis showed highly significant differences among stages and a highly significant linear effect due to days. Further analyses showed the linear day effects were not the same for all three stages.

In the present study, which was conducted as a more controlled experiment than the earlier study, a similar age preference sequence

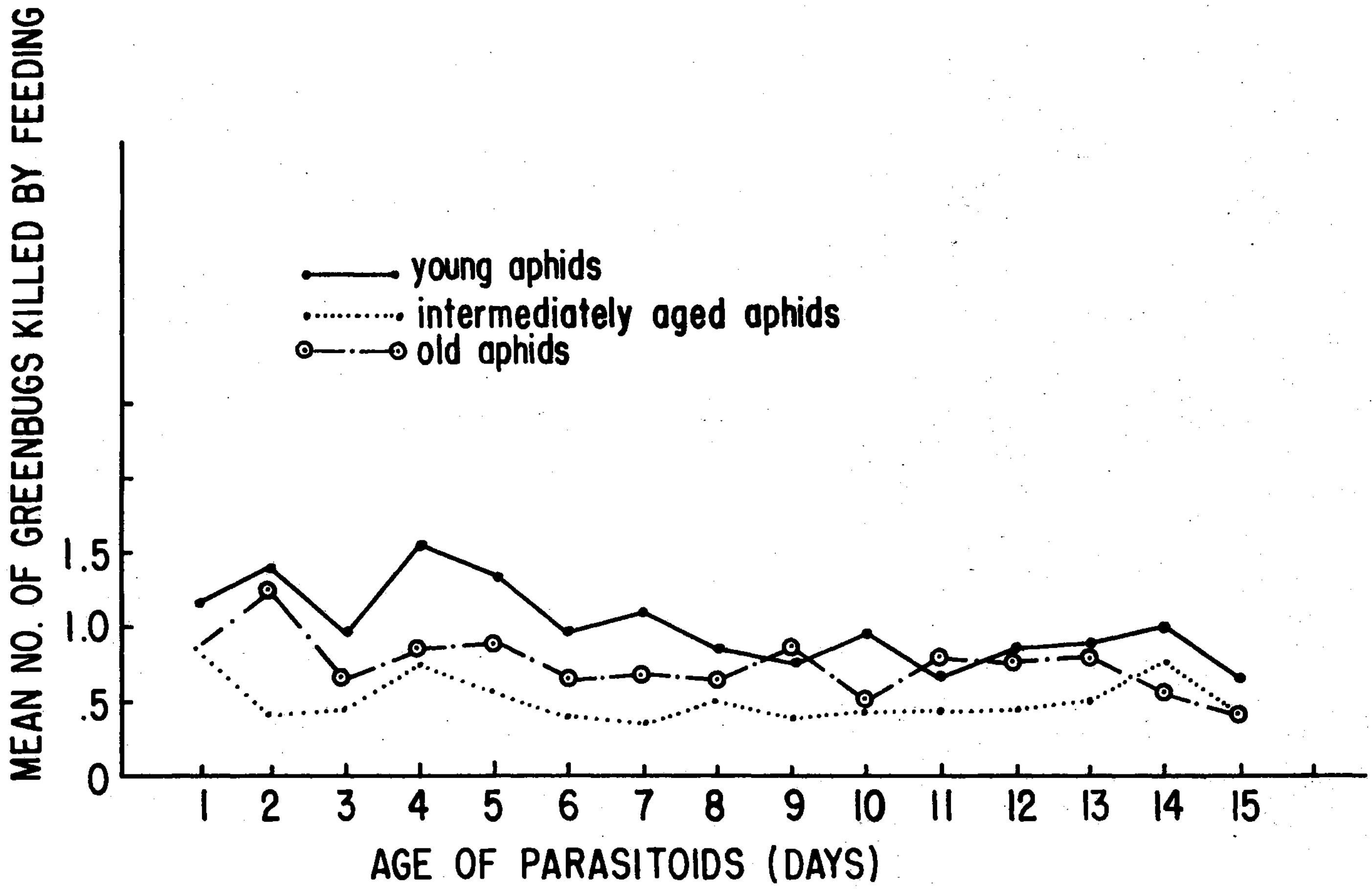

Figure 5. Daily mean number of greenbugs killed by the feeding of 20 ♀ _A. asychis_ the first 15 days of their lives as determined in the laboratory

was found, although the ratios differed. A 3:2:1 ratio was observed in 1972, compared to a 7:6:4 ratio in the 1973 study for the young, old, and intermediate ages, respectively.

Esmaili and Wilde (1972) reported that as A. asychis stung those aphids to be fed upon, the aphids swelled and a state of paralysis developed. In 30 consecutive observations it was noticed that in addition to the paralytic effects, aphids removed before A. asychis could feed never recovered and were dead within a few hours. No mummies developed from any of the aphids stung for feeding. Apparently, feeding hastens death in the aphids, although the ultimate source of death is either the injection of some foreign substance into the aphid from the ovipositor or mechanical damage caused by the ovipositor. More research is needed to determine the exact cause of death.

Ovipositional Preference and Emergence

Table I presents a summary of the data taken in the ovipositional preference study. Again, A. asychis shows an age preference pattern when ovipositing, but one different from that encountered in the feeding preference study. As can be seen, a 1 > 3 > 5 day preference was shown. The time from oviposition to mummification was 5-6 days for all ages of aphids used. The parasitoid required another 4-7 days to complete development and emerge. Thus, 10-13 days were required for the complete development of A. asychis, with most requiring 11 or 12 days. As reported by Cate et al. (1973), males usually appeared earlier than females.

TABLE I

THE NUMBER OF MUMMIES PRODUCED/HOST AGE CLASS, THE TIME REQUIRED FOR MUMMIFICATION OF THE APHID AND EMERGENCE OF THE PARASITOID, AND PERCENT EMERGENCE OF *A. ASYCHIS* UNDER LABORATORY CONDITIONS

Age of Parasitized aphids (hours)	Number of Mummies Produced Per Age a/	Elapsed Time from Oviposition to Mummification (days)	Elapsed Time From Mummification to Emergence (days)	% b/ Emergence	% c/ Female Emergence
12	100	5-6	4-7	95	54.7
60-66	44	5-6	4-7	93	56
100-106	26	5-6	4-7	85	95.4

a/ Three replicates conducted in a randomized block design, a total of 360 aphids used/age class/replicate

b/ $$\% \text{ Emergence} = \frac{(\text{Number Emerged/Age}) \times 100}{\text{Number of mummies produced/age}}$$

c/ $$\% \text{ Female Emergence} = \frac{\text{Number Females Emerged/Age}}{\text{Number Emerged/Age}} \times 100$$

Percent emergence (91%) was slightly higher than reported by Cate et al. (1973). Since in this experiment no exceptionally old aphids were used and there appeared to be a slight decrease in percent emergence in the older, parasitized aphids, one could speculate that in a population of aphids of all possible ages, a slightly lower percent emergence would be encountered than was found in this study using no older aphids. The high percentage of females (95%) in the older aphids is curious. Of the 26 mummies produced in the 5 day old aphid age class, 22 parasitoids emerged, of which 21 were female. More research is required to determine whether this is an exception or the general rule.

Effect of Parasitization on Greenbug Fecundity

Preliminary investigations suggested that some ovipositional insertions by *A. asychis* were not successful in producing a mummy. To insure as much consistency as possible, greenbugs stung for less than 30 seconds were not used in this study. Although this precaution was taken, several of the stung aphids never produced a mummy (Table II). Generally, the smaller aphids were more easily stung by the parasitoid. The older aphids may physically manipulate themselves and the parasitoid after insertion of the ovipositor so that successful oviposition is extremely difficult.

Statistical analyses were conducted to make comparisons of fecundity of stung and unstung aphids and stung parasitized (forming a mummy) aphids with those stung but not forming mummies. A significant difference was found at all ages when stung and unstung aphids were compared (at 99% level). There was also a significant

TABLE II

AVERAGE GREENBUG FECUNDITY AS AFFECTED BY OVIPOSITION BY *A. ASYCHIS* [a]

Age of aphid When Stung (days)	Average No. of Progeny/Stung, Unparasitized aphid	Average No. of Progeny/Unstung aphid [b]	Average No. of Progeny/Parasitized aphid	Total No. of Parasitized aphids That Reproduced
1	6.00 (7)	6.10	0 (23)	0
2	10.10 (7)	7.37	0 (23)	0
3	11.56 (9)	11.60	0 (21)	0
4	16.50 (11)	12.47	0.58 (19)	4
5	19.20 (15)	16.90	1.94 (15)	9
6	22.90 (17)	21.50	4.70 (13)	12
7	21.60 (10)	22.70	7.35 (20)	20

a/ Values in ( ) are the number of aphids on which the mean in the same column is based.

$$\text{The mean} = \frac{\text{Total progeny for 6 days after stinging}}{\text{The number in ( )}}.$$

b/

$$\text{These means} = \frac{\text{Total number of progeny for 6 days corresponding to same 6 days in treatment}}{\text{Number of aphids (= 30)}}.$$

difference between the fecundity of stung parasitized and stung unparasitized aphids. Comparisons of stung unparasitized and normal unstung aphids revealed little differences in fecundity. Therefore, it appears that oviposition, not stinging, is the major source of decreasing greenbug fecundity.

Aphids parasitized during the first 3 days of life produced no progeny. Reproduction never occurred more than 3 days after parasitization in any of the age groups.

In all age groups mummification was completed in 5 or 6 days and the remainder of the developmental period culminating in emergence required an additional 5-6 days. This supports data presented earlier that _A_. _asychis_ emerge 11-12 days after oviposition.

Do the conclusions reached in the ovipositional preference study represent a true preference pattern or a pattern more closely associated with success of oviposition? It probably represents a pattern of preference because oviposition success of the 1, 2, and 3 day old aphids in the fecundity study was ca. the same (Table II), whereas a large difference was found in the number of mummies produced by 1 and 3 day old aphids in the oviposition preference study (Table I).

Though the vials were excellent tools, allowing easy handling of the aphids and supporting them up to 6 days, conditions in them are conducive to fungal growth. This growth usually occurred after formation of the mummy and, in many cases, prevented emergence. For this reason, emergence (60%) was slightly lower than normal.

In future studies utilizing vials, the mummies should be removed to insure normal emergence.

To determine the relationship between longevity of a parasitoid and the number of aphids it parasitized, a fourth degree polynomial was fitted to the data presented in Cate et al. (1973). The model used was:

$$Y_{ij} = B_0 + B_1X_i + B_2X_i^2 + B_3X_i^3 + B_4X_i^4 + E_{ij} ,$$

where

Y_{ij} = the total number of aphids parasitized by the jth parasitoid by ith day,

X_i = the age of parasitoid at death,

E_{ij} = random error associated with Y_{ij}.

Since the results showed only a linear effect, a straight line was fitted (the plot of the line is shown in Figure 6). The prediction equation is:

$$\hat{Y} = -17.759 + 10.18X .$$

Another relationship was studied to predict the percent of aphids parasitized by the jth parasitoid on the ith day. A fourth degree polynomial was tried. Its form was:

$$Z_{ij} = B_0 + B_1X_i + B_2X_i^2 + B_3X_i^3 + B_4X_i^4 + E_{ij}$$

where

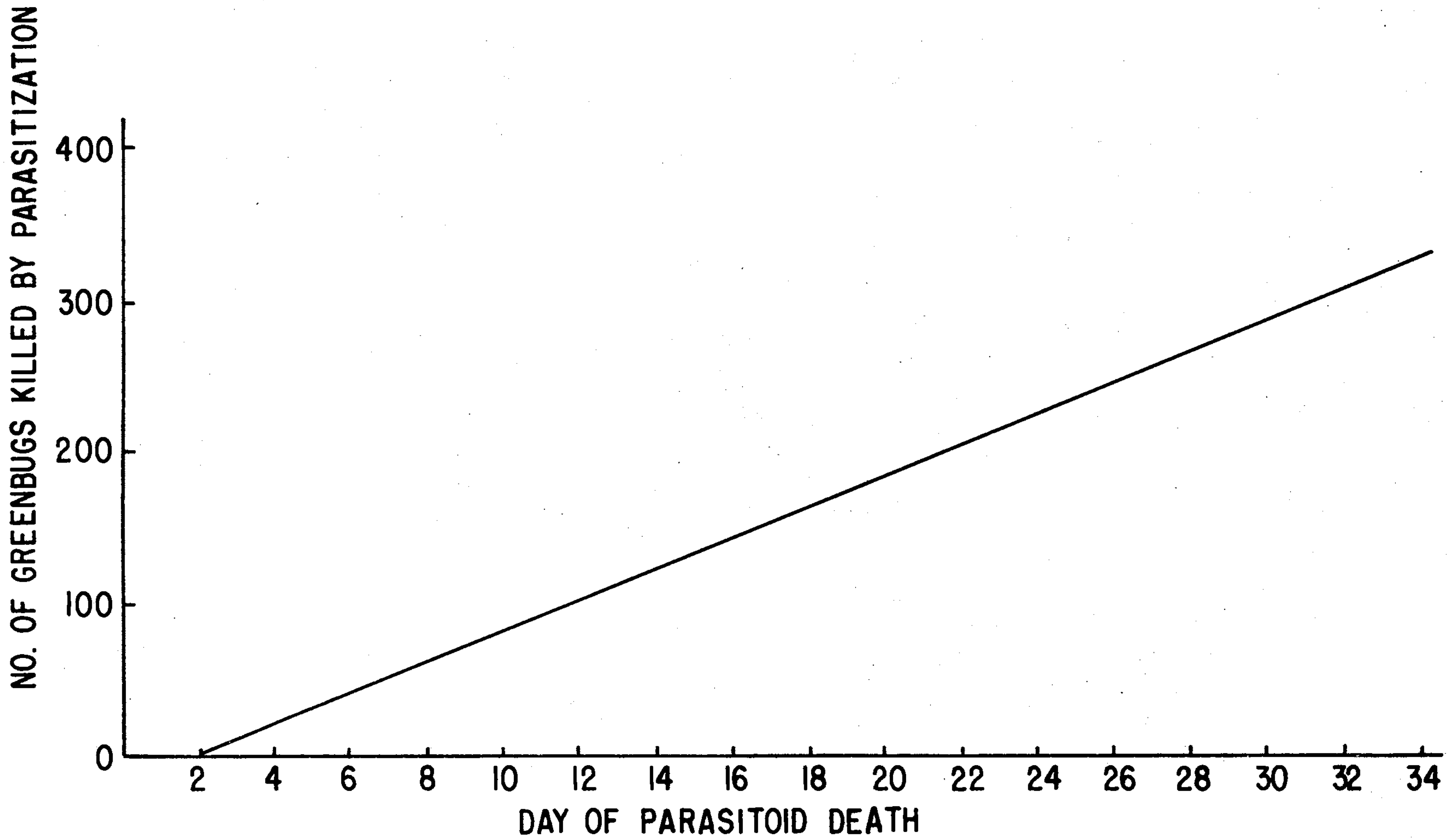

Figure 6. The cumulative number of mummies produced by female A. asychis dying on a certain day

$$Z_{ij} = \text{Percent aphids parasitized on the ith day by the jth parasitoid} = \frac{\begin{pmatrix}\text{Number of aphids parasitized} \\ \text{on ith day by the jth para-} \\ \text{sitoid } (= A_{ij})\end{pmatrix} \times 100}{\text{Total number aphids parasitized during the parasitoid's lifetime } (= \Sigma A_{ij})}$$

X_i = the day on which the aphids were parasitized, and

E_{ij} = random error associated with Z_{ij} .

It was found that only a linear effect was significant. Thus, as parasitoid age increases there is linear decrease in the percent of total oviposition. Figure 7 presents a plot of the line. The equation for this line is:

$$\hat{Z} = 6.753 - 0.22X .$$

From the results of these studies A. asychis appears to have potential as a biological control agent. In addition to those means of control of greenbug populations previously mentioned, the parasitoid is effective in reducing the reproductive potential of the parasitized aphids.

It would seem that A. asychis shows excellent possibilities in inundative programs of mass release for the regulation of greenbug populations. It attacks all ages of aphids, both for parasitization and feeding. Oviposition decreased reproduction in older parasitized aphids and entirely eliminated production of progeny in aphids 3 days old or younger. Adult parasitoids may be kept in cold storage for extended periods with few harmful effects (Archer and Eikenbary 1973). Because of its long lifespan A. asychis can be retained in the laboratory to maintain cultures and subsequently shipped to the field

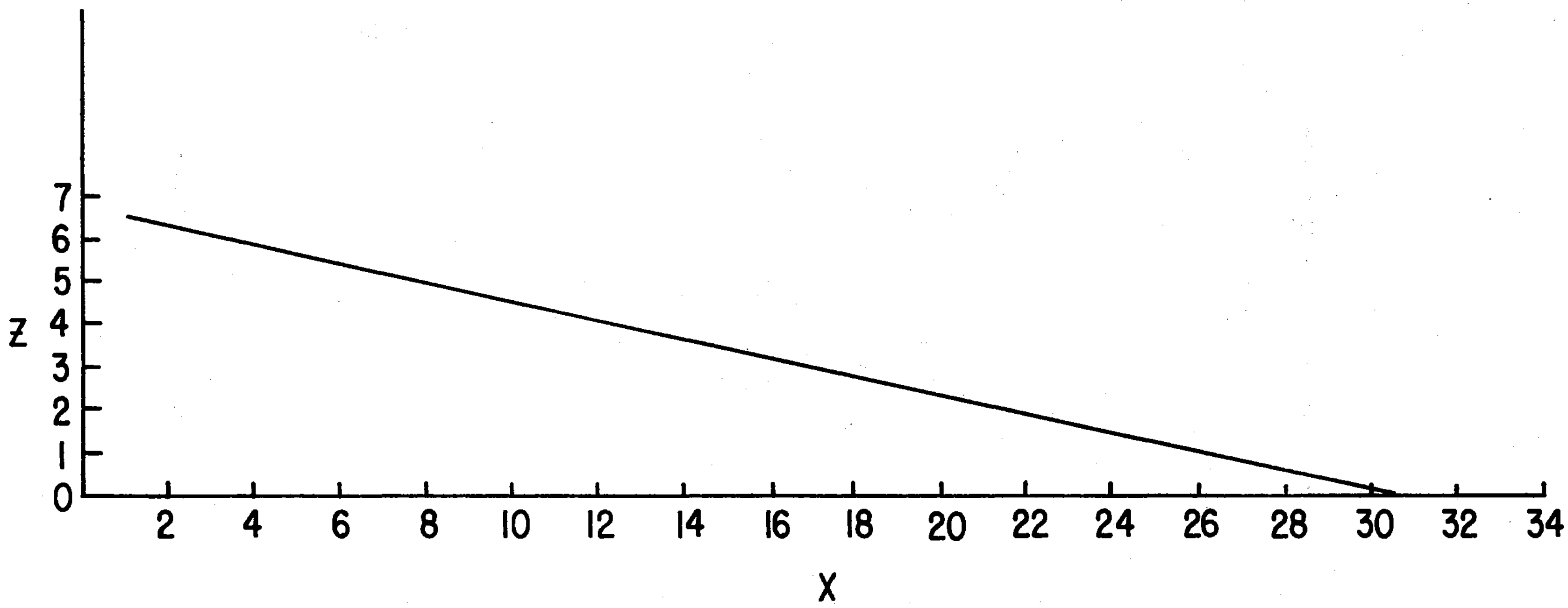

Figure 7. The daily % of the total number of mummies produced by 20 ♀ A. asychis [a/]

[a/] X = Day on which the aphids were parasitized

Z = % aphids parasitized on the ith day by the jth parasitoid when less than 10 days old and still produce ca. 50% of their offspring. From laboratory studies it appears that _A. asychis_ has potential on greenbug populations in any stage of development, but sould probably show its greatest impact on those populations with large numbers of very young greenbugs. More research should be conducted concerning storage, rearing, acclimation, and release of the parasitoid. Overall, the future of _A. asychis_ as a biological control agent of greenbugs looks bright.

CHAPTER IV

A TECHNIQUE UTILIZING RADIOISOPTOPES TO DEMONSTRATE HOST FEEDING BY THE PARASITOID APHELINUS ASYCHIS

Aphelinus asychis (Walker) kills several of its hosts apparently by feeding from the wound inflicted by the parasitoid's ovipositor (Esmaili and Wilde 1972, Cate et al. 1973). This relationship has been suggested as occurring in other host-parasitoid relationships as well, but with only observational data as proof (Marchal 1909, Rockwood 1917, Hartley 1922, and DeBach 1943). The purpose of these experiments was to demonstrate, with the aid of "tagged" molecules (^{14}C-carboxyl-inulin and ^{14}C-glucose), that A. asychis feeds upon greenbugs and in particular the contents of its hosts' alimentary canals.

Materials and Methods

The greenbug, Schizaphis graminum (Rondani), was chosen as the host for this study because A. asychis was visually observed to apparently feed upon several (an average of over 20) greenbugs during its lifespan (Cate et al. 1973). A chemically defined diet and the techniques for raising aphids were similar to those of Cress and Chada (1971). Uniformly labelled ^{14}C-glucose and ^{14}C-carboxyl-inulin

(New England Nuclear) were incorporated into the diet as radioactive markers. Four concentrations of each radioactive compound were prepared by adding 1 ml of distilled water to 50μCi of each of the marked compounds. Subsequently, 22 ml of the radioactive water was added to 0.5, 0.75, 1.0, and 1.25 ml of the greenbug diet, resulting in diets marked with ca. 25,000, 19,000, 15,000, and 12,000 CPM/μl.

Preliminary investigations revealed that the aphids initiate feeding readily in darkness. Therefore, adult aphids (starved for 24 h) were transferred to the radioactive diet in early evening. After 12 h of feeding the greenbugs were removed and placed singly into gelatin capsules with a semi-starved (without food for 24 h) female parasitoid. The parasitoids were removed immediately (within 10 min.) after completion of feeding and subsequently frozen. Later, five of the frozen parasitoids/aphid diet were thoroughly homogenized after placement into 15 ml of a liquid scintillant (Wharton et al. 1965) and measured for radioactivity with a Beckman LS100 Liquid Scintillation Counter ^{R}. Controls were conducted in the same manner except the parasitoids were not allowed to feed from the stung aphid.

To determine if any radioactivity was present in the homocoel of greenbugs after feeding from either of the diets, the lets of 5 adult aphids were removed and placed into the liquid scintillant along with any hemolymph that emanated from the severed legs. Radioactivity was measured as previously described.

Results and Discussion

Although there was an apparent increase in radioactivity in parasitoids feeding upon greenbugs from diets of increasing radioactivity (Table III), an analysis of variance showed no significant difference due to concentrations (Calculated $F = 1.69$). Considerable variations in parasitoid radioactivity are possibly due to: (1) differences in the size of host aphids, (2) differences in the amount of food ingested by the aphids during the 12 h feeding period, (3) differences in the size and age of the parasitoids, and (4) differences in the amount of foodstuffs consumed from the aphid. In an attempt to minimize these sources of variation, aphids were selected for equal size and uniformly allowed to feed from the radioactive diet for 12 h. Selected parasitoids were of approximate equal size. However, the parasitoids were not removed from the capsule until they had terminated feeding, which could have been a major source of variation because the parasitoids' duration of feeding varied greatly (as much as 30 min.).

A comparison of the control versus the treatment insects in Table III shows far more radioactivity in the treatment parasitoids. A closer analysis of the control data shows slightly more radioactivity than background (35 CPM), suggesting that a small amount of contamination occurred during the stinging process and/or some other random contact with the aphic or its excreta. The combined data of the control and experimental insects leave little doubt that _A. asychis_ demonstrates host feeding.

TABLE III

RADIOACTIVITY PRESENT IN THE PARASITOID A. ASYCHIS AFTER FEEDING ON RADIOACTIVE GREENBUGS

Greenbug diet a/	Average CPM of Parasitoids fed on b/			
	Glucose fed greenbugs	Control	Inulin fed greenbugs c/	Control c/
25,000	626	36	812	32
19,000	572	63	—	—
15,000	542	50	—	—
12,000	442	42	532	66

a/ Expressed in CPM of ^{14}C-glucose or ^{14}C-inulin/diet

b/ 4 replicates, 5 parasitoids/replicate conducted in a randomized block design, standard deviation = 118.1

c/ Blanks = no data obtained

A second study to determine if the parasitoid was feeding on contents of the aphid's alimentary canal was initiated using ^{14}C-carboxyl-inulin rather than ^{14}C-glucose in the aphid diet. Inulin has been shown to be inert and not moved across membranes in most organisms (Wharton et al. 1965). Since no significant differences in parasitoid radioactivity were found when aphids were feeding on different concentrations of ^{14}C-glucose, only two concentrations of ^{14}C-carboxyl-inulin diets (corresponding to the low and high ^{14}C-glucose diet concentrations) were replicated in the parasitoid feeding-inulin study. Significant radioactivity was recorded at both concentrations with higher means for the inulin than the glucose (Table III). The diets were checked to determine their actual radioactivity and found to be approximately equal.

To verify whether the greenbug restricted inulin to the lumen of its alimentary canal after feeding from the ^{14}C-carboxyl-inulin diet, hemolymph and dissected legs were assayed for radioactivity and compared with similar samples taken from aphids feeding from the ^{14}C-glucose diet. Each group of greenbugs was offered the highest concentration of inulin or glucose. A mean of 338 CPM was found in the glucose fed aphid samples, compared to only 55 CPM in the inulin fed samples. The slight difference between the background counts (35 CPM) and the inulin samples may be due to the error of the scintillation counter (up to 35% in samples of this magnitude) and slight contamination that might be expected on the tarsi of the greenbugs due to excreta on the parafilm through which they fed. Hence, these data suggest that carboxyl-inulin remains within the alimentary canal with no movement across the gut membrane for the 12 h period in our study. These data combined with those of Table III indicate that some of the food ingested by A. asychis is taken from contents of the greenbugs' alimentary canal.

The higher CPM assayed in the parasitoids feeding from inulin fed aphids compared to the glucose fed aphids may be because of metabolism and movement of glucose across the gut producing a lower concentration for the parasitoid to ingest, whereas inulin is probably more restricted to the alimentary canal producing a source of higher radioactivity for ingestion by the parasitoid.

Cate et al. (paper in manuscript) found that the greenbugs died after being stung by semi-starved parasitoids even if feeding did not occur. No acceptable explanation for this earlier observation could be given. The results of this study suggest that the parasitoid probably disrupts the gut with its ovipositor. This may be the ultimate cause of aphid death associated with parasitoid feeding.

This technique offers possibilities for use as a marker for future releases of *A. asychis*. The parental generation could be marked in the laboratory, released, and subsequent field samples taken and checked. Longevity of the parasitoid in the field might be more closely monitored in this way. However, more research needs to be conducted to determine the effects of isotopes on the parasitoid, their potential in marking eggs and the F_1 generation, and longevity within the parasitoid.

CHAPTER V

SUMMARY AND CONCLUSIONS

From laboratory studies conducted in 1972-73 it was concluded that *A. asychis* may regulate greenbug populations in 3 ways: (1) by parasitism, (2) by host feeding, and (3) by reducing the reproductive potential and in some cases completely eliminating reproduction of parasitized greenbugs. *A. asychis* was found to parasitize an average of 233 greenbugs in a mean lifespan of ca. 19 days. The greenbug required 5-6 days to form a mummy and the parasitoid emerged from the mummy in an additional 5-6 days. This gives a total developmental period from oviposition to emergence of 10-12 days. Parasitoids emerged from ca. 81% of the mummies. Of this total percent emergence, female emergence comprised 56%. Males usually emerged ca. 1 day before the females.

The parasitoid's preference for age of greenbugs for parasitizing was young > middle age > old. All ages may be successfully parasitized; however, the parasitoid meets greater resistance from the greenbug as the aphid approaches maturity. The greenbug has several means of resistance: (1) by falling from the plant; (2) relocation on the plant; (3) "bobbing" its abdomen; and (4) overpowering the parasitoid while the parasitoid is in the stinging process. All these factors play important roles in the preference pattern of *A. asychis*.

Host feeding by adult A. asychis was proven utilizing greenbug diets tagged with ^{14}C-glucose. Further experiments with ^{14}C-carboxyl-inulin indicated that A. asychis ingested a part of its diet from the greenbug's alimentary canal. In an average lifespan A. asychis may kill an average of ca. 30 greenbugs by feeding. The young aphids are preferred for feeding with the older generally preferred to the middle aged aphids.

Aphid reproduction is significantly decreased after parasitization by A. asychis. Aphids parasitized when less than 3 days old never reproduced. Stung unparasitized aphid reproduction is not significantly affected.

From the results of these experiments, it seems that A. asychis offers a great potential as a regulator of greenbug populations. It attacks all ages of greenbugs for oviposition and feeding although it shows a preference for the younger aphids in both cases. From the standpoint of reduction of greenbug fecundity it appears that this preference for young aphids is advantageous because reproduction is eliminated in young, parasitized greenbugs. A. asychis maintains the ability to parasitize aphids and produce female progeny throughout its lifespan. Due to the long lifespan of A. asychis it may be used in the laboratory to maintain cultures and later transported to the field for inundative releases. Further experiments need to be conducted to determine its ability to survive in the southwest before a complete evaluation of A. asychis can be reported. However, from all evidence the future of A. asychis as a biological control agent of the greenbug on sorghum looks bright.

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VITA

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