PARASITE-HOST INTERACTION OF NATIVE PARASITES AND *APHELINUS ASYCHIS* ON APHIDS OF SORGHUM

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PREFACE

The cancellation of many chemical use patterns on various crops and the increasing concern over environmental contamination due to pesticides have caused an urgency for the development of other agents to supplement, if not to replace, conventional insecticides. The degree of insect damage once considered acceptable is no longer tolerable, and attempts to develop more effective insecticides have been self-defeating to some degree. Residue problems are of increasing importance, especially when chemicals are used to treat agricultural crops; chemicals are also detrimental to many beneficial insects (parasites and predators). Inadvertent misplacement of chemicals to streams, rivers, lakes, ponds, or areas other than the intended target is also not uncommon. The development of resistance among insects to insecticides and the potential danger to the user of some of the modern chemicals are additional disadvantages.

All of these factors make the utilization of biological control measures most attractive. Parasites, predators, and microbial pathogens are agents that may be used in this way, and in past years many of them have been used successfully.

However, in the present research on the greenbug, only one group of biological agents, that of parasites, will be utilized and studied.

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INTRODUCTION

The greenbug, Schizaphis graminum (Rondani), is considered the most injurious insect attacking small grains in the Great Plains area with losses of many millions of dollars to the small grains in outbreak years. In the summer of 1968, severe greenbug outbreaks occurred for the first time on sorghum crops in Oklahoma and surrounding states. There was great concern among entomologists in the states involved. The outcome was the establishment of a state and regional project to investigate biological control of this pest.

Previous work on natural or biological control of the greenbug on wheat and other small grains in the United States is very limited, while work on natural and biological control of the greenbug on grain sorghum is virtually nonexistent since this problem developed during the summer of 1968.

Thus the present research was undertaken to (1) determine the species of native parasites attacking aphids on sorghum in Oklahoma and evaluate their role in controlling the greenbug; (2) investigate the bionomics of Aphelinus asychis, an imported parasite of the greenbug, in the field; and (3) develop a method for rearing large numbers of A. asychis for field releases.

PARASITES REARED FROM THE GREENBUG AND CORN LEAF APHID IN OKLAHOMA IN 1968 AND 1969

The greenbug, Schizaphis graminum (Rondani), is a serious pest of winter-grown small grains in the central and southwestern states. It was described in Italy in 1852 (Hunter and Glenn 1909), and first recorded in the United States in Virginia in 1882 (Webster and Phillips 1912). The greenbug caused considerable damage to wheat and oats in certain areas of Indiana, Tennessee, North Carolina, and Texas in 1890. Oklahoma recorded the 1st outbreak on small grains in 1901. Damaging infestations have since occurred in 1907, 1916, 1939, and 1942 when more than 61 million bushels of grain were lost (Daniels et al. 1956). Record losses occurred again in 1949-51 in the Panhandle. The greenbug is still a major pest on small grains.

In the summer of 1968, severe greenbug outbreaks occurred for the 1st time on sorghum crops in Oklahoma, Arizona, California, New Mexico, Texas, Colorado, Kansas, Nebraska, and South Dakota (Wood et al. 1969, Harvey and Hackerott 1969a). Greenbugs originating on sorghum are considered to be a different biotype from those collected on wheat and were designated as C-biotype (Harvey and Hackerott 1969a, b). The corn leaf aphid, Rhopalosiphum maidis (Fitch), and oat-bird cherry aphid, R. padi (L.), also occur on sorghums, but usually do not cause economic damage. Thus, the greenbug poses a serious threat to sorghum crops as well as small grains, since the growing seasons of the crops overlap.

The purpose of this research was to determine the species of parasites that attack these aphids in Oklahoma and to evaluate their role in controlling the greenbug. Three primary parasites (Lysiphlebus testaceipes (Cresson), Aphelinus nigritus Howard, A. varipes (Foerster) and 4 secondary parasites (Aphidencyrtus aphidivorus (Mayr), Pachyneuron siphonophorae (Ashmead), Asaphes lucens (Provancher), and Charips sp.) were reared from the host aphids. According to Webster and Phillips (1912), the following parasites were reared from the greenbug collected on wheat: Aphidius (= Lysiphlebus) testaceipes (Cresson), A. confusus Ashmead, Aphelinus mali Haldemann, A. nigritus Howard, A. semiflavus Howard, Aphidencyrtus aphidiphagus Ashmead, Pachyneuron sp., Megorismus sp., and Allotria sp. The parasite Aphidius avenaphis Fitch was reared from the greenbug in the insectary. Simpson et al. (1959) reported rearing A. semiflavus from the greenbug in Kansas. Bibby (1959) found the rusty plum aphid, Hysteroneura setariae (Thomas), on grain sorghum, highly parasitized by A. nigritus. Since the early report by Webster and Phillips (1912), we have found little published material on the native parasites of the greenbug. Most investigators mention only L. testaceipes in connection with greenbug outbreaks.

Materials and Methods

A limited survey was made in 1968 for parasites associated with the greenbug. A more extensive survey was made in 1969 for parasites of the greenbug, corn leaf aphid, and oat-bird cherry aphid. All 3 species are found on sorghum in Oklahoma. Parasitized aphids (mummies) were collected regularly from the middle of June through August from sorghum fields in the major growing areas of the State.

A mouth aspirator was used to remove the mummies from the sorghum leaves. Collections from each field were placed in individual vials and properly labeled as to date and location. After being returned to the laboratory, the mummies were counted, separated to species, and checked for previous parasite emergence. Those showing emergence holes were discarded and not included as part of the collection. The mummies were then placed individually in size 3 gelatin capsules and stored at laboratory temperature ($75^{o} \pm 5^{o}$ F) for emergence. Parasite emergence was checked and recorded daily.

Parasites were identified by Dr. B. D. Burks of the Insect Identification and Parasite Introduction Research Branch, Entomology Research Division, USDA, Beltsville, Md.

Results and Discussion

Tables 1 and 2 list the parasites reared from the 3 aphids. The host plant, host insect, color of mummies, year, county location, and remarks accompany each parasite. Seven species representing the families Braconidae, Eulophidae, Encyrtidae, Pteromalidae, and Cynipidae were recovered. The parasites belonging to the Braconidae and Eulophidae are primary parasites, and all others are considered to be secondary or hyperparasites.

The braconid L. testaceipes was the most abundant parasite of the greenbug and is easily recognized in the field by its gold-colored mummies. It was also reared from corn leaf and oat-bird cherry aphids. Only 3 oat-bird cherry aphid mummies were collected, and these were parasitized by L. testaceipes. This aphid was found mainly on broom corn during the summer. However, it is found primarily during the

cooler months on small grains. The presence of L. testaceipes was not evident until early August, and in several fields under observation the greenbug had already caused extensive damage before the parasite population increased. L. testaceipes is also reported as being the most efficient parasite of the greenbug on wheat (Hunter and Glenn 1909, Webster 1909, Webster and Phillips 1912, Daniels et al. 1956, Wood 1958).

A. nigritus was the second most abundant parasite. It was present early in the season (middle of June to middle of July) on corn leaf aphids in forage sorghum. From the middle of July until the end of August it was found primarily parasitizing greenbugs on grain sorghum. Another parasite reared from the greenbug and corn leaf aphid was A. varipes. Though present in small numbers, it could prove to be an important parasite of the greenbug. Both Aphelinus parasites were recovered for the 1st time from field collections in Oklahoma (Fig. 1). Bibby (1959) reported A. nigritus parasitizing the rusty plum aphid on grain sorghum in Arizona. Previously, it had been found in Kansas, Minnesota, New Mexico, and South Carolina (Webster and Phillips 1912). One of us (Wood 1958) found A. nigritus in a greenhouse at Stillwater, Okla. After colonization, it was released in the field, but no recoveries were made.

A. varipes is an introduced parasite that has been recorded from most of the European countries (Férrière 1965), where it has been found parasitizing several species of aphids. In 1962, R. van den Bosch collected A. varipes from Aphis gossypii Glover in Egypt and made rather large releases in the vicinity of Riverside, Calif. (personal communication). The parasite was recovered at a release site late in

1962, but no subsequent recovery attempts were made near Riverside, so there is no knowledge concerning the fate of A. varipes in southern California. In the fall of 1968, members of the European Parasite Laboratory of the Insect Identification and Parasite Introduction Research Branch, ERD, USDA, found A. varipes in mummies of the corn leaf aphid collected in southern France. Laboratory studies in France indicated that the greenbug was a good host for A. varipes, so a program for introduction of this parasite into Oklahoma was suggested. As a result, entomologists of the USDA and the Department of Entomology at Oklahoma State University, Stillwater, received a shipment of mummies on Jan. 18, 1969, that yielded 78 adults. These were increased on greenbug-infested barley in the greenhouse until March 20, when about 1000 mummies were placed in a field cage over wheat having a greenbug and an oat-bird cherry aphid population as high as 2500/ft of row. On Apr. 4 an additional lot of 8500 mummies was put in the cloth-covered cage similar to the one used by van den Bosch et al. (1959). Observations did not indicate as high parasitization by A. varipes as by the native L. testaceipes, so the test was discontinued about 3 weeks after the last release, and the cage was removed. However, A. varipes was recovered during the summer from 5 counties in Oklahoma (Fig. 1), namely Payne, Grady, Okfuskee, Texas, and Cimarron. Since it is just becoming established, its impact on the greenbug cannot be surmised from our 1969 survey.

It is not known how A. varipes disseminated so rapidly and over such distance in 1 season. For instance, this parasite was released at Stillwater in March and April and collected in the Panhandle of Oklahoma, 250 miles from the liberation site, 2-3 months later. It is

possible that the parasite may have flown to a new area, settled, reproduced, and through a series of generations and movements reached the Panhandle. Most likely, however, the dispersal of the parasite was due to parasitism of winged hosts or just before the aphids became winged prior to flight, and these aphids were blown by the wind in a northwestern direction from the liberation site. A. varipes was found ca. 75 miles southeasterly from the liberation site. While A. varipes was released by van den Bosch in California in 1962, it was not collected in our survey in 1968. Therefore, our recovery of this parasite was most likely from our liberations. The dispersal of this parasite might have been better except that grain sorghums and other plant hosts were separated by several miles.

The percent parasitism of the hosts in the field is difficult to calculate, because parasitized greenbugs and corn leaf aphids have the habit of dropping or crawling off the plant and into cracks and crevices in the soil at the base of the plant when parasitized by A. varipes. This characteristic was observed in laboratory studies. It can be expected that this same trait would occur in nature. However, no attempt was made to find parasitized aphids at the base of the plant. Also, no attempt was made to rear the apparently unparasitized aphids on a host plant. This could have provided additional parasitized hosts, as parasitized aphids take about 6 days to discolor (turn black) and be observed for collection.

A. varipes has some qualities that make it exceptionally desirable to have as a control agent in the ecosystem. For instance, its habit of going into the whorl of the sorghum plant and parasitizing the corn leaf aphid is highly beneficial, as this aphid is commonly found in the

whorl early in the season until the crop is nearly ready to be harvested. This would allow A. varipes to maintain itself in a sorghum field and be available for parasitism of the greenbug as it becomes established. Also, by this parasite parasitizing the corn leaf aphid in the whorl of the plant, there would be the added advantage of protecting the parasite to some extent from insecticides applied to the sorghum fields for control of the greenbug. Presently it is difficult to kill the corn leaf aphids in the whorl of the plant, so this fact tends to insure hosts and a home for A. varipes. This parasite will receive close attention in 1970.

The 2 most prevalent hyperparasites were A. aphidivorus and P. siphonophorae. A. aphidivorus emerged only from black mummies indicating that it was a hyperparasite on Aphelinus or a tertiary parasite upon its own species. P. siphonophorae emerged from black and gold mummies. This fact indicated that it was a hyperparasite on Aphelinus and L. testaceipes or possibly a tertiary parasite upon its own species or even A. aphidivorus. Both of these parasites have been reared from different aphids; they are generally considered secondary parasites (Webster and Phillips 1912, Schlinger 1960, Schlinger and Hall 1960).

Fig. 1 shows the areas that were surveyed. A number of counties were surveyed in which no parasitized aphids or greenbugs were found. This does not mean that the greenbug or its parasites were not present; both were present probably in most all areas, but in such small numbers they could not be found.

BIONOMICS OF *APHELINUS ASYCHIS*, AN INTRODUCED PARASITE OF THE SORGHUM GREENBUG

Traditionally, the greenbug, *Schizaphis graminum* (Rondani), has been known as a serious pest of winter-grown small grains in the central and southwestern states. However, in the summer of 1968, severe greenbug outbreaks occurred for the 1st time on sorghum crops in Oklahoma, Arizona, California, New Mexico, Texas, Colorado, Kansas, Nebraska, and South Dakota (Wood et al. 1969, Harvey and Hackerott 1969a). According to Harvey and Hackerott (1969a,b), greenbugs that originated on sorghums were a different biotype from those found on wheat, and were designated as C-biotype. Localized damaging infestations of the "C" greenbug were recorded in Oklahoma again in 1969 and 1970; but for the most part the sorghum producers were aware of their presence and reduced the greenbug population with insecticide applications before they reached damaging proportions. Because sorghum has a relatively low cash value per acre, it is not economically feasible to make more than 1 application of insecticide for greenbug control. In addition to the cost, there are also problems of residue and the destruction of beneficial insects. Thus, in 1969, research was initiated on biological control of the greenbug. Several species of imported parasites were received from the Insect Identification and Parasite Introduction Research Branch, USDA, Moorestown, N. J., one of which was *Aphelinus asychis* (Walker). This parasite was introduced from Iran.

During summer and fall of 1969, preliminary field investigations were conducted with A. asychis and 4 species of aphids commonly found on sorghum or small grains. The purpose of this work was to determine the host preference, adult longevity, developmental time, fecundity, and sex ratio of A. asychis in cages in the field.

Materials and Methods

To determine host preference of the parasite, 4x3-ft cages, 5½ ft high, covered on the sides and top with organdy cloth, were placed in the field over grain sorghum (Fig. 2). The lower half of 1 end of each cage was fixed so we could enter to introduce and inspect parasites and remove parasitized aphids (mummies) easily. Metal rods, ca. 2 ft long and ½ in. in diam, bent in the shape of a "7," were placed on the inside of the cages with the lip over the wooden framework and driven into the soil to anchor the cages. Additional cage stability was provided by 2 opposing guy ropes running from the cage top to the ground.

Nine cages were used in this experiment. Three cages, a check and 2 treatments, were placed on the Oklahoma State University Agronomy Research Station at Perkins, Okla. Six cages for 2 other tests, each involving a check and 2 treatments, were placed on the Teel farm at Hooker, in the Oklahoma Panhandle. The sorghum at Perkins and Hooker was irrigated and was ca. 2 ft high when caged.

Each cage enclosed 7-10 sorghum plants. Before each test the caged plants were examined closely and any predators (in any stage) present were removed. No aphid parasites were observed. The main predators encountered were lady bird beetles (in all stages of development), green lacewings (eggs and larvae), and a few syrphid

larvae. After hand removal of all predators, the cages were infested with 4 aphid species, namely C-biotype greenbug, corn leaf aphid, Rhopalosiphum maidis (Fitch), oat-bird cherry aphid, R. padi (L.), and the yellow sugarcane aphid, Sipha flava (Forbes).

All aphids were laboratory reared on Rogers barley grown in 6-in. plastic pots, except the yellow sugarcane aphid which was cultured on sorghum. The aphids were transported to the field on the potted plants. The plants in the cages located at Perkins were infested on July 20 with 150-200 aphids of each of the 4 species. The middle plants in each cage were infested with aphids by cutting infested plants or sections of plants, depending on aphids present, from the laboratory-reared culture and placing them in the leaf axils of the host plant. Once the infested plant material began to dry out, the aphids ceased feeding and moved over to the fresh host plants. This movement usually took place within a day. Each aphid species was oriented, as near as possible, on the sorghum plants according to the way in which it occurs naturally. For instance, the corn leaf aphid is found mainly in the plant whorl, hence material of it was placed there. The greenbugs and oat-bird cherry aphids were placed on the top side of the bottom leaves; then they moved immediately to the underside of the leaves where the 1st colonies are usually found. The yellow sugarcane aphids were placed on leaves about middle way on the plant and this species also moved to the underside of the leaves. The day following aphid inoculation, parasites were introduced into the cages. Because of the relatively short distance from the laboratory to the Perkins Research Station, it was possible to release newly emerged mated female parasites

which were transported to the field in 2-dr vials and released at the base of the plants.

The cages located at Hooker, ca. 300 miles from Stillwater, were infested with the same number of aphids of each species, except greenbugs, and in the same manner as discussed hereinbefore for those at Perkins. The natural greenbug population had begun to increase at the time the cages were set out, so it was not necessary to infest caged plants with that aphid. When the 1st test was initiated at Hooker on July 24, the greenbug population was estimated at 300-400/cage. At the initiation of the 2nd test the following week, the greenbug population was ca. 600-700/cage. Mated female parasites 2-3 days old were released the day after aphid infestation. These were about the youngest mated parasites that one could release at this distance from the rearing laboratory because of the time allotted for sexing, mating, and transportation to the field. The parasites were usually kept cool while being transported to the field by placing them in an ice chest with Canned Ice (R.M. Hollingshead Corp., Santa Clara, Calif.). However, before being released, the parasites were allowed to become acclimatized.

The host preference of A. asychis was measured by comparing the number of parasitized aphids of each of the 4 species. At Perkins, we tried to remove all mummies before any 1st generation parasites emerged. Additional mated females were released into the test cages several days later, to compensate for the removal of the parasitized aphids. At Hooker, the number of mummies collected from each cage in Test 1 was: 1st week 25, 2nd week 60, and 3rd week 70. In Test 2 the number of mummies collected from each cage was: 1st week 25, and 2nd week 50.

The mummies were removed from the sorghum plants by means of a mouth aspirator. Collections from each cage were placed in individual vials and properly labeled. When we returned to the laboratory, we placed the mummies in a refrigerator to retard parasite emergence until they could be identified and the numbers of each determined.

The 2nd portion of this research involved a study to obtain data on adult longevity, developmental time, fecundity, and sex ratio of the parasite under field conditions. The methods and procedures were similar to those used by Raney et al. (1971) in laboratory studies. Ten potted sorghum plants were hand infested with twenty-five 2nd- and 3rd-stage nymphs of the greenbug (C-biotype) and then caged individually. Sufficient time was allowed for the aphids to distribute themselves on the plants, and then 1 female A. asychis was released in each cage. The caged plants were then placed in a plot of grain sorghum on the Experiment Station at Stillwater and checked daily to determine the longevity of the parasite. Once mummies began to appear, the plants were brought into the laboratory each day to remove the mummies and were returned immediately to the field. The daily collection of mummies from each plant was placed in a 1oz plastic container, properly labeled, and returned to the field for parasite emergence. Upon emergence the parasites were sexed and recorded daily. The developmental time was estimated from the dates of parasite introduction, mummy appearance, and parasite emergence. Fecundity was determined according to the number of mummies obtained during the life span of the parasite. Temperature data were obtained from a standard weather shelter located in a weather station adjacent to the plot of sorghum.

Results and Discussion

The host preference of A. asychis under field conditions when caged with 4 species of aphids on grain sorghum is presented in Table 3. At each field test location the data indicated that the preferred host was the greenbug followed by the corn leaf aphid. Raney et al. (1971) reported similar results obtained from laboratory studies conducted at various constant and constant-alternating temperatures using the greenbug, corn leaf aphid, and yellow sugarcane aphid. We found just a few mummies of the oat-bird cherry aphid and yellow sugarcane aphid. In the field, neither of these aphid species increased greatly on grain sorghum, possibly a result of the effect of high temperature, as both species are usually associated with cooler temperatures that prevail during fall, winter, and early spring (Chada et al. 1965, Jackson et al. 1970). Raney et al. (1971) also observed that the yellow sugarcane aphid reproduced better at the constant-alternating temperature of 21.1-32.2^{o} C than at a constant 32.2^{o} C.

The number of nonparasitized and parasitized greenbugs was far greater than that of the corn leaf aphid. Hence our data may not have been a true measurement of host preference as the greenbug became the most abundant species. Small numbers of corn leaf aphids were observed interspersed among the greenbugs on the lower leaves, but for the most part they were usually found in the whorl of the plant (Almand et al. 1969, Jackson et al. 1970). Because the parasites were released at the base of the plants where there was an abundance of greenbugs, the relatively small number of corn leaf aphid mummies obtained may have resulted because the parasites did not tend to enter the plant whorls.

However, laboratory tests and observations indicated that A. asychis would enter and parasitize corn leaf aphids in the plant whorl when it was the only available host. In the field, it was noticed also that the greenbugs and corn leaf aphids migrated up the plant to the whorl and/or head of sorghum, depending upon the age of the plant, as the lower leaves withered. Thus the majority of the corn leaf aphid mummies was obtained from the sorghum head. While many of the aphids that moved up the plant may have been parasitized previously, A. asychis was observed also parasitizing aphids in the sorghum head. The few corn leaf aphid mummies that were obtained from the tests at Hooker were interspersed with greenbugs and collected from leaves as the plants died before heading.

The objective of the host preference study was to get an indication of the desirability of native aphid species as hosts for A. asychis. More detailed studies involving different ratios between parasite and host are necessary and must be conducted to determine or predict the degree of control exhibited by A. asychis.

Table 4 presents data on adult longevity, fecundity, percent emergence, and sex ratio of reared A. asychis. Fig. 3 shows the daily minimum and maximum temperatures for the months of August, September, October, and November. The developmental period of A. asychis is shown also for the months indicated.

The mean longevity of A. asychis adults was 19.7 days with a range of 9-38 days. The parasites were introduced into the first 5 cages on Aug. 21. Within the period of their life span the temperature dropped to a low of 10.0^{o} C on Sept. 24 and was highest (37.8^{o} C) the day of parasite introduction. The parasites in the second 5 cages were

introduced on Sept. 16. The temperature range during the period of our study of life span of all parasites was minus 10.0-37.8° C, but the low and high means approximated 15.6-26.7° C, respectively. A. asychis remained motionless when the temperature dropped to 12.8° C or below but became active again as the temperature began to rise. Lundie (1924) reported that Aphelinus mali Haldeman lived from 14 to 21 days under variable temperatures, and a few lived as long as 42 days. In laboratory studies, Raney et al. (1971) reported the mean longevity of A. asychis to be ca. 14 days at the constant-alternating temperature 21.1-32.2° C (12 hr at each temperature).

The fecundity (Table 4) of A. asychis ranged from 33 to 159 mummies with a mean of 94.5. Lundie (1924) reported that 48-140 progeny were produced by A. mali at variable temperatures. Smith (1923) reported that the fecundity of 6 females of Aphelinus lapsiligni Howard ranged from 40 to 91 mummies with a mean of 65.6. Parasite emergence from mummies ranged from 76 to 100%, with a mean of 86%.

The means of the developmental periods (egg deposition to adult emergence) of A. asychis under variable field temperatures are presented in Fig. 3. During our study mean developmental periods of 13.5, 17.0, and 40.5 days were observed. The shortest mean developmental period (13.5 days) occurred prior to Sept. 16. The temperature during this period ranged from 12.8 to 37.8° C, with a mean of ca. 26.7° C. In laboratory studies, Manglitz and Schalk (1970) reported that the developmental time (egg to adult) of Aphelinus semiflavus Howard at a mean temperature of 26° C (night 22°, day 31° C) ranged from 11 to 17 days, with a mean of 12.65 days. In our study, as the temperature decreased, the mean developmental time increased considerably, requiring

40.5 days for eggs deposited on or around Oct. 8. The temperature dropped to freezing or below on 14 of the 40.5 days but the freezes appeared to have no effects on percent emergence. Lundie (1924) reported that the life cycle of A. mali varied from 19 to 43 days, and that there was a marked tendency for length of the cycle to increase as colder weather set in. The first parasites began emerging on Sept. 2 and emergence continued until Nov. 23. The minimum temperature was 2^{o} C on the day the last parasite emerged (Fig. 3). However, when the daily minimum was minus 4^{o} C (Nov. 21), 6 adults (4 females, 2 males) emerged.

The female:male ratio was ca. 1:1 if the 3 cages in which only males emerged were excluded. The emergence of only males indicated that the parasites in the 3 cages were not mated. Their fecundity, however, was considerably higher than that of several mated females. The production of only male offspring from virgin female parasites was also recorded by Raney et al. (1971).

In laboratory and field studies, A. asychis was observed ovipositing in apterous and alate aphids, and adult parasites were reared from each type of host. Some aphids also developed wings after being parasitized. Table 4 gives the number and percent of apterous and alate mummified aphids collected from the plants in the field cages. Both males and females were reared from the alate forms. From 2 to 17% of the mummified aphids were alate. This percent might increase considerably in sorghum fields where a higher greenbug population could be supported. Parasitism of aphids prior to wing development and alate aphids coupled with prevailing winds may be an effective means of natural spread and subsequent establishment of A. asychis because it

does not fly readily. The rapid spread of Praon palitans Muesebeck, an imported parasite of the spotted alfalfa aphid, was by means of alate aphids. P. palitans was carried in proportionately greater numbers by parasitized alate aphids than either Trioxys utilis Muesebeck or A. semiflavus, also imported parasites. P. palitans was carried ca. 40 miles in this manner from several colonization sites in California and became established (van den Bosch et al. 1959).

COLONIZATION AND ACCRETIVE RELEASES OF *APHELINUS ASYCHIS*, AN IMPORTED PARASITE OF THE GREENBUG

After the severe greenbug outbreak on sorghum in 1968 in Oklahoma and surrounding states, the Insect Identification and Parasite Introduction Research Branch, ERD, USDA, initiated a search in the Middle East and southern Europe for effective natural enemies of this pest. One of the parasites introduced was *Aphelinus asychis* (Walker). Laboratory and field tests in 1969 indicated that *A. asychis* preferred the C-biotype greenbug over other aphid species associated with sorghum (Raney et al. 1971; Jackson and Eikenbary 1970). Thus, efforts were aimed at developing a method for the mass production and subsequent release of this parasite in the field.

A similar situation prevailed in California in 1954 with the spotted alfalfa aphid outbreaks. Three hymenopterous parasites of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), were imported into California in 1955 from the Mediterranean and Middle East regions (van den Bosch 1957) and successfully mass produced in the insectaries at Albany and Riverside (Finney et al. 1960). Ultimately all 3 species became widely established (van den Bosch 1959).

This section reports on the laboratory rearing and field colonization of *A. asychis*.

Materials and Methods

Rearing of A. asychis

A rearing room equipped with growth lights (12-hr photoperiod) and maintained at 23° - 26° C and 40 - 50% relative humidity was utilized for mass culturing A. asychis. Sorghum seed (variety 894) was sown in galvanized metal flats (20 x 14 x 3 3/4 inches) and maintained in a greenhouse. When the sorghum plants were approximately 12-18 inches tall (Fig. 4), the flats were transported to the rearing laboratory and infested with greenbugs, Schizaphis graminum (Rond.), which were at different stages of nymphal development. Greenbugs for infestation purposes were obtained from laboratory and greenhouse cultures maintained on sorghum plants grown in 6-inch plastic pots. Initially the rearing room was filled to capacity (40 flats) and then approximately 200 to 300 parasites were liberated among the infested plants. Every 3 weeks thereafter the flats were moved in and out on a rotation basis (i.e., 20 flats were removed and taken to the field and 20 flats containing plants about 15 inches high were added to the rearing room). As the plants in the 1st set of flats began to die, the greenbugs moved to the fresh plant material and began reproducing. Also, the new plant material was inoculated with greenbugs from greenhouse cultures.

Under rearing conditions mentioned previously, black mummified aphids began to appear on the 6th day. Adult parasite emergence began on the 12th day. The F_1 parasite generation was usually allowed to emerge to increase the adult parasite population in the rearing room and subsequently attain a higher number of mummies prior to emergence of the 2nd generation as the greenbug population increased considerably

during this period. After 3 weeks the greenbugs had usually killed the plants (Fig. 5).

Several days prior to adult emergence of the second parasite generation, the plant material with attached mummies (Fig. 6) was cut level with the soil, placed in cardboard boxes, and transported to the field for liberation of emerging parasites.

In the process of cutting and handling the plant material, most adults (F_1 generation) and some mummies were dislodged and fell onto the sand in top of the flats as well as on the tables supporting the flats. Using mouth aspirators approximately 200 to 400 adult females were collected from the flats. These parasites were used to reinoculate the new flats that were usually moved into the rearing room that same day or at least by the following day. The harvested flats containing some mummies and adults were then taken to the same field in which other mummies had already been distributed and placed at random among the rows. They were left for 3 to 4 days to allow the adults (sometimes estimated as high as 1,000/flat) time to move to the infested host plants.

Collection of A. asychis Adults

Cages shown in Fig. 7 were also utilized for the collection of adult A. asychis. Twelve 6-inch pots of sorghum were placed in each cage and about 1,000 greenbugs and 25-50 parasites were introduced. When parasites began emerging, quart jars were screwed into stationary rings on top of the cages and black muslin cloth placed over the cages and fitted snugly around the rings so that overhead lights shone through into the cage (Fig. 8). The parasites were readily attracted to the

light and easily collected in the jars. However, in the presence of diffused light, A. asychis is usually negative phototactic and remains on the host plants. The number of adults collected per cage varied from 500 to several thousand depending upon the initial number of greenbugs and parasites introduced. These adult collections supplemented the distribution of mummies and other adults obtained from the flats. This method was later abandoned because higher numbers of adults were obtained from the flats.

Parasite Releases

Liberations of adults and mummies (pupal stage of parasite) were made at 5 locations surrounding Stillwater in north central Oklahoma (Table 5). Two releases, one in Payne County approximately 5 miles west and the other in Noble County 15 miles north of Stillwater were made in April on young wheat. At the release site in Payne County the adults and mummies were placed in an organdy-covered cage (6 x 3 x 4 ft) over the young wheat which had a very light infestation of greenbugs, oat-bird cherry aphids, Rhopalosiphum padi (L.), and English grain aphids, Macrosiphum granarium (Kirby). We planned to leave the parasites caged until a sufficient build-up was evident. However, 2 days after placing the parasites in the cage, a cold wave moved into Oklahoma bringing sleet, snow, and freezing rains. When the temperature began to warm up, about 10 days later, the cage was checked and only a small number of A. asychis were observed. Since the aphid population was also at a low level, the cage was removed. The other liberation on wheat and all liberations on sorghum were made by distributing the dried plant material (mummies attached) and flats (adults present) among the infested plants.

Collection of Parasitized Aphids

Following field releases, weekly surveys and collections of parasitized aphids were made at the release sites. Two of us spent an hour at each release site/week collecting mummies. Parasitized aphids were returned to the laboratory, placed individually in size 3 gelatin capsules, and held for adult emergence.

Results and Discussion

Rearing of A. asychis

A. asychis was mass cultured on greenbugs from March to September 1970 and inoculative releases were made on wheat and sorghum during this same period. The production of A. asychis averaged about 7,000 mummies/flat.

Parasite Releases

Altogether, a total of 18,000 A. asychis adults and over a half-million mummies were released in an effort to achieve parasite establishment. However, the greenbug population was so low at most of the locations that repeated releases were unwarranted, except as noted (Table 5). Repeated releases were made on the 2 plots of grain sorghum at the Agronomy Research Station primarily because of slightly higher greenbug populations there than at other fields checked prior to the release. Two releases were also made at Lake Carl Blackwell on a late planting of sorghum with the anticipation of a greenbug build-up. However this build-up never materialized.

A. asychis adults were observed at both release sites on wheat approximately 2 weeks after liberation, but no mummies were evident and

only a few greenbugs were noticed. These were found around the crown of the plants. The next week no A. asychis adults, mummies, or greenbugs were observed. The wheat had begun to head so additional observations were abandoned.

The following month (May) parasites were released in a field of forage sorghum showing only scattered colonies of greenbugs and just a few yellow sugarcane aphids, Sipha flava (Forbes), but a medium population of corn leaf aphids, Rhopalosiphum maidis (Fitch), in the plant whorls. Subsequent checks for parasites and mummies were negative. A contributing factor which greatly influenced and eliminated any hopes of parasite recovery was the harvesting of this field for ensilage (3 separate cuttings). This reduced the aphid population to practically zero. The cutting of forage sorghum for ensilage is a common practice in this area. However, releases were made in hopes that some parasites might survive and disperse to other areas.

Further liberations were abandoned until the end of July when greenbugs began to appear in fields planted to grain sorghum. The main reason for the absence of releases during the month of June was the lack of suitable fields. As mentioned previously, most forage sorghum was harvested for ensilage. Therefore, there was a period of about 2 months before greenbug infestations were evident on grain sorghum.

The 1st release of A. asychis on grain sorghum was made at Ingalls, Okla. No insecticides had been applied to the land since the current owner bought it, which was 25 years ago. Greenbug colonies were easily found on practically every leaf and appeared on the increase. However, following the release, the weather was unusually hot and dry making the plants unpalatable to the greenbugs. This factor, coupled with

increased predation by coccinellids and lacewings, caused a rapid decline of the greenbugs. After several periodic inspections no parasites or mummies were observed.

Subsequent releases were made on grain sorghum at the Agronomy Research Station and Lake Carl Blackwell and continued until the middle of September. Again, the factors mentioned above still prevailed and were unfavorable for greenbug production. However, the greenbug population was considerably higher at the Agronomy Research Station plots, before and after the releases, than at any previous release site. Therefore, this enabled us to make considerably more observations. Mummified aphids were also collected from this location and held for adult emergence. Table 6 gives a summary of the parasites reared from these collections. Our primary concern was the recovery of A. asychis adults. Therefore, only black mummified aphids formed by A. asychis or other members of the family Eulophidae were collected. Although gold-colored mummies formed by Lysiphlebus testaceipes (Cresson) (Braconidae) (Jackson et al. 1970) were numerous, such data are not reported here. Only 25 A. asychis adults (15 females, 10 males) were reared from the collected mummies; and 27 adults were observed in the field on different occasions. The majority of the adults emerged from mummies collected the 1st week following liberations in plots 1 and 2. Two more A. asychis (1 female, 1 male) were reared from material collected in plot 1 on August 28. These parasites were probably the F_1 generation adults emerging from a 3rd release made in this plot on August 21. Shands et al. (1965) released a total of about 25,000 adult hymenopterous parasites (4 species) during 1957, 1958, and 1962 in an attempt to effect parasite establishment and subsequent control of

aphids infesting potatoes. Only 6 specimens of Aphelinus semiflavus Howard were reared from collections of parasitized aphids. In the initial stages of the spotted alfalfa aphid parasite colonization program, van den Bosch et al. (1959) made a number of small parasite releases in commercial alfalfa fields in an effort to achieve parasite establishment. Despite numerous releases in areas containing heavy aphid infestations, no parasites were recovered after several months of intensive searching. Therefore, van den Bosch et al. (1959) abandoned the method of random parasite distribution and established a number of "permanent" colonization plots in alfalfa fields. As a result of this change, Praon palitans Muesebeck, Trioxys utilis Muesebeck, and Aphelinus semiflavus Howard became widely established in the field and played an important role in the biological control of the spotted alfalfa aphid.

Aphelinus nigritus Howard, a native, primary parasite of the greenbug and corn leaf aphid in Oklahoma, and 3 hyperparasites, previously recorded (Jackson et al. 1970), were also reared from the collected mummies (Table 6). Pachyneuron siphonophorae (Ashmead) and Aphidencyrtus aphidivorus (Mayr) were the most prevalent hyperparasites; their presence, to a certain extent, probably limited the recovery of additional A. asychis adults.

Overall, the localized establishment of A. asychis is very doubtful from these field releases. Hunter and Glenn (1909) reported that hot, dry weather was unfavorable and even fatal to greenbugs. In our study, we also thought that this factor was primarily responsible for the low greenbug populations in most fields during the summer months. Temperatures averaged 1^{o} - 2^{o} above normal and were 38^{o} C or higher on

12 days during August. This factor along with predation (coccinellids and lacewings) and hyperparasite activity might have affected parasite survival. Had conditions been more favorable, our results might have been more encouraging. However, much information was gained from this endeavor that may be applied successfully in future studies.

SUMMARY

Parasites were reared from the greenbug, Schizaphis graminum (Rondani); the corn leaf aphid, Rhopalosiphum maidis (Fitch); and the oat-bird cherry aphid, R. padi (L.) collected in 1968 and 1969 from sorghum in Oklahoma. Seven parasites representing the families Braconidae, Eulophidae, Encyrtidae, Pteromalidae, and Cynipidae were recovered. Lysiphlebus testaceipes (Cresson) was the most abundant parasite of the greenbug but was not evident in the field until August. Aphelinus nigritus (Howard) and A. varipes (Foerster) were recovered in June and July from the corn leaf aphid; in August they were found parasitizing the greenbug. Both parasites were recovered for the first time from field collections in Oklahoma. The most abundant secondary parasite was Aphidencyrtus aphidivorus (Mayr). Other secondary parasites reared were Pachyneuron siphonophorae (Ashmead), Charips sp., and Asaphes lucens (Provancher).

Field studies of A. asychis, an introduced parasite of the greenbug, were conducted during summer and fall of 1969 to determine host preference, adult longevity, developmental time, fecundity, and sex ratio of the parasite in cages in the field. Based on the species and number of aphids parasitized, A. asychis showed a definite preference for the C-biotype greenbug over the corn leaf aphid, oat-bird cherry aphid, and yellow sugarcane aphid. The mean longevity of A. asychis adults was 19.7 days. The field temperature during the study ranged from minus 10.0 to 37.8^{0} C. Fecundity ranged from 33 to 159 mummies

per female parasite, with a mean of 94.5; the sex ratio of emerging adults was about 1.1. The developmental time (egg to adult) varied inversely with the temperature. A. asychis parasitized both apterous and alate aphids.

In the laboratory under controlled conditions (12-hr photoperiod, 23^{o}-26^{o} C), A. asychis was mass cultured on greenbugs from March to September of 1970, and inoculative releases were made on wheat and sorghum during this same period. A total of 18,000 A. asychis adults and over a half-million mummies were released at 5 locations near Stillwater in an effort to achieve parasite establishment. Plots 1 and 2 on the Agronomy Research Station were the only locations in which mummies were found. Twenty-seven adults were observed in these plots on different occasions. Only 25 A. asychis adults (15 females, 10 males) were reared from collected mummies. The establishment of A. asychis is very doubtful from these field releases.

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APPENDIX

Table 1. Parasites reared from greenbug, corn leaf aphid, and oat-bird cherry aphid. 1968-69.

Parasite	Host plant	Host insect[a]	Color mummies	1968	1969	County	Remarks
Braconidae *L. testaceipes*	Broom corn, grain sorghum	GB, CLA, & OCA	Gold	X	X	Texas, Payne Cimarron, Beaver, Grady	Most abundant parasite
Eulophidae *A. varipes*	Broom corn, forage & grain sorghum	GB, CLA	Black		X	Payne, Okfuskee, Grady, Texas, Cimarron	Introduced parasite from France, 3rd most abundant parasite
A. nigritus	Broom corn, forage & grain sorghum	GB, CLA	Black		X	Payne, Texas, Cimarron	2nd most abundant parasite
Encyrtidae *A. aphidivorus*	Forage & grain sorghum	GB, CLA	Black		X	Payne, Grady, Texas	Most abundant hyperparasite
Pteromalidae *P. siphonophorae*	Forage & grain sorghum	GB, CLA	Black, gold	X	X	Payne, Grady, Texas, Beaver, Cimarron	2nd most abundant hyperparasite
A. lucens	—	GB		X			Single record hyperparasite
Cynipidae *Charips* sp.	Forage & grain sorghum	GB	Black, gold	X	X	Payne, Grady, Texas	A hyperparasite

[a]GB = greenbug; CLA = corn leaf aphid; OCA = oat-bird cherry aphid.

Table 2. Insect parasites of aphids on sorghum in Oklahoma. 1969.[a]

Date of collection	No. parasitized aphids collected	No. parasites emerging from parasitized aphids	% emergence	% *L. testaceipes*	% *Aphelinus* spp.	% *A. aphidivorus*	% *P. siphonophorae*	% *Charips* sp.
			Collected from greenbug					
June 13-30	1	1	100	0.0	0.0	0.0	0.0	100
July 1-15	31	17	55	.0	.0	32.3	16.1	6.5
July 16-31	94	49	52	5.3	38.3	4.3	2.1	2.1
Aug. 1-15	332	182	55	19.6	16.3	11.1	7.2	0.6
Aug. 16-31	550	264	48	43.2	2.0	2.0	1.0	.0
			Collected from corn leaf aphid					
June 13-30	43	27	63	.0	58.0	5.0	.0	.0
July 1-15	130	91	70	.0	64.0	5.4	.8	.0
July 16-31	22	5	23	.0	18.2	4.5	.0	.0
Aug. 1-15	17	12	71	11.8	29.4	17.6	11.8	.0
Aug. 16-31	24	18	75	62.5	8.3	4.2	.0	.0

[a]60 fields were surveyed.

Table 3. Preference of A. asychis under field conditions when caged with 4 species of aphids on grain sorghum. Perkins and Hooker, Okla. 1969.

Cage	Approx. no. aphids introduced initially/ cage	No. female para-sites/ cage	No. parasitized aphids collected[a]			
			OCA	CLA	GB	YSCA
		Perkins[b]				
		Parasites introduced July 20				
1 (check)	600[c]	0	0	0	0	0
2	600	145	0	70	399	1
3	600	300	0	84	507	3
		Hooker[d]				
		Test 1: Parasites introduced July 24				
1 (check)	800[e]	0	0	0	0	0
2	800	50	2	4	149	0
3	800	75	4	5	146	0
		Test 2: Parasites introduced July 31				
1 (check)	1100[e]	0	0	0	0	0
2	1100	50	0	1	74	0
3	1100	75	0	5	70	0

[a]OCA = oat-bird cherry aphid; CLA = corn leaf aphid; GB = greenbug; YSCA = yellow sugarcane aphid.

[b]All mummies removed on Aug. 2 and 18.

[c]150 aphids of each species.

[d]Samples of mummies were collected weekly until plants died - 1st week 25, 2nd week 60, & 3rd week 70 in Test 1; Test 2 - 1st week 25 and 2nd week 50.

[e]150 aphids each of OCA, CLA, & YSCA; GB already present, 300-400/ cage in Test 1, 600-700/cage in Test 2.

Table 4. Longevity, fecundity, percent emergence, and sex ratio of A. asychis at variable field temperatures with the greenbug as host. Stillwater, Okla. 1969.

Cage[a]	Adult longevity (days)	Fecundity (no. mummies)	% of mummies with wings	% emergence	Sex ratio	
					M	F
1	26	93	12.9	76.0	71	0[b]
2	13	40	10.0	80.0	9	23
3	9	41	17.1	100.0	13	28
4	38	159	3.8	89.0	59	82
5	10	101	2.0	84.0	24	61
6	14	81	4.9	93.0	75	0[b]
7	29	33	6.1	94.0	18	13
8	14	137	3.7	80.0	109	0[b]
9	20	149	8.1	88.0	44	87
10	24	111	4.5	87.0	57	40
Mean	19.7	94.5	6.2	86.0	31.7	47.7

[a]Cages 1-5 parasites introduced Aug. 21 and cages 6-10 parasites introduced Sept. 16.

[b]Parasite not mated when placed in cage, excluded from mean.

Table 5. Liberation of A. asychis adults and mummies near Stillwater, Okla. 1970

Release date	Host plant[a]	Species of aphids present on release date[b]	Aphid population at time of release[c]	Location	Number liberated	
					Adults	Mummies
4-7	wheat	GB	very light	15 mi. N. Stillwater	3,000	50,000
4-7	wheat	GB, OCA, & EG	very light	Lake Carl Blackwell	500	20,000
5-27	for. sorghum	CLA, GB, & YSCA	medium	5 mi. N. Stillwater	1,000	40,000
7-27	gr. sorghum	GB & CLA	light	Ingalls	0	35,000
7-29	gr. sorghum	GB & CLA	light	Agr. Res. Sta. Plot 1	500	30,000
8-3	gr. sorghum	GB & CLA	light	Agr. Res. Sta. Plot 1	500	49,000
8-3	gr. sorghum	GB & CLA	medium	Agr. Res. Sta. Plot 2	500	0
8-7	gr. sorghum	GB & CLA	medium	Agr. Res. Sta. Plot 2	9,000	52,500
8-21	gr. sorghum	GB & CLA	light	Agr. Res. Sta. Plot 1	0	48,000
8-25	gr. sorghum	GB & CLA	very light	Lake Carl Blackwell	3,000	180,000
9-7	gr. sorghum	GB & CLA	very light	Lake Carl Blackwell	0	70,000

[a]for. = forage; gr. = grain.

[b]GB = greenbug; OCA = oat-bird cherry aphid; EG = English grain aphid; CLA = corn leaf aphid; YSCA = yellow sugarcane aphid. When more than 1 sp. occurs, their listing is in the order of decreasing abundance.

[c]Very light infestation = 10 aphids/plant; light infestation = 10-200 aphids/plant; medium infestation = 200-2,000 aphids/plant.

Table 6. Summary of parasites reared from parasitized greenbugs collected on grain sorghum at the Agronomy Research Station, Stillwater, Okla. 1970.

Date	Plot no.	No. parasitized aphids collected	No. parasites emerging from aphids	% emergence	Primary parasites[a]		Secondary parasites		
					% *A. asychis*	% *A. nigritis*	% *P. siphonophorae*	% *A. aphidivorus*	% *Charips* sp.
8-7	1	18	12	67	28	33	0	6	0
8-10	1	14	11	79	7	71	0	0	0
8-10	2	68	57	84	25	35	9	13	1
8-17	1	43	30	70	0	30	23	14	2
8-17	2	90	25	28	0	4	17	6	1
8-27	2	16	5	31	0	0	13	13	6
8-28	1	35	14	40	6	6	20	6	3
9-10	1	38	5	13	0	3	8	3	0
9-11	1	39	0	0	0	0	0	0	0

[a]Only black mummified aphids formed by *Aphelinus* spp. were collected.

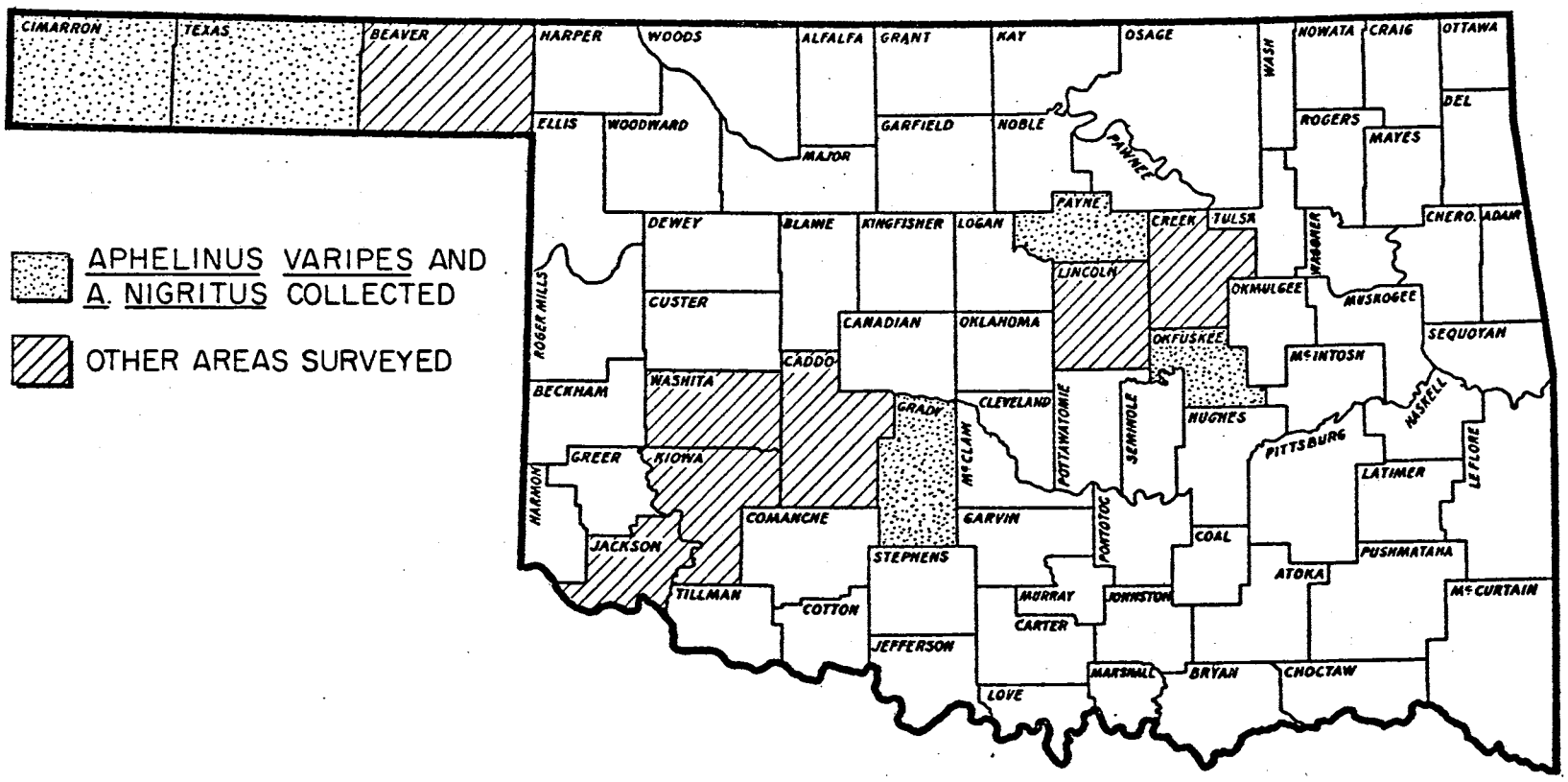

Figure 1. Counties surveyed for parasites of the aphids on sorghum in Oklahoma in 1968 and 1969. A. varipes was released in March and April in Payne County and recovered in the Panhandle area in July and August 1969.

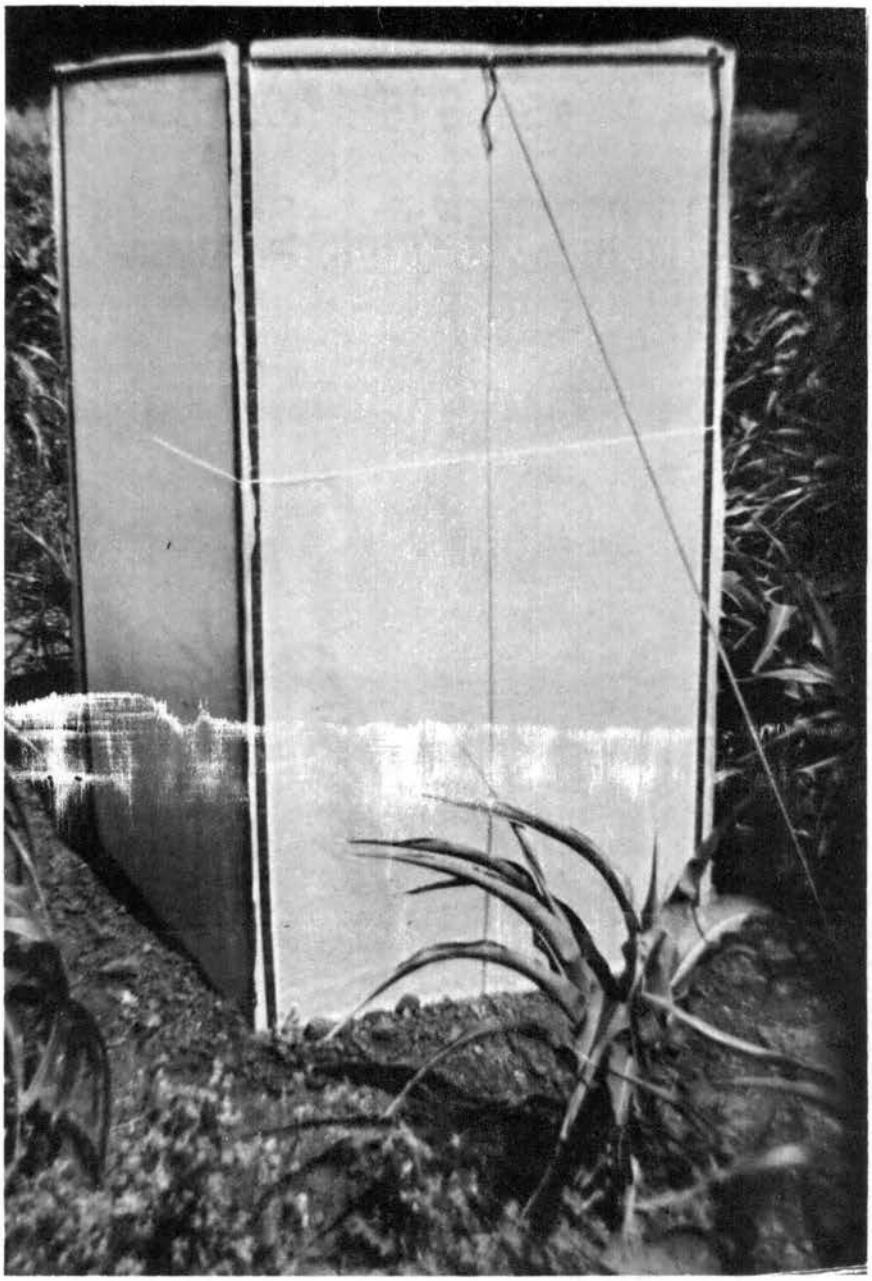

Figure 2. Cage similar to those utilized in field studies to determine the host preference of A. asychis.

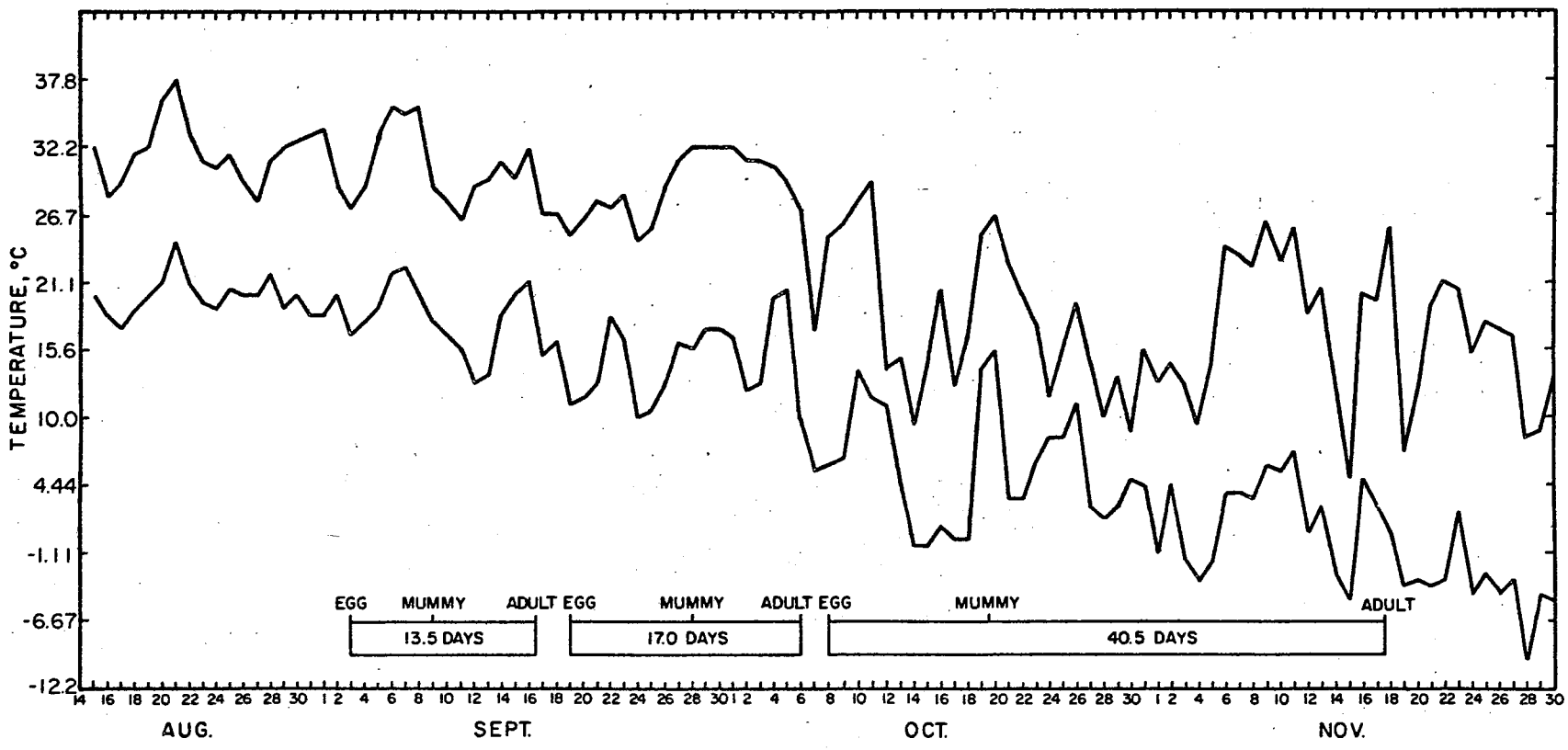

Figure 3. The effect of minimum and maximum temperatures on the developmental time of A. asychis.

Figure 4. Appearance of sorghum plants and metal flat used in the rearing of greenbugs and A. asychis.

Figure 5. Appearance of sorghum plants about 3 weeks after being subjected to greenbugs.

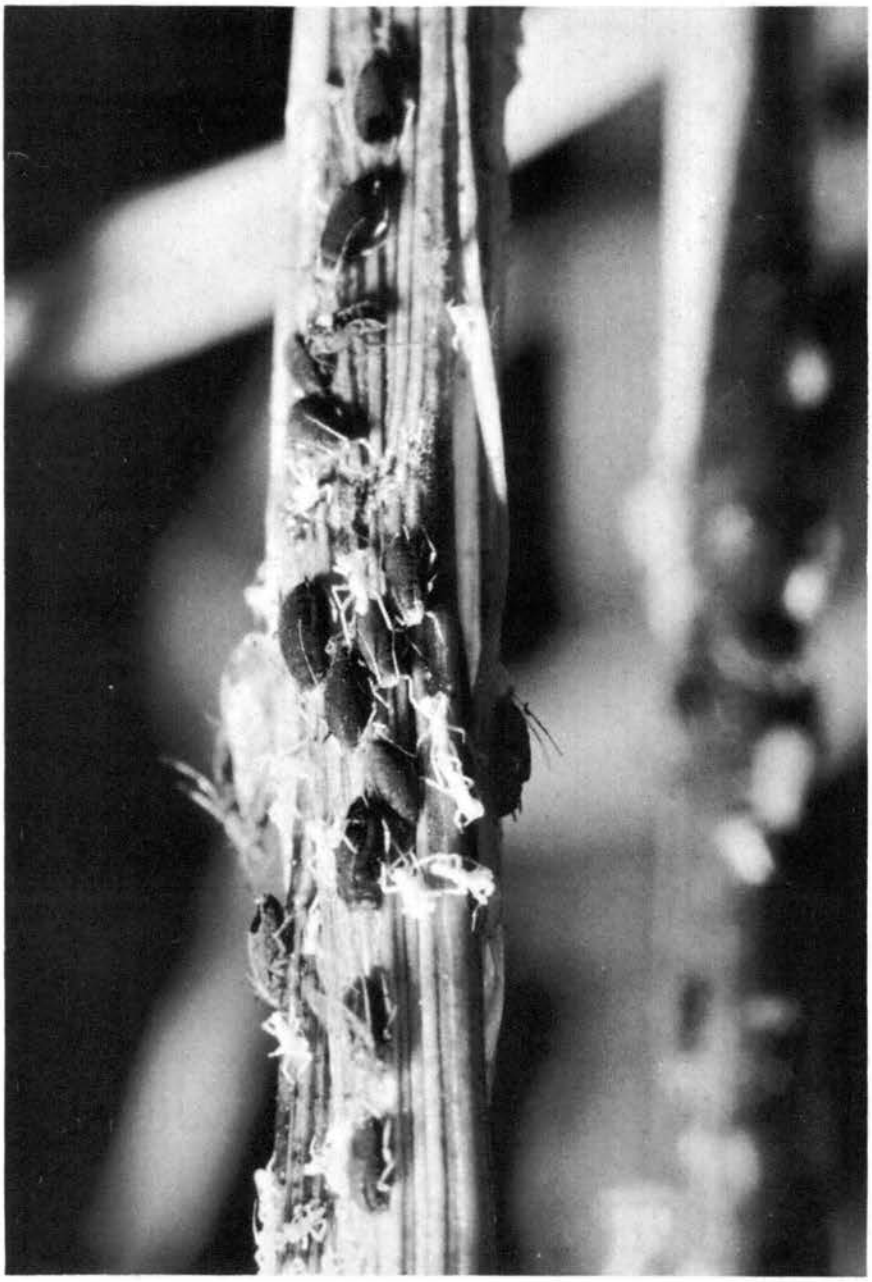

Figure 6. Small section of a sorghum stem showing the black mummified (parasitized) greenbugs.

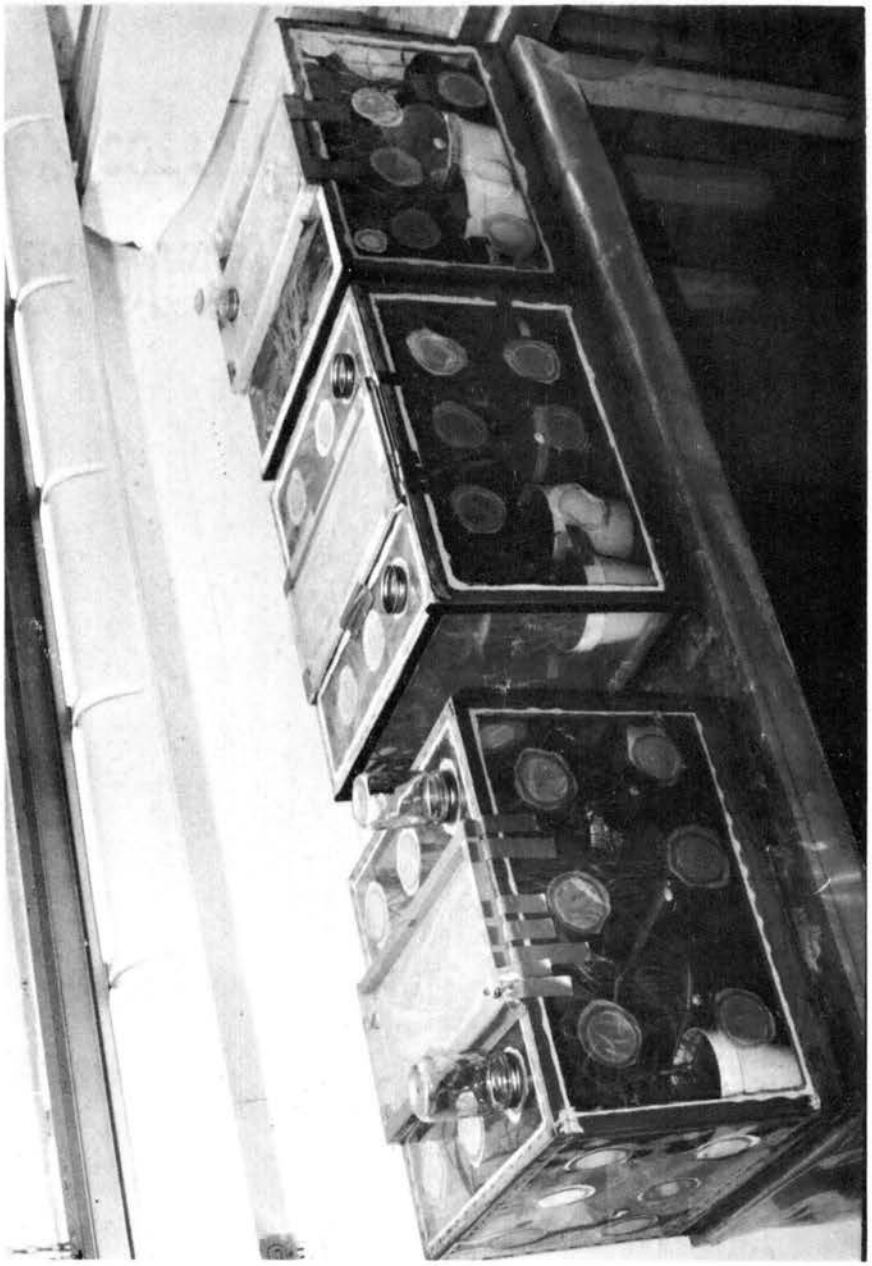

Figure 7. Cage utilized in the production and collection of adult A. asychis.

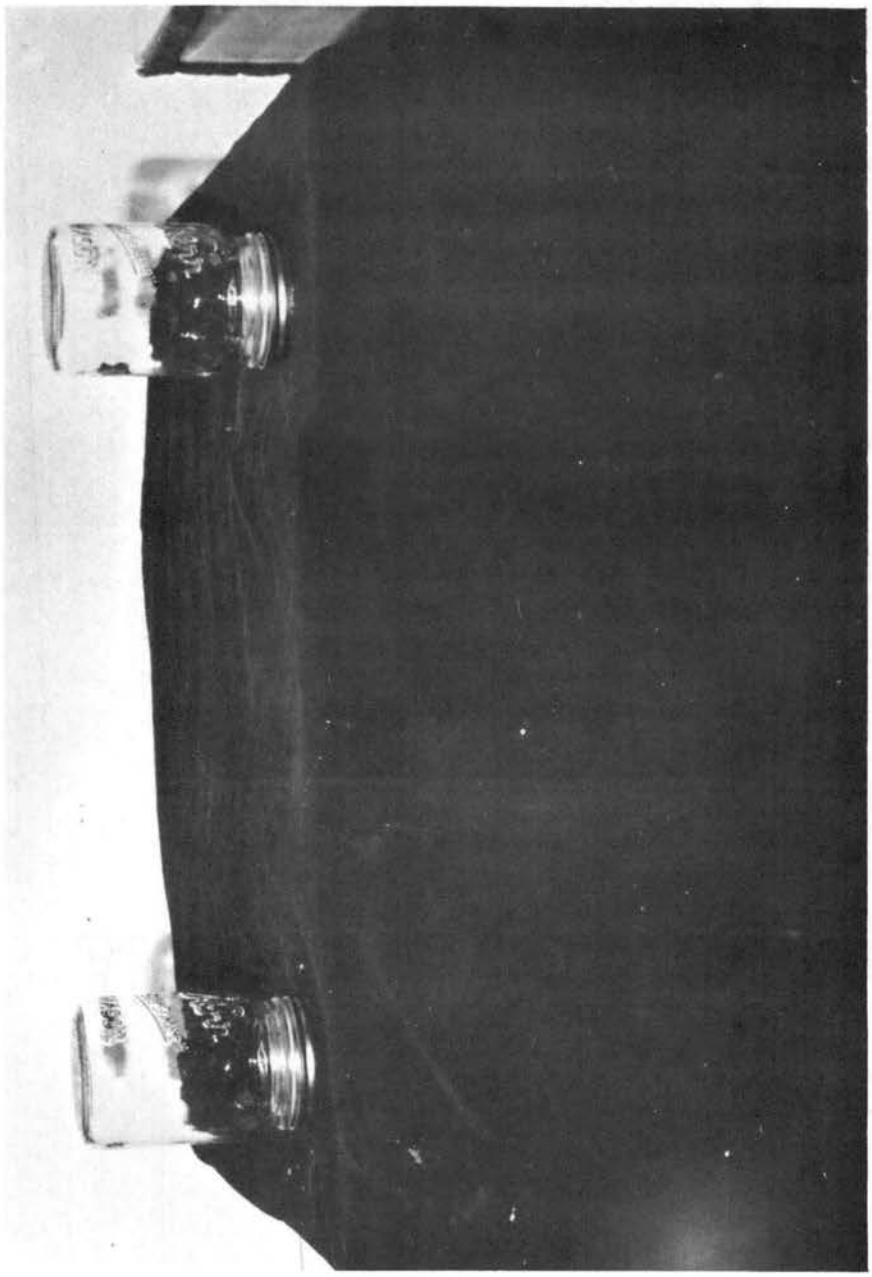

Figure 8. Preparation of cage prior to collecting A. asychis adults.

VITA

Herman Brown Jackson, Jr.

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Doctor of Philosophy

Thesis: PARASITE-HOST INTERACTION OF NATIVE PARASITES AND APHELINUS ASYCHIS ON APHIDS OF SORGHUM

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