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SYSTEMATICS AND BIOLOGY OF THE CROP ASSOCIATED SPECIES OF
POLYNEMA (HYMENOPTERA:MYMARIDAE) IN SOUTHERN ARIZONA

The University of Arizona

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SYSTEMATICS AND BIOLOGY OF THE
CROP ASSOCIATED SPECIES OF POLYNEMA
(HYMENOPTERA:MYMARIDAE) IN SOUTHERN ARIZONA

by
Kenneth Ryan Lakin

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF ENTOMOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Kenneth Ryan Lakin
entitled SYSTEMATICS AND BIOLOGY OF THE CROP ASSOCIATED SPECIES
OF POLYNEMA (HYMENOPTERA: MYMARIDAE) IN SOUTHERN ARIZONA

and recommend that it be accepted as fulfilling the dissertation requirement
for the Degree of DOCTOR OF PHILOSOPHY.

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_____	_____ Date

Final approval and acceptance of this dissertation is contingent upon the
candidate's submission of the final copy of the dissertation to the Graduate
College.

I hereby certify that I have read this dissertation prepared under my
direction and recommend that it be accepted as fulfilling the dissertation
requirement.

<u>Floyd B. Werner</u> Dissertation Director	<u>8 August 1985</u> Date
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DEDICATION

To Dr. Charles G. Jackson, friend, mentor, and boss, I dedicate this dissertation.

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ABSTRACT

Egg parasitoid wasps of the genus Polynema (Hymenoptera: Mymaridae) were collected over a 4-year period from crop-associated areas in southern Arizona. Seven morphologically distinct species were collected with 3 species identified. Evidence was found for the existence of an additional sibling species. Taxonomic characters currently used for this genus were evaluated for stability when the parasitoids develop in alternate hosts and some were found to be unreliable. A key to the Polynema species from Arizona was composed utilizing new characters.

Biological studies were carried out on 5 of these species to determine host species, longevity, developmental time, and potential fecundity. Three of these species exhibit the potential for sympatric parasitization of the eggs of the threecornered alfalfa hopper. A 2-year study of the seasonal distribution of these parasitoids in alfalfa fields in southern Arizona was carried out.

INTRODUCTION & LITERATURE REVIEW

The hymenopterous family Mymaridae consists of minute wasps whose adult body length ranges from about 0.3 to 4.0 mm. Biological studies of this family are few, and due to the small sizes of the wasps, host records are incomplete with some open to question. However, it is probable that insect eggs are the only hosts for their parasitic larvae.

Mymarids have a world-wide distribution. Their environmental range extends from the arctic regions around Point Barrow, Alaska, through the temperate regions and into the Tropics (Annecke and Doutt 1961). They have been found airborne at altitudes of 1,500 m and are probably distributed by wind (Glick 1939). As a family, mymarids have a wide range of hosts. Seventeen families within 8 insect orders are recorded as hosts for species found within the U.S. (Burks 1979). Some have invaded fresh water habitats with parasitization of the eggs of such insects as Gerris remigis Say, Notonecta sp. and Dytiscus sp. by Caraphractus cinctus Walker (D. Jackson 1958) and Gerris sp. by Anaphes gerrisophaga (Doutt 1949).

There are several characteristics which readily distinguish Mymaridae from other chalcidoids: the head has 3 main sutures (frontals, median, and supraorbitals), the

base of the hind wing is stalked (lacks membrane), the vein on the front wing ends in the first third of the wing (Arescon is an exception), and there are hypochaetae on the forewing.

Taxonomic considerations

Mymarid classification follows two basic schools of thought. The system proposed by Annecke and Doutt (1961) separates the family into two subfamilies, Alaptinae and Mymarinae, on the basis of abdominal attachment to the thorax. The subfamilies are subdivided into 5 tribes by the number of tarsal segments and by the degree of petiole development. The second system divides the family into two subfamilies on the basis of the number of tarsal segments, without subdivision into tribes (Debauche 1948, and Peck et al. 1964). Yoshimoto (1975) proposed 5 subfamilies based upon Annecke and Doutt's system, with the addition of Cretaceous amber-embedded fossils and a divergent group, the mymarommids. This last group differs from mymarids, as commonly defined, by having a 2-segmented petiole, the hind wings reduced to haltere-like structures, and in lacking elongate sensilla on the female's antennae and the 3 main head sutures common to all other mymarids. Debauche (1948), Peck et al. (1964) and Schauff (1984) consider Mymarommidae to be a separate family while Annecke and

Doutt (1961) consider the mymaromids to bear only generic distinction within the subfamily Mymarinae.

The evolutionary origin of mymarids is in just as much dispute. Although mymarids are placed in the superfamily Chalcidoidea, they lack some basic characteristics found throughout the group. First, the pronotum of mymarids frequently reaches the tegula, a condition not found in any other chalcidoid. Secondly, mymarid larvae resemble scelionid (superfamily Proctotrupoidea) larvae more than larval forms found in Chalcidoidea. The third major difference is that the mymarid antennal toruli are separated by 3-5 times their own diameter, while the toruli of most other chalcidoids are separated by 1X their diameter. Yoshimoto (1975) believes that Mymaridae evolved from the eurytomid-torymid line before the evolution of the line that led to chalcids, pteromalids, tetracampids and eulophids. Burks (1979) feels that mymarids evolved from the same line that produced Eulophidae.

Genus Polynema Haliday

Polynema Haliday, 1833: 347. Type-species: Ichneumon ovulorum Linnaeus, desig. by Westwood, 1840.

Eutriche Nees, 1834: 196. Type-species: Eutriche gracilis Nees. Monotypic.

Callitriche Agassiz, 1847: 173, 439. Emendation. Preoccupied.

Doriclytus Foerster, 1847: 226. Type species: Doriclytus vitripennis Foerster. Monotypic.

Cosmocoma Foerster, 1856: 117, 120. Unnecessary new name.

Walkerella Westwood, 1878: 584. Type-species:
Walkerella temeraria Westwood. Monotypic.

Maidiella Soyka, 1946: 178. Type-species: Maidiella
neofuscipes Soyka. Orig. desig.

Novickyella Soyke, 1946: 197. Type-species:
Novickyella gracilor Soyka. Orig. desig.

Barypolynema Ogloblin, 1946: 282. Type-species:
Barypolynema reticulatum Ogloblin. Orig. desig.

The genus Polynema is cosmopolitan, with 25 species described from the U.S. As with the other members of the family Mymaridae, these wasps are egg parasitoids of insects and parasitize members of the families Cicadellidae, Delphacidae, Lestidae, Membracidae, Miridae, Nabidae, and Reduviidae. Diagnostic characters for the genus include confluent marginal and stigmal wing veins, four-segmented tarsi, and a petiolate abdomen. Until a recent revision of Holarctic mymarid genera (Schauff 1984), the presence of a propodeal medial carina was also considered to be prerequisite for the genus. Schauff concluded that this character is too variable to be of diagnostic value. The only key for the U.S. species of Polynema was privately published by Girault (1929) and is based upon characters such as the number of rows of setae on the wing, length of setae on the margin of the wing, wing color, antennal and leg color, and comparative lengths of antennal segments.

Soyka (1956) published a monograph of European species of Polynema and closely related genera. His keys are based upon relative length of female antennal segments, not allowing for intraspecific variation. Soyka described 81 species, each represented by a solitary female wasp.

M. W. R. de V. Graham (1982) considered the presence (or absence) of antennal scape crossridges and the length of female abdominal tergites 1 and 2 to be characters worth considering to differentiate among the Polynema species. Among individuals of P. atratum Haliday, M. W. R. de V. Graham found a wide range in the color of antennal and leg segments.

In some species of Hymenoptera, a change of host induces morphological changes in the developing parasitoids. An example of this was investigated in detail by Salt (1937). The eggs of the alderfly, Sialis lutaria Linn., are naturally parasitized by Trichogramma semblidis (Aurivillius) with the resulting male progeny being apterous while the females are winged. When females of T. semblidis are put in contact with known hosts of the cryptic species, Trichogramma evanescens Westwood, they readily oviposit and successful parasitism occurs. The male progeny emerging from these hosts differ from alderfly-reared males by antennal and leg characteristics, in addition to having fully developed wings.

A pteromalid parasitoid, Rhopalicus tutela Walker, was found to exhibit morphological differences dependent upon whether the bark beetles Hylurgops palliatus Cyll. or Ips typographus L. served as host (Eck 1978). Not only did the size of the progeny differ but also proportions and shapes which were more pronounced in the females.

Host species have been reported for less than half of the Polynema species described for the U.S., and limited biological information is available for only four of these wasps. It is not uncommon for a mymarid species to have more than one natural host. Anagrus epos Girault and Anaphes conotracheli Girault both have 6 different recorded hosts. Most mymarids for which more than one host is recorded specialize in one family; however, A. conotracheli has been reported to parasitize eggs of 3 orders: Diptera, Coleoptera, and Hymenoptera. The effect of host on its morphology has not been reported.

Mymarid biology

Reproductive potential of mymarids has been investigated using 3 different methods of determination. One involves a count of the ovarian eggs. The second records the total number of eggs laid/female while the third reports the actual number of progeny per female. The reported number of ovarian eggs ranges from a mean of 11.4 per female in a Gonatocerus sp. (Chandra 1980) to 100 eggs

found in one Caraphraactus cinctus female (D. Jackson 1958), but Jackson included no mean or sample size. The number of progeny per female also has a great range, with the maximum number per female of 153 being recorded for C. cinctus (D. Jackson 1966, again with no sample size or mean given). Sahad (1982b) reports an average of 28 ovarian eggs per newly emerged female Gonatocerus cincticipitis Sahad (range, 23-36) while the number of eggs laid throughout the life of this species averaged 49, with a range of 28-77 (Sahad 1982 a).

Reproductive potential within the genus Polynema has been reported under laboratory conditions only for P. boreum Girault, 23.6 +/-12.3 in Nabis americanoferus Carayon eggs (Lakin et al. 1984). Due to mymarids' short life-spans, reproduction must take place soon after adult emergence. The percentage of eggs laid in the first day after emergence ranges from 50% for a Gonatocerus sp. (Sahad 1982a) to 80% for Anagrus flaveolus Wat. (Chandra 1980). Polynema boreum does not achieve 50% progeny production until the female is 5 days old (Lakin et al., 1984).

Although different temperature regimes significantly alter Anaphes diana (Girault) longevity, fecundity remains unaffected (Bloem & Yeargan 1982). But a 24, 48, or 72h delay in access to a host caused an appreciable decrease in fecundity of A. diana (Yeargan and Shuck 1981).

The number of adults emerging from each host egg varies, depending on species of wasp and host.

Caraphractus cinctus parasitizes a variety of host species, which in turn exhibit substantial differences in egg size. The smaller host eggs, those of Agabus spp., may yield 2-3 adults, while the largest may yield up to 56 emerging adults (D. Jackson 1958). As with most mymarid genera, Polynema biology has been infrequently and insufficiently studied. Records for members within this genus indicate that only one adult per host egg is the normal occurrence (Balduf 1928, Kiss 1984, and Lakin et al. 1984).

Anaphes diana, a parasitoid of Sitona spp. eggs, exhibits a definite preference for host eggs that are 2 days old (Leibee et al. 1979). At a temperature of 25° C, the eggs of Lygus hesperus Knight require about 8 days to hatch. When presented with L. hesperus eggs of a known age (1-8 days old), A. diana completed development in over 80% of the eggs presented that were 6 days old or less. Seven-day old eggs exhibited only an 18.9% parasitization, while no 8-day old eggs were parasitized.

There is a paucity of information on the time for development from oviposition to emergence of adult mymarids. Most laboratory studies are run at temperatures of 25-27° C with developmental times ranging from 8-18 days, depending on species (Anderson and Paschke 1969), MacGill 1934, Chandra 1980, and Stoner and Surber 1971).

Again, the only published reports on the developmental time for a Polynema species is for P. boreum, 17 days at 27° C (Lakin et al. 1984).

Egg parasitoids can be highly successful in the control of pest species. The International Biological Programme (Delucchi 1976) presented a report on the world-wide problem of the rice stem-borers, a complex of 15 lepidopterous pests including Chilo suppressalis (Walk.), C. polychrysa (Meyrick), Tryporyza incertulas (Walk.), T. innotata (Walk.) and Sesamia inferens (Walk.). These pests prefer cultivated rice to the wild species of rice. In Sarawak, a state in the Federation of Malaysia, egg to pupal mortality is reported to be up to 99% for 3 of the most important species of rice stem-borers. The rice paddies are not treated with insecticide and the larval borer population rarely exceeds 33,000 per acre. However, Japan and the Philippines regularly apply insecticides and experience larval borer populations of 80,000 to 200,000 per acre. Thailand reports that parasitism is higher for the rice stem-borer egg stage than for all of the other stages.

The maximum benefits of the egg parasitoids in an IPM program will be gained only when each case is well studied. For example, in California vineyards, the grape leafhopper, Erythroneura elegantula Osborn, can become a problem because of feeding damage to foliage, honeydew

excretion on grapes producing cosmetic damage and decreasing production by the sheer number of leafhoppers. This species has been controlled by a minute mymarid wasp, Anagrus epos Girault, which is an egg parasitoid of this and other leafhoppers. By the encouragement of alternate hosts for the parasitoid, effective control of the grape leafhopper has been achieved. Around 1982, the variegated grape leafhopper, E. variabilis Beamer, became a problem farther north in California's vineyards. Initially, it was hoped that A. epos would also control this pest, but it did not. Investigations revealed the reason. The eggs of the variegated grape leafhopper were found inserted in the plant tissue in or around the veins, while those of the grape leafhopper are inserted into the tissue between the veins. Since A. epos searches primarily in the area between the veins, most of the eggs of the variegated grape leafhopper escape parasitization (Kido et al. 1984).

Although members of the genus Polynema parasitize the eggs of insect pests such as Lygus lineolaris (Palisot de Beauvois), and Circulifer tenellus (Baker), there have been no studies into their potential as biological components in a pest control program.

This paper involves investigation of the genus Polynema over a 4 year period in predominantly agricultural areas of southern Arizona. Whenever possible the species were cultured to facilitate laboratory investigations.

Laboratory information was then combined with field-collected data to present as accurate as possible a picture of host-parasitoid relationships.

MATERIALS AND METHODS

All of the wasps and host insects in this study were collected in southern Arizona. The Polynema boreum used to determine longevity, and all other species of Polynema, were collected near Marana, in Pima County. The P. boreum used in other biological studies also included individuals collected at Yuma.

Field Collection and Sorting Techniques

Specimens taken in the years 1983 and 1984 were collected by a D-Vac® vacuum sampler in alfalfa (Medicago sativa L.) fields and nearby weed patches (H. Graham et al. 1984). In 1983, two different techniques for using the D-Vac were tried. One (vertical) involved holding the 33.5 cm diameter sampling head of the D-Vac over the plant being sampled, about 5 to 25 cm above ground level, depending on plant height, for no more than 15 seconds. The other method involved sweeping the intake hose through the tops of the plants in an arc of about 160 degrees around the collector. For each technique, 50 samples, either sweeps or vertical samples, were taken per replicate, with 5 replicates per week, as weather conditions allowed. By May, 1983, it was determined that the vertical method yielded more mymarids despite the fact that a much smaller area was

sampled. Consequently, as of that date, the vertical method was the only one used. The D-Vac samples were placed in ice chests cooled by Blue Ice™, then transported to the laboratory for examination. Upon return to the laboratory, the samples were fumigated with ethyl acetate to kill the insects. Separation of mymarids from the other insects and from larger pieces of plant material was accomplished by sifting the sample contents through a series of geological soil sieves. These samples were stored in a freezer until they could be examined and the wasps removed. The wasps were stored by sample and replicate in glass vials containing 70% ethyl alcohol until they could be identified.

When live insects were needed for biological investigations, they were collected by D-Vac as an additional sample. If it was not possible to check for mymarids on the day of collection, the D-Vac bag was stored in a refrigerator (at 6° C) overnight. Collection of the mymarids from the samples was done in a light-box (62cm x 47cm x 43cm). The bottom of the box was replaced by a single layer of organdy cloth while the top was removed. The box was placed on its side with the organdy end next to a fluorescent light source containing a 15-watt ultraviolet and a 15-watt coolwhite tube. Room lights were turned off and the D-Vac sample (or a portion of it) placed in the light box. Most of the insects in the sample were

attracted to the light showing through the organdy. They then were collected by means of a mouth aspirator.

Laboratory Host Plants

Plants used for the rearing of insects were raised from seed in greenhouses. Alfalfa (mixed cultivars) and careless-weed (Amaranthus palmeri Wats.) were used for oviposition sites for the potential insect hosts. These plants were started in peat seedling pots and later transplanted to 3.8-1 peat pots. At weekly intervals, the alfalfa was sprayed with a mixture of 4.9 ml each of 50% malathion and 25% diazinon in 946 ml of water to aid in the control of thrips and whiteflies. If an alfalfa plant was needed, it was omitted from the spray program one week prior to its use. The alfalfa was cut back to approximately 4 cm above the ground when the stems started blooming. Thicker, older alfalfa stems were made available to threecornered alfalfa hoppers (Spissistilus festinus (Say)) for oviposition sites, since these plants were better able to withstand feeding damage. Careless-weed leaves on primary branches tended to grow too large for the stem cages used. However, smaller leaves that could easily fit the stem cages occurred on very young plants and on the new growth of plants which had been allowed to mature, then severely cut back.

Laboratory Cages

In order to obtain host eggs and to maintain their viability for the time required for parasitoid development, portions of stems on potted plants were made available for host oviposition. Sleeve cages were utilized to confine the host species for oviposition. These cages were constructed in two sizes depending on the size of the plant stem and leaves to be contained. Smaller diameter cages, used with alfalfa, were constructed of dialysis tubing with an inside diameter of 1.6-cm and cut 15-cm long. The lower opening of the cage was sealed with a cylindrical foam rubber plug ca. 1-cm deep. The plug was cut 1/2 way across the diameter, so that it would fit around the plant stem and effectively seal the cage. The upper end of the cage, which projected beyond the stem's terminal, was sealed with an uncut plug similar to the lower plug. The wasps emerging from parasitized eggs were very phototactic initially, and many crawled up into the foam plug where they either escaped or were trapped. To prevent this from occurring, the inside portion of the top plug and the outside of the dialysis tubing was colored black with a felt tip marker. Plants with large leaves, such as carelessweed required a sleeve cage with a larger inside diameter. These cages were made from a 20-cm length of dialysis tubing with an inside diameter of 5-cm, and closed with foam rubber plugs ca. 2-cm thick. Both types of cages were

supported by a heavy gauge, maleable wire with a loop bent in the top through which the cage protruded. The bottom of the wire was inserted into the soil in the flower pot. Use of several cages on one host plant allowed for the concurrent running of several replications in minimal space.

Rearing conditions for *Polynema boreum*

The rearing and experimental conditions for *Polynema boreum* were somewhat different from those of the other mymarids. This is mostly due to the acceptance of an artificial substrate for oviposition by the known host, *Nabis americanoferus* Carayon. Oviposition packets ca. 5-cm square were made from a sheet of Saran Wrap® heat sealed to a Parafilm® sheet with a gelcarin filling, originally developed for rearing *Lygus* species (Patana 1982). These packets were exposed to nabids for 1-2 days, depending upon the number of eggs which had been stored in a refrigerator (at 3.9° C) for up to three months and still be acceptable to the wasps for oviposition with some successful development occurring. However, only egg packets which had been stored for no more than two weeks were used for biological studies. To determine developmental time and daily progeny production, a new packet of eggs was presented daily to each female wasp. The packet from the previous day was removed and placed in a labeled 100 x 15 mm petri dish. To prevent dessication, a filter paper disk was added to

the petri dish and kept moist throughout the wasp's developmental periods. The petri dishes were placed in an environmental chamber at 27 +/- 1° C, 35% RH, and a 14-10 hour (L-D) photoperiod. The petri dishes were checked daily for the presence of emerged adults, and sex and number recorded.

Cages used for Polynema boreum consisted of 47 ml clear plastic cups with snap-on plastic lids. The bases of the cups were removed and a layer of organdy cloth glued over the opening. Nourishment for the wasps was provided by a 7.4-ml glass vial containing a 10% sucrose solution. The vial was plugged with a sponge wick and inserted through a hole punched in the side of the cage.

Confinement of the other parasitoid species for biological studies was accomplished by use of cages constructed from 31-ml clear plastic cups. The bottoms of the cups were removed and replaced with organdy cloth. A hole was punched in the soft plastic lids with a #3 cork borer to receive a 2.5-ml glass vial containing a 10% solution of sucrose in distilled water and a sponge wick.

Taxonomic Studies

The taxonomic studies of the mymarids were on specimens mounted on microscope slides. Where many slides were needed for a short term, Hoyer's mounting medium was used. This allowed for mounting of specimens which had

been preserved in alcohol and could be done in one step. Unfortunately, this mounting medium does not maintain a high optical quality for prolonged periods of time, even when the coverglass is ringed with a sealer substance. More permanent slides were prepared using a method supplied by Dr. M. E. Schauff, U.S. National Museum, (personal communication). This method was more time consuming, and had a greater chance of damaging the specimen; however, once the technique was learned, it produced quality slides of long duration. The procedure is as follows:

1) KOH (sat.) bath. This can be with hot KOH for 3 to 5 minutes and must be closely watched, or it can be with room temperature KOH and can be left overnight. Experience determined how long each species could be left in the KOH bath before the wings started to curl.

THE TIME FOR EACH OF THE FOLLOWING STEPS IS NOT CRITICAL BUT SHOULD BE AT LEAST 10 MINUTES.

- 2) Distilled water bath
- 3) 10% ethyl alcohol
- 4) 25% ethyl alcohol
- 5) 50% ethyl alcohol
- 6) 75% ethyl alcohol
- 7) 95% ethyl alcohol
- 8) 100% (absolute) ethyl alcohol
- 9) 90% absolute ethyl alcohol & 10% clove oil
- 10) 75% absolute ethyl alcohol & 25% clove oil

- 11) 50% absolute ethly alcohol & 50% clove oil
- 12) 100% clove oil
- 13) 66% clove oil & 33% Balsam mounting medium
- 14) 10% clove oil & 90% Balsam mounting medium (This last step is the actual mounting medium.)

To prevent the weight of a microscope cover glass from crushing the specimens, pieces of broken cover glass were placed in the mounting medium to help support the weight of the cover glass. Most specimens were mounted so that they could be viewed dorsally. When using the clove oil-balsam technique, the slide drying process was shortened by geating the slides on a slide-warmer tray; however, this frequently produced bubbles in the Hoyer's medium. For Hoyer's best results were obtained by allowing the slides to dry while flat in a slide box. At times more mounting medium had to be added adjacent to the cover glass to compensate for contraction of the mounting medium. This was true for both of the techniques.

Measurements were made through a compound microscope using a Lasico Auto-scaler[®] ocular micrometer with computations and printout on a Texas Instruments TI-5040[®] adding machine. Using a 10X objective lens in conjunction with the 10X eyepiece gave a total magnification of 100X. The micrometer was calibrated against a stage micrometer

slide and a correction factor entered into the system's memory.

Measurements of leg and antennal segments were made along the longitudinal axis of each segment. The starting and ending point for each segment was a line perpendicular to the longitudinal axis of that segment. These points needed to take into account the frequently irregular terminal portions of each segment and did so by using the membranous attachment portion as the reference point. When the membranous attachment site was not totally perpendicular to the longitudinal axis, a point half way between the extremes of attachment points was used as the reference point.

A potential problem when measuring a structure mounted on a microscope slide is that of orientation not parallel to the ocular micrometer, thus producing a measurement less than the true length. To compensate for this problem, segments were measured only when both ends of that segment were in the same focus, and preferably where several consecutive segments were in focus. With the shallow depth of field of compound microscopes, I felt that when several adjacent segments were in focus, the structure was fairly flat and reasonably accurate measurements could be taken. Occasionally, a portion of one antenna would meet this criterion while the rest of the structure would show a noticeable curving. When this occurred, the rest of the

measurements would be taken from the other antenna on the same insect. All leg segment measurements were taken from the same side of an insect unless a structure was either covered or not flat enough to provide accurate measurements.

Material destined for S.E.M. photography should be dry; however, with insects the size of mymarids, no special techniques are needed. Specimens which have been air dried or preserved in 95% ethyl alcohol serve equally well. Since members of the genus Polynema are more heavily sclerotized than many other mymarids, there is negligible shrinking of the specimens, thus no need for critical point drying. To attach the wasps to the S.E.M. plug, Scotch Brand Double Stick Tape® was used. The plug was then placed in a Polaron E-5100™ sputterer, where a 30-nanometer thick layer of 60-40 gold-paladium was applied. All S.E.M. observations and photographs utilized an I.S.I. DS-130™ scanning electron microscope and Polaroid Type 55™ positive/negative film.

Biological Studies

Laboratory determination of biological data for the species of Polynema was carried out in an environmental chamber made from a converted Whirlpool™ refrigerator. Heating and cooling were thermostatically controlled and two 20-watt fluorescent lights on timers provided light.

Conditions within this chamber were as follows: 26° +/- 1° C, 55-95% relative humidity, and photoperiod of 14-10 (L-D) hours.

Determination of host species was carried out by inference. When Polynema species were recovered live from field-collected material, the adult insects in the sample which might serve as potential hosts were removed and saved. Selection for testing as potential hosts included criteria such as the number collected, size, and method of oviposition. Since most of the recorded mymarid hosts insert their eggs into plant tissue, this behavior in potential hosts was considered important. Adults of all insect species considered to be potential hosts were then placed by species in sleeve cages containing the type of plant from which the sample was taken. After 1 or 2 days they were removed and a female Polynema from the same sample was placed in the sleeve cage for approximately 24 hours. If parasitoids emerged from the host eggs being tested, replications were attempted. Emergence of few parasitoids could be indicative of one or more of the following: 1) the field-collected wasp may not have parasitized many eggs although the host was acceptable, 2) the potential hosts may have been true hosts but may not have laid many eggs, or 3) the host may not have been the true field host but was marginally acceptable by the parasitoid under laboratory conditions. Possibility 1 was tested by

replication, possibility 2 was checked by whether or not many of the potential host eggs hatched. If the first two possibilities for low parasitism were tested and found not to be applicable, then the third was accepted as probable.

Identification of host insects was made by comparison of collected specimens with specimens identified by Dr. M. Nielson, USDA, ARS, retired (leafhoppers) and Dr. F. Werner, Department of Entomology, University of Arizona (nabids). Dr. C. T. Mason of the University of Arizona Herbarium identified the unknown species of plants.

Determination of the longevity of the parasitoids was carried out in the 2.5-ml observation cages described previously. Five adults which had emerged within the previous 24 hours were placed in each cage, provided a solution of 10% sucrose, and observed daily for mortality.

The methods for determining developmental time varied slightly. For most of the species, this test was carried out in a sleeve cage. The female parasitoid was removed 24 hours after being placed in the sleeve cage, after which the plant was returned to the environmental chamber. To check for parasitoid emergence, the cage was opened in a darkened room and adjacent to a clear plastic box (37cm x 16cm x 27cm) placed in front of a fluorescent light fixture containing a 15-watt ultraviolet and a 15-watt coolwhite fluorescent light. The plant section which had been caged was closely examined and wasps found on the

plant and those attracted into the plastic box were aspirated, then sexed and counted.

Potential fecundity for each wasp species was assessed by counting the number of eggs within the abdomen of a 0 to 24-hour-old wasp which had not been exposed to host eggs. The live wasp was placed on a slide in an insect saline solution under a cover glass. Pressure on the cover glass caused the female's abdomen to rupture, exposing the eggs so that they could be counted.

The study of the daily progeny production of P. boreum was undertaken on two different hosts, Nabis alternatus Parshley and N. americanoferus. Each day ca. 20 host eggs were offered per P. boreum female, from an age of day 1 until her death. A male P. boreum was kept with each female throughout her life.

A substantial size variation in some field-collected Polynema species was noted. Some of the smaller Polynema, other than for size, appeared to be indistinguishable from other parasitoids from the threecornered alfalfa hopper eggs. This led to speculation that the utilization of an alternate host with smaller eggs might be the cause. During the times when the smaller individuals appeared, there was also an abundance of leafhoppers of the genus Aceratagallia. Laboratory testing showed that eggs of this leafhopper genus were readily accepted as hosts by the smaller wasps. The hypothesis that host egg size

determined adult size was tested by simply rearing large field-collected wasps on eggs of the proven host, the threecornered alfalfa hopper, then exposing the progeny to leafhopper eggs. Conversely, small field-collected Polynema were reared on the eggs of Aceratagallia species, then the progeny were exposed to eggs of the threecornered alfalfa hopper.

Weekly D-Vac samples were made in alfalfa throughout the years of 1983 and 1984, as weather and farming practices allowed. Polynema species within the samples were identified, mounted on microscope slides when necessary, and analyzed for patterns of seasonal abundance.

RESULTS AND DISCUSSION

Systematic studies

A checklist of *Polynema* species field-collected in southern Arizona

Polynema boreum Girault

Host Insects:

Nabis americanoferus Carayon
N. alternatus Parshley
N. capsiformis (Germar)
(Heteroptera: Nabidae)

Host Plants: Collected from numerous species of plants.

Dates collected: Collected throughout the year. Higher population levels occur in late spring and early summer.

Polynema imitatrix Gahan

Host Insects: Spissistilus festinus (Say)
(threecornered alfalfa hopper)
(Homoptera: Membracidae)

Host Plants: Found in abundance on alfalfa and in smaller numbers on associated weeds.

Dates Collected: Collected throughout the year. Highest population levels occur from late July through October.

Polynema medicae (Annecke and Doutt)

Host Insects: Spissistilus festinus
Aceratagallia spp.
(Homoptera: Cicadellidae)

Host Plants: Found in abundance on alfalfa and in smaller numbers on weeds.

Dates Collected: Collected throughout the year. Highest population levels occur from late July through October.

Polynema sp. a

Host Insects: Unknown

Host Plants: Astragalus sp.

Dates collected: Spring

Polynema sp. b

Host Insects: Ollarianus strictus Ball
(Homoptera: Cicadellidae)

Host Plants: Found in largest numbers on careless-weed and other species of weeds, infrequently collected from alfalfa.

Dates Collected: Collected in small numbers in alfalfa throughout the year. Highest population levels occur in May (in alfalfa).

Polynema sp. c

Host Insects: Spissistilus festinus
Aceratagallia spp.

Host Plants: Found in greatest numbers on alfalfa with some individuals collected on associated weeds.

Dates Collected: Collected throughout the year. Highest population levels occur from late July through October.

Polynema sp. d

Host Insects: Spissistilus festinus
(LABORATORY HOST, NO EVIDENCE FOR FIELD
ASSOCIATION)

Host Plants: First collected on sunflower
(Helianthus sp.), infrequently collected
in alfalfa.

Dates Collected: Found in alfalfa D-Vac samples
from May through September with highest
population levels in July and August.

Polynema sp. e

Host Insects: Aceratagallia obsucra Omar
field collected on white horse-nettle
(Solanum elaeagnifolium Cav.)
Aceratagallia spp. (LABORATORY)

Host Plants: First and commonly collected on white
horse-nettle, infrequently collected on
alfalfa.

Dates Collected: Spring and early summer.

Identification of the genera of mymarids is fairly simple if the specimens are mounted on microscope slides and an adequate compound microscope is available. There are 3 recent keys written in English which enable one to identify a mymarid: Annecke and Doutt (1961), Peck et al. (1964), and Schauff (1984). The two later keys are illustrated and Schauff goes into detail describing differences among the genera. The key to the genera of Mymaridae by Schauff has been condensed in this paper to include only those genera collected from southern Arizona.

A Key to the Genera of Field-Collected Mymarids of Southern Arizona

(condensed from Schauff, 1984)

- 1) Tarsi 5 segmented ----- 2
 Tarsi 4 segmented ----- 4
- 2) Wings remain narrow beyond venation (usually
 5-10 X as long as wide) ----- 3
 Wings broad beyond venation (2-4 X as long as
 wide); anterior edge of pronotum without
 transverse carina; propodeum smooth or
 nearly so ----- Gonatocerus
- 3) Abdomen sessile, phragma projecting into
 gaster; 10 antennal segments in female,
 12 segments in male ----- Dicopus
 Abdomen petiolate, phragma not projecting into
 gaster; 10 antennal segments in female,
 9 or 12 segments in male; forewing with
 slight crescent shape ----- Campoptera
- 4) Abdomen without distinct petiole ----- 5
 Abdomen distinctly petiolate ----- 7
- 5) Abdomen with hypopygium well developed,
 reaching end of abdomen, mandibles reduced,
 not overlapping ----- Erythmelus
 Abdomen with hypopygium absent or not reaching
 end of abdomen, mandibles well developed,
 overlapping ----- 6
- 6) Forewing with a line of setae descending from
 beneath venation to posterior wing margin,
 antennal club 1 or 2 segmented, abdomen
 distinctly narrowed between propodeum
 and gaster ----- Anaphes
 Forewing without such line of setae, abdomen
 joined broadly at base with thorax ----- Anagrus
- 7) Propodeum smooth or with single median carina
 of varying length; venation extremely
 reduced, marginal and stigmal vein
 confluent ----- Polynema
 Propodeum with inverted V-shaped carina;
 venation developed, marginal and stigmal
 veins separate ----- Acmopolynema

The only key for U.S. species of Polynema was privately published by Girault in 1929 and had been difficult to obtain until republished in the Memoirs of the American Entomological Institute #28 (Gordh et al. 1979). It includes 18 of the 25 Polynema species which have been described for the U.S. Parts of the key are useful while other parts assume knowledge of mymarid morphology eg. "Fore wings only moderately broad, their marginal cilia longer." Girault also utilized color to separate species. Color should be used cautiously, as noted in the discussion of the possible sibling species, Polynema imitatrix and Polynema sp. d, and by M. W. R. de V. Graham (1982). Another character which Girault used is the number of rows of cilia on the disc of the forewing. From Figure 1, a Polynema wing, one can see the difficulty in determining the exact number of rows of cilia. Although Girault used approximate numbers of rows :--- about 15 or more lines discal cilia ---", cilia are present on both surfaces of the wings and frequently are simultaneously in focus, a possible cause of confusion.

The only other key for the species of Polynema is Soyka (1956). This is a key for the species of European Polynema in a 115 page monograph. M. W. R. de V. Graham (1982) reported that Soyka relied heavily on relative length of the antennal segments, describing 81 species of

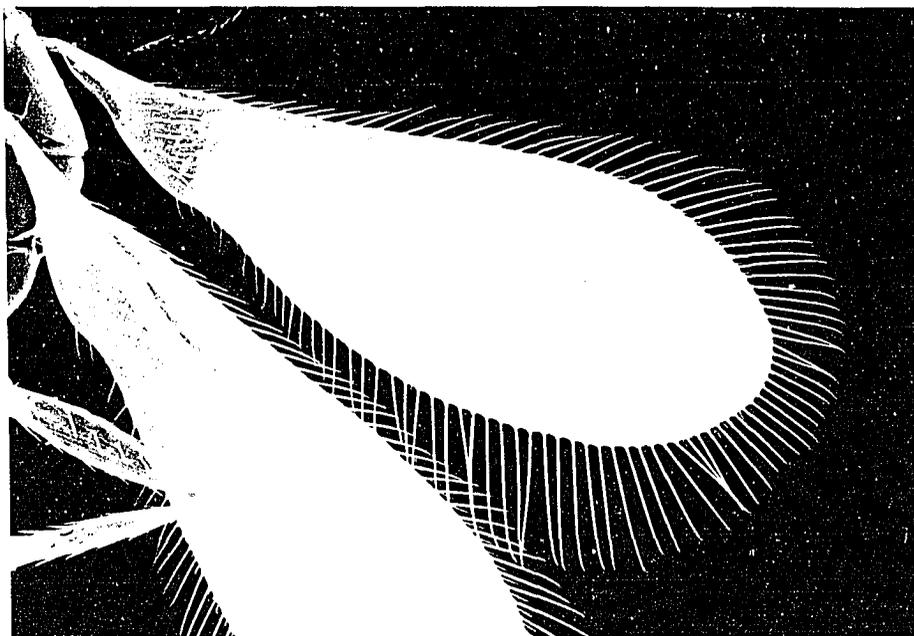


Figure 1. Forewing of Polynema sp. b.

Polynema, each from a single female. Graham strongly criticized Soyka for not allowing for variation within species.

A problem encountered with slide-mounted material is that some wasps are mounted with a lateral view while others present a dorsal view. If relative antennal segment lengths could be used in identification of mymarids, we would have a character visible no matter how the wasps are mounted. The question arises whether these lengths would remain stable if the wasp were recovered from two or more species of hosts. Tables 1-3 provide these antennal segment proportions taken from Polynema species collected in southern Arizona.

In the following sections on host determination and seasonal distribution, the probability of concurrent, sympatric parasitization of two host species in different insect families by the same species of Polynema has been demonstrated. This seemed to be an excellent proving ground for testing the stability of relative antennal lengths. Would the relative lengths of the segments remain consistent even though the wasps were reared in different host species which yielded different sized adult wasps? The ratio of forewing length to width, and the ratio of the maximum wing width to the maximum length of the wing margin cilia were also determined. One wasp, P. medicae, was measured under 4 different situations: 1) parents reared

from the threecornered alfalfa hopper producing progeny in alfalfa hopper eggs, 2) parents reared from alfalfa hopper eggs producing progeny in Aceratagallia spp. eggs, 3) parents from Aceratagallia spp. eggs producing progeny in Aceratagallia spp. eggs, and 4) parents from Aceratagallia spp. producing progeny in alfalfa hopper eggs (Table 1). A second wasp species, Polynema sp. c, was also tested for the effect of host species on relative body measurements. Due to the logistic problem of not enough host eggs being available, only two host combinations were tested: 1) parents from threecornered alfalfa hopper eggs producing progeny in the eggs of the threecornered alfalfa hopper, and 2) parents from the eggs of the alfalfa hopper producing progeny in the eggs of Aceratagallia spp. leafhoppers. Each antennal segment was measured, then the measurements converted to a percentage of the entire antennal length. For each wasp species/host species evaluated, the mean percentage for each antennal segment is given (Table 2). An ANOVA was run for each antennal segment within the wasp species/host species group. Six out of the 10 female antennal segments were found significantly different (.05) when analyzed among the 4 host relationships for P. medicae. Both of the wing measurement ratios were also found to be significantly different within each group. When antennal segment measurements were analyzed between two host relationships for Polynema sp. c, 6 out of 10

Table 1. ANOVA analysis of the consistency of female Polynema medicae appendage measurements. Antennal segments given are considered as a percentage of the total antennal length. The numbers are means from a sample.

STRUCTURE	<u>Polynema medicae</u>			
	HOSTS <u>a/</u>			
	1	2	3	4
<u>ANTENNAL SEGMENTS:</u>				
Sample Size	4	5	7	6
Scape	17.7	16.9	16.9	17.1
Pedicel	b/ ** 10.2	11.3	10.8	9.9
Funicle: 1	* 8.2	7.8	8.1	8.8
2	** 13.1	12.2	10.9	13.1
3	** 7.6	6.5	6.7	7.5
4	6.0	5.8	5.7	5.8
5	6.4	6.0	6.3	6.6
6	** 7.3	7.5	7.6	7.2
Club	24.9	26.0	27.0	24.0
<u>WING MEASUREMENT RATIOS</u>				
Sample Size	6	5	7	6
Length:Width	** 5.8	5.9	5.7	5.4
Width:Marginal Cilia Length	** 0.8	0.8	0.7	0.8

- a/ 1 = parents reared from the threecornered alfalfa hopper producing progeny in alfalfa hopper eggs
 2 = parents reared from alfalfa hopper eggs producing progeny in Aceratagallia spp. eggs
 3 = parents from Aceratagallia spp. eggs producing progeny in Aceratagallia spp. eggs
 4 = parents from Aceratagallia spp. eggs producing progeny in alfalfa hopper eggs
b/ * = significant difference among hosts at $p < .05$
 ** = significant difference among hosts at $p < .01$

Table 2. ANOVA analysis of the consistency of female Polynema sp. c appendage measurements. Antennal segments given are considered as a percentage of the total antennal length. The numbers are means from a sample.

		<u>Polynema</u> sp. c HOSTS <u>a/</u>	
		1	2
<u>ANTENNAL SEGMENTS:</u>			
Sample Size		9	9
Scape		16.7	16.9
Pedicel		9.1	9.6
Funicle: 1	b/ *	6.1	5.5
	2	**	13.7
	3	**	8.7
	4	*	6.9
	5		7.4
	6	**	9.3
Club		22.1	25.6
<u>WING MEASUREMENT RATIOS</u>			
Sample Size		9	10
Length:Width		3.9	3.9
Width:Marginal Cilia Length	**	1.4	1.2

a/ 1 = parents from threecornered alfalfa hopper eggs produced progeny in alfalfa hopper eggs

2 = parents from the eggs of the alfalfa hopper produced progeny in Aceratagallia eggs

b/ * = significant difference between hosts at $p < .05$

** = significant difference between hosts at $p < .01$

segments were found to differ significantly; however, only 1 out of 2 of the wing measurement ratios was found to be significantly different (Table 2). These results demonstrate that the measurements of appendages and their segments may vary significantly when different host species are utilized by a wasp species. It should be noted that approximate relationships among the segments were not greatly affected by changing hosts. Thus the usage of terms such as subequal or much greater than, still are valid; however, the use of precise measurements and ratios to define a species is highly questionable.

A morphometric comparison between Norway representatives of Anaphes cultripennis Debauche and the Belgium-collected type specimen showed interesting differences (Sveum and Solem 1980). The first and last female antennal segments differed substantially between the Belgium type and the Norway-collected wasps, while the rest of the segments were within the same range. Wing length to width ratios did not differ, but the Norway body lengths were greater. Host species for the parasitoids were not given. The authors attributed these differences to Bergman's and Allen's rules. Ray (1960) reported similar results for other poikilotherms. Appendage measurements for 4 other species of Polynema collected from southern Arizona are given in Table 3.

Table 3. Appendage measurements for females from 4 species of Polynema. Antennal segments given are considered as a percentage of the total antennal length. The numbers below are means from a sample.

	<u>imitatrix</u>	<u>Polynema</u> <u>sp. d</u>	<u>species</u> <u>boreum</u>	<u>sp. b</u>
<u>ANTENNAL SEGMENTS:</u>				
sample size	5	9	8	5
scape	14.5	15.8	13.0	15.3
pedicel	8.1	7.9	7.7	10.4
funicle: 1	7.4	7.5	7.4	6.7
2	14.0	13.5	15.1	10.9
3	10.3	9.6	10.2	8.1
4	7.0	6.7	7.3	6.6
5	7.5	7.9	7.1	6.2
6	9.8	9.1	9.8	8.1
club	23.0	22.1	22.4	27.6
<u>WING MEASUREMENT</u> <u>RATIOS</u>				
Sample Size	9	9	8	8
Length:Width	3.7	3.5	3.9	3.0
Width:Marginal Cilia Length	1.4	1.7	1.7	2.2

As we have seen, ratios of wing measurements do not remain consistently stable when the species of the host changes. Still, general wing shapes remain stable. For example, the wing shape of Polynema medicae retained its very distinctive narrow base whether development occurred in the eggs of alfalfa hoppers or of leafhoppers. The wing shapes of Polynema imitatrix and Polynema sp. d are essentially identical (Figure 2). The relationship between these two species will be discussed in the section on sympatric species. Wing patterns of Polynema species which are not egg parasitoids of the threecornered alfalfa hopper are shown in Figure 3. A wing characteristic which may be of value in separating Polynema species is the cilia pattern near the base.

Antennal segments also show a degree of consistency in that one segment will be longer in relation to other segments. Approximate size comparisons between female antennal segments are dependable enough to serve as usable taxonomic characters, eg. segment A is ca. 2 X as long as segment B. Figures 4 and 5 show the antennal segments of Polynema species collected in southern Arizona. The length to diameter ratio of male antennal segments has not been investigated by this author, but it has been assumed that these antennal measurements also may exhibit significant variation.

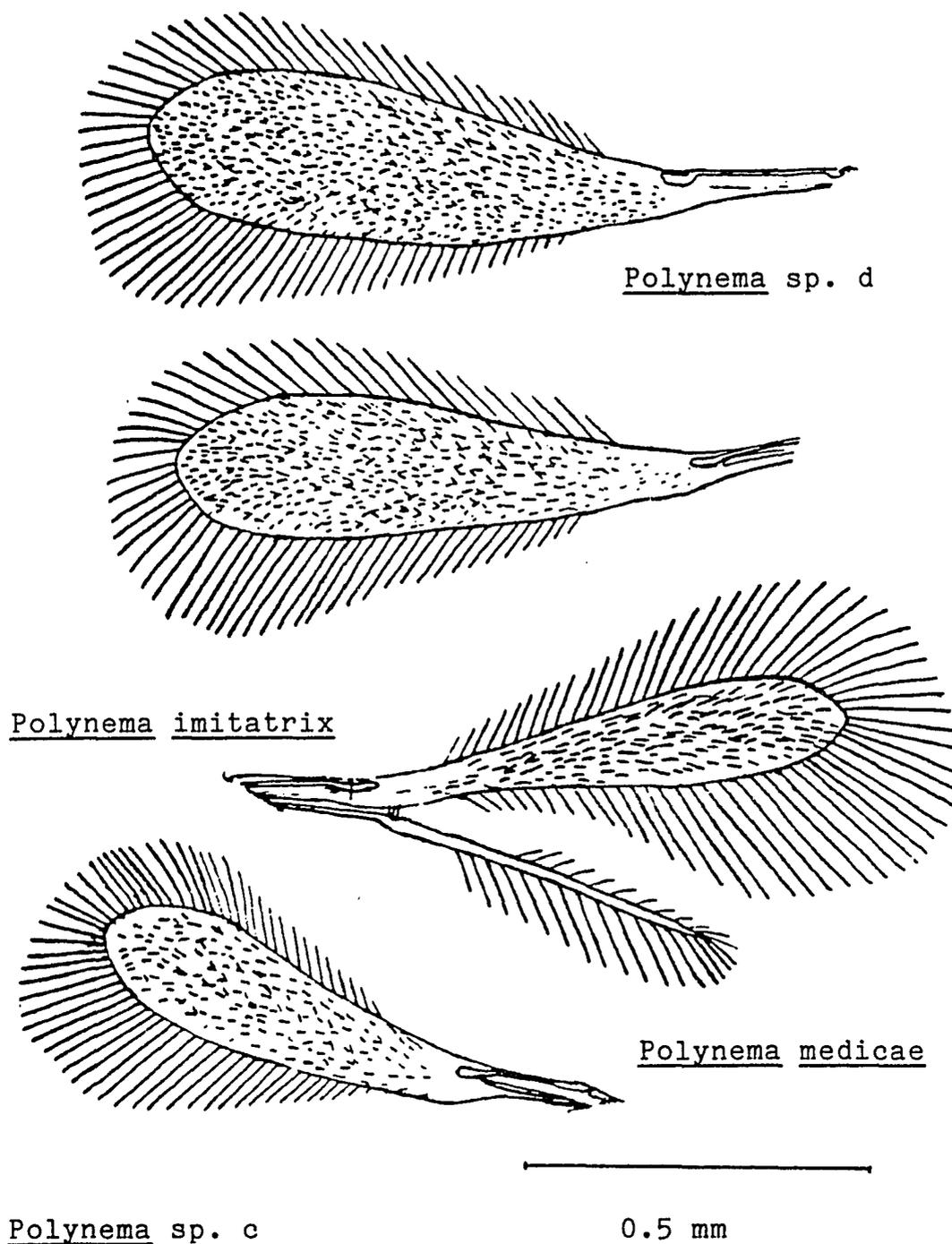


Figure 2. Fore-wing patterns of Polynema species collected from southern Arizona which are capable of parasitizing the eggs of the threecornered alfalfa hopper.

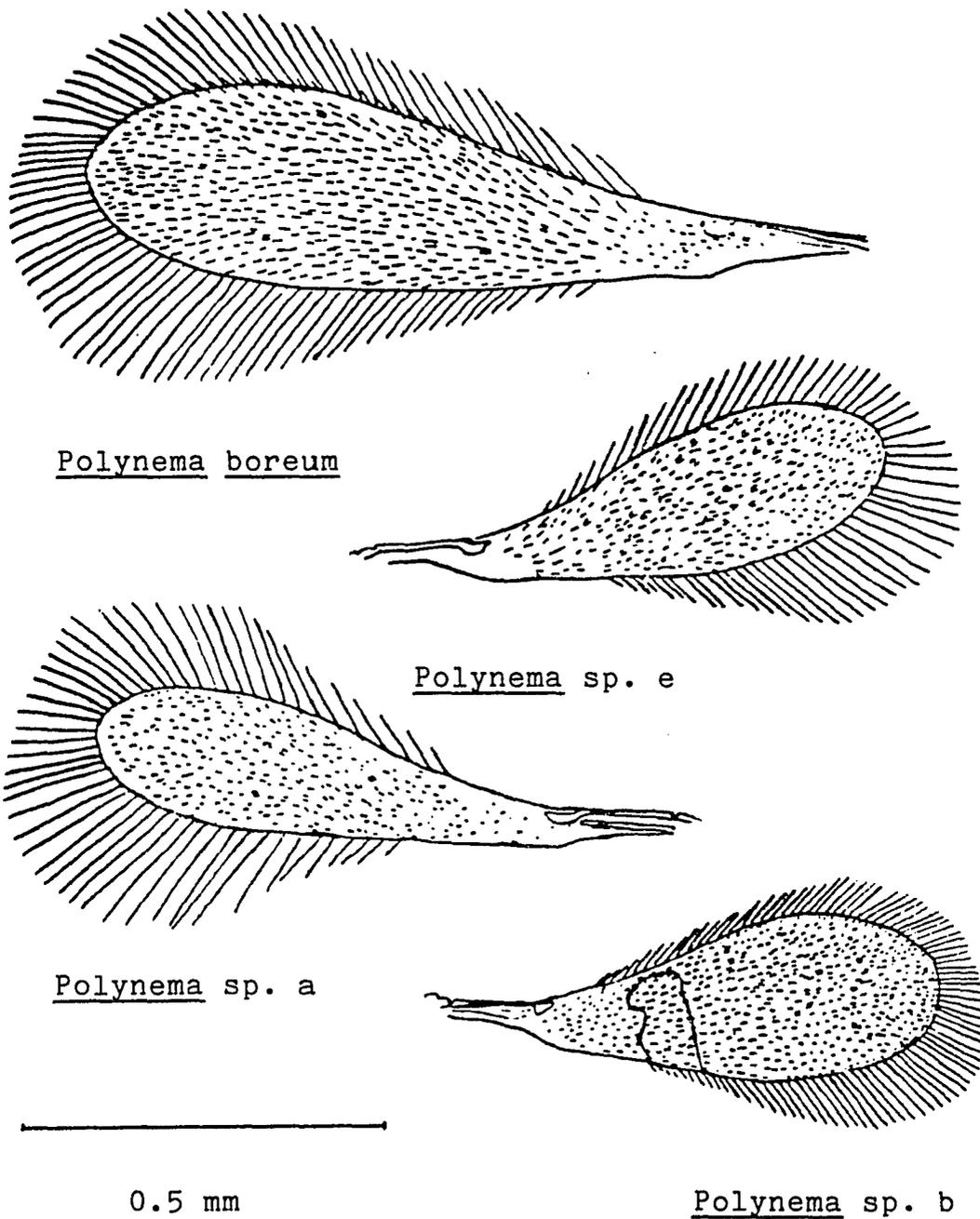


Figure 3. Fore-wing patterns of additional Polynema species collected from southern Arizona.

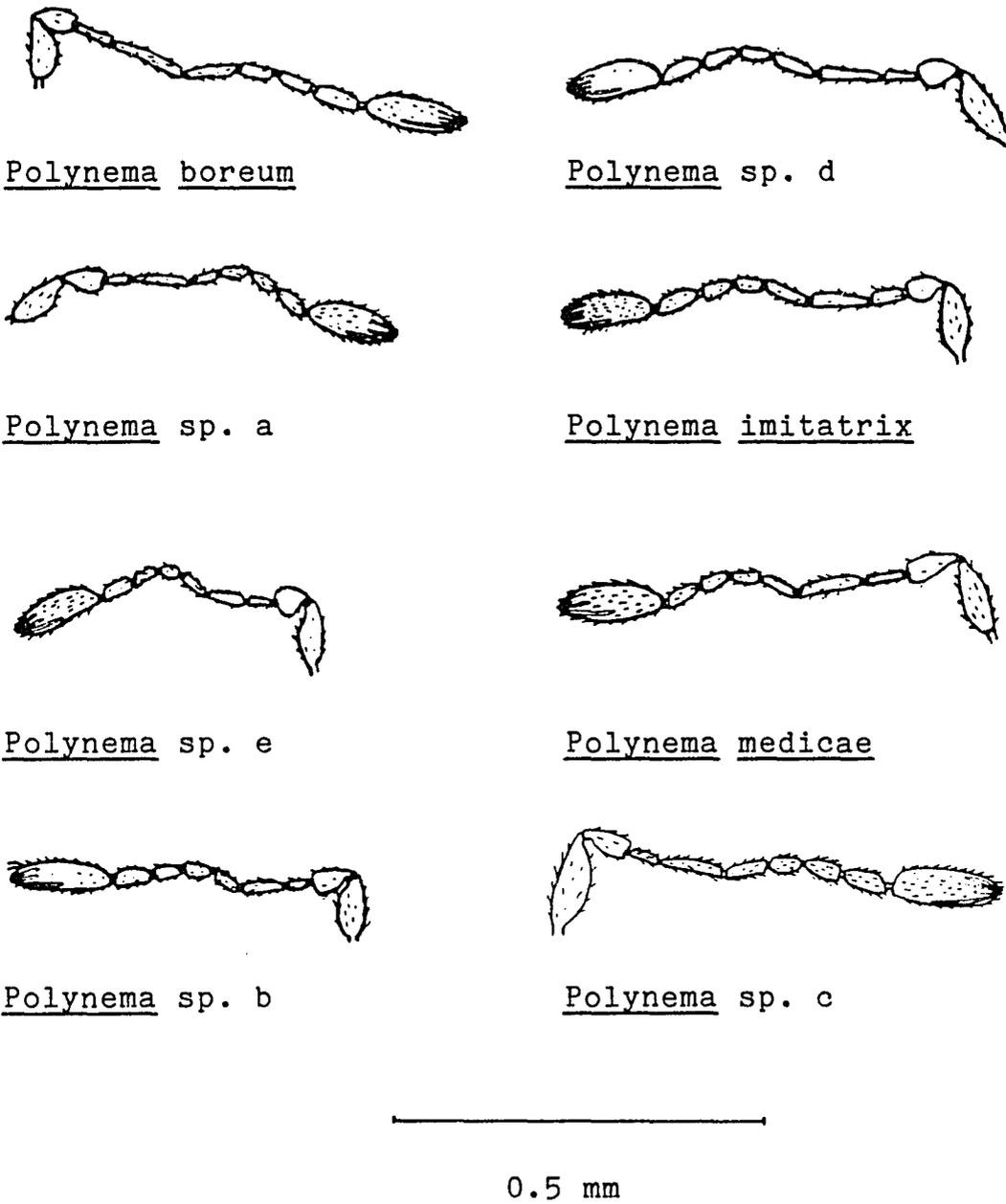


Figure 4. Antennal types of female Polynema species collected from southern Arizona.

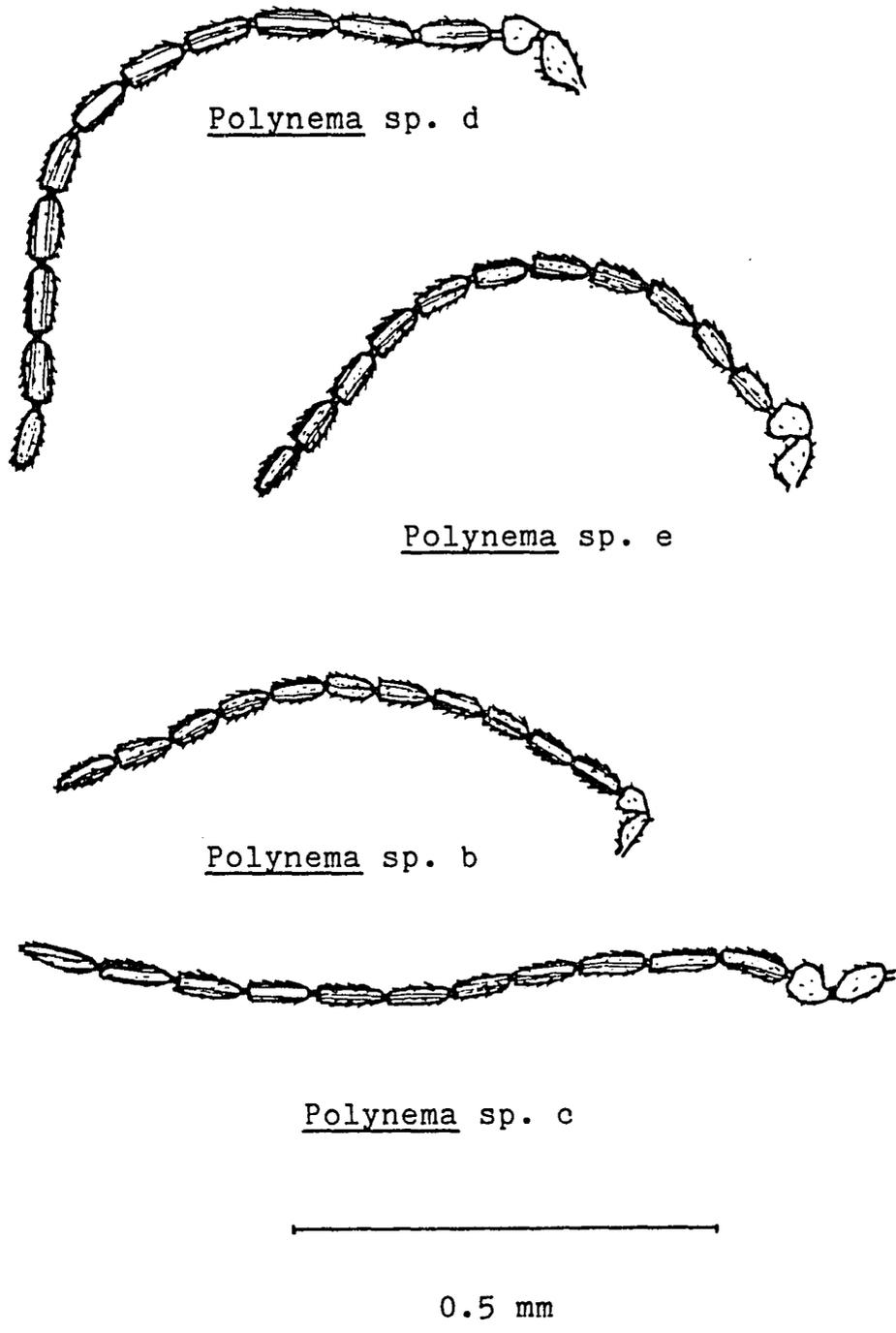
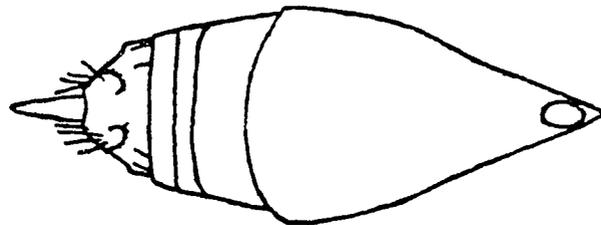


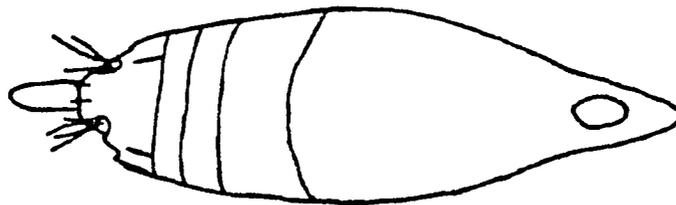
Figure 5. Antennal types of male Polynema species collected from southern Arizona.

The morphological measurement variations which have been discussed have been those from appendages. An area which needs to be investigated is that of consistency of measurement ratios on the major body segments, ie. head, thorax, and gaster. The approximate ratio of length to width of the first tergite on the female gaster holds promise for use as a character for the separation of species (Figures 6 & 7). This character would not be affected by distension of the abdomen, a variation frequently caused by the method of killing the specimen.

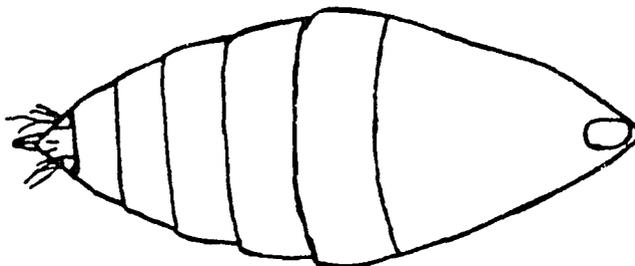
Since it seems that precise morphological measurements of appendages cannot be relied upon to serve as a definitive way to distinguish among mymarids, other morphological characters need to be found. This author has found the notaulus to be one such character for Polynema species. The two types are shown in Figure 8. One type of notaulus ends anteriorly in a pit before reaching the pronotum while the other type of notaulus forms an angle, widens a small amount, then continues on to the pronotum. Using the notaulus types as a basis for initial separation of species, a key for the Polynema of southern Arizona has been prepared.



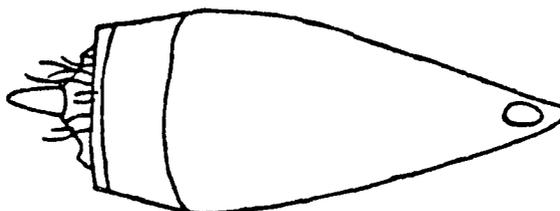
Polynema imitatrix



Polynema sp. d

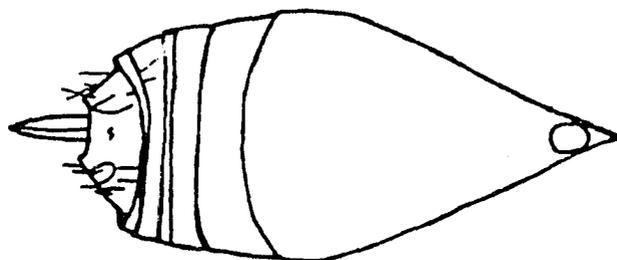


Polynema sp. c

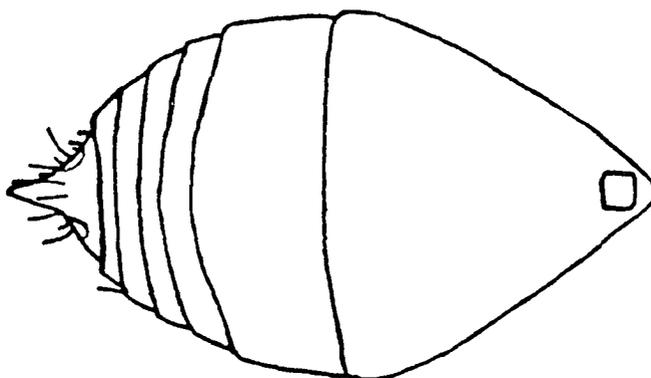


Polynema medicae

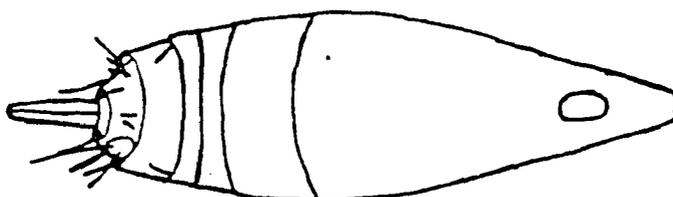
Figure 6. Dorsal view of the gaster of female Polynema species collected from southern Arizona and which are parasitoids of threecornered alfalfa hopper eggs.



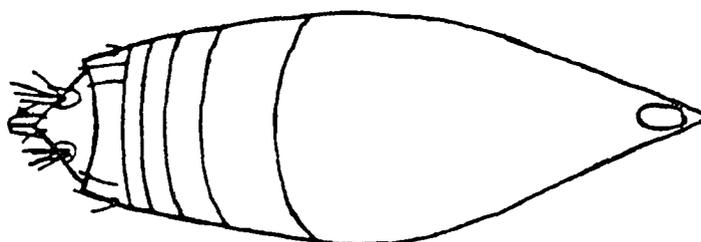
Polynema sp. a



Polynema sp. b



Polynema sp. e

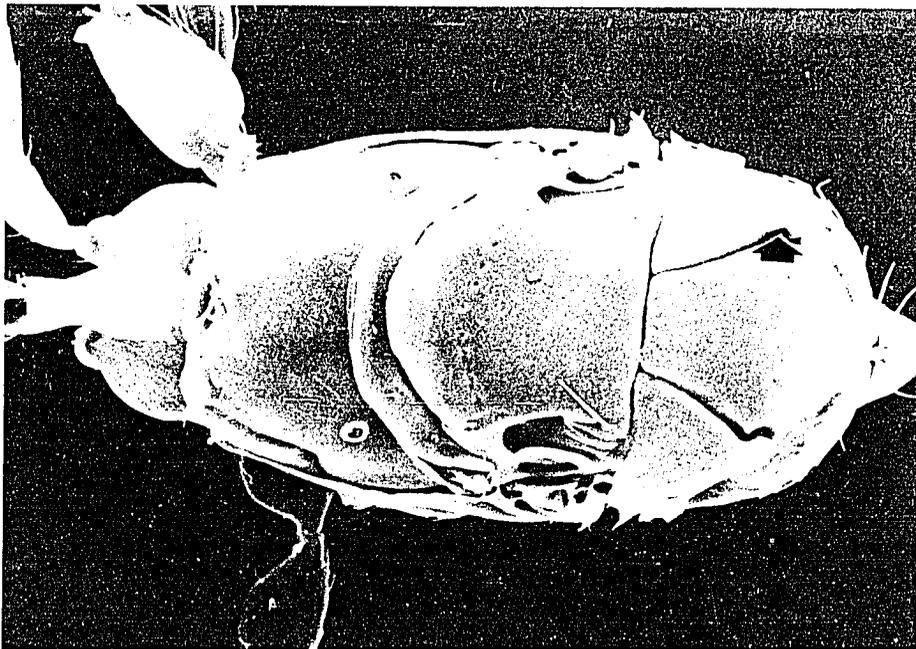


Polynema boreum

Figure 7. Dorsal view of the gaster of female Polynema species collected from southern Arizona.



a



b

Figure 8 a & b. Dorsal view of Polynema spp. thorax. In 8 a the notaulus ends anteriorly in a pit while in 8 b, it forms an angle then continues to the pronotum.

Key to the Species of Polynema Found in Agricultural Areas
of Southern Arizona

(for use with prepared microscope slides)

- 1) Anterior ending of notaulus in a pit (Fig. 8a) ----- 2
 Anterior ending of notaulus angled (Fig. 8b) ----- 5
- 2) Propodeal median carina at least 1.4 length
 of propodeum ----- 3
 Propodeal median carina very short or absent ----- 4
- 3) Dusky patch present on forewing (sometimes
 faint); second female funicle segment
 subequal to pedicel
 ----- Polynema imitatrix & P. sp. d
- 4) Forewing about 4 1/2 X as long as wide (Fig.
 3); female funicle segment #2 subequal
 to funicle segment #6, much shorter than
 scape; antenna concolorous brown; propodeal
 median carina short, often represented
 as a tooth ----- Polynema sp. e
 Forewing about 6 X as long as wide (Fig. 2);
 female funicle segment #2 about 1.5-2 X
 longer than funicle segment #6; scape
 and pedicel yellow; propodeal median
 carina absent ----- Polynema medicae
- 5) Scape with cross striae on mesal surface of
 propodeal median carina about 1/2 length
 of propodeum; female scape shorter than
 funicle segment #2 ----- Polynema boreum
 Scape smooth or with a few longitudinal striae;
 propodeal median carina much less than 1/2
 length of propodeum, may be represented as
 a tooth; female scape longer than funicle
 segment #2 ----- 6
- 6) Female first tergite length subequal to
 width; scape, pedicel, petiole and legs
 yellow ----- Polynema sp. c
 Female first tergite length ca. 1.5 X width;
 antennae concolorous brown, petiole and
 legs brown ----- Polynema sp. a

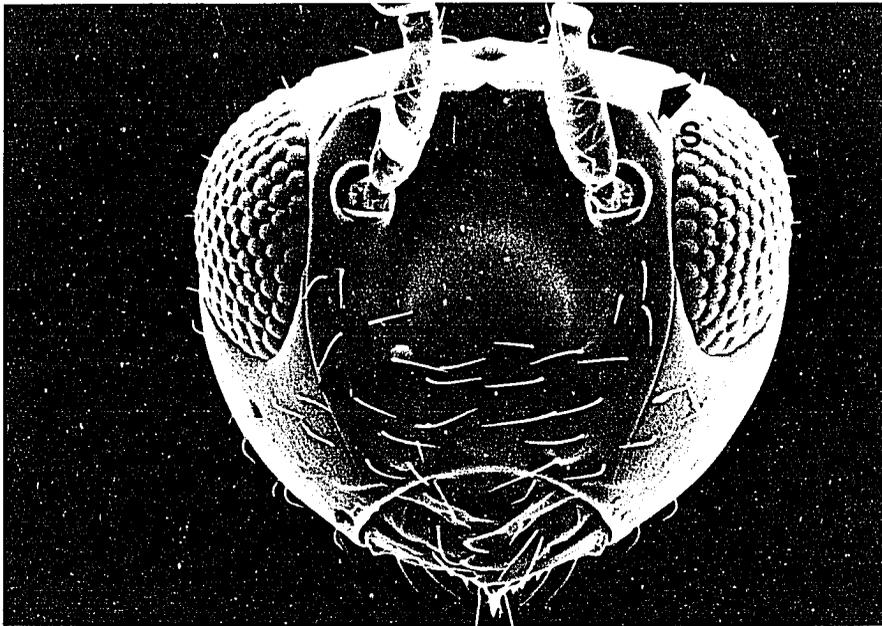
Polynema sp. b has exhibited a character which I

have not observed on any other Polynema collected in

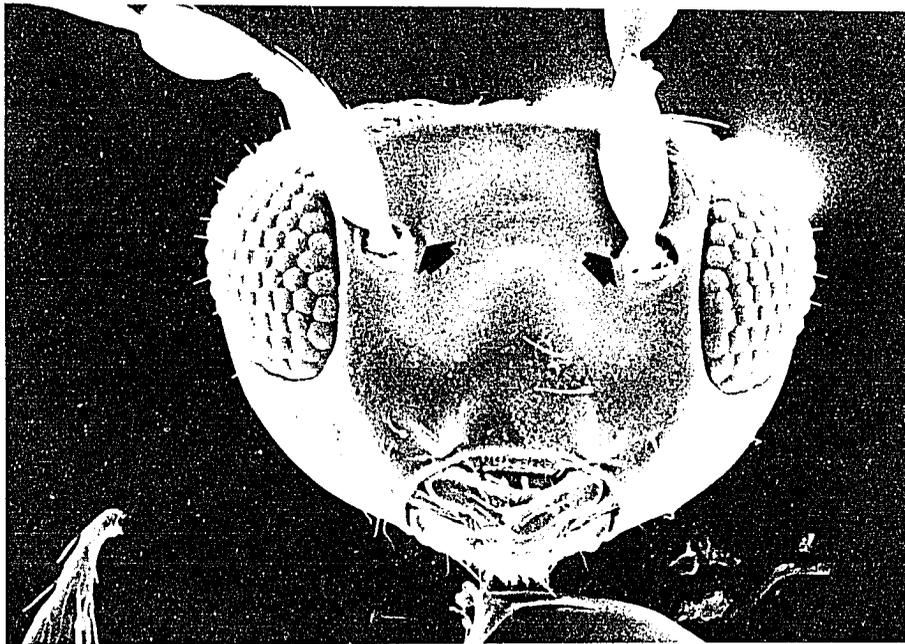
Arizona (Figure 9). Between the toruli are two apodemes. These resemble the apodemes found adjacent to the frontoclypeal suture. When the heads of Polynema medicae were teased apart and examined using a scanning electron microscope, the apodemes along the frontoclypeal suture were found to be the anterior tentorial pits. To this date, a S.E.M. examination of the interior of Polynema sp. b heads has not been performed. However, in my estimation, the torular apodemes represent a dorsal branch of the tentorium. Should this be the case, the presence of these apodemes adjacent to the toruli may serve as an additional character for subdividing the genus Polynema.

Sympatric sibling species

Polynema systematics suffers from the scarcity of usable characters. Because of this and the large numbers collected, in both species and actual numbers, confusion between species is likely. One such case occurs between two species collected in alfalfa fields around Tucson. One type of wasp, P. imitatrix, seasonally occurs in large numbers in alfalfa fields while the other, Polynema sp. d, is infrequently recovered from alfalfa. Examination of S.E.M. photographs reveals no discernible differences. When antennal segments and wing ratios of these wasps reared on alfalfa hopper eggs are analyzed using ANOVA, there are fewer differences than those occurring between



a



b

Figure 9 a & b. a) Frontal view of Polynema boreum showing the 3 main head sutures: frontal, median, and the supraorbitals. b) Frontal view of Polynema sp. b showing pits adjacent and medial to the toruli.

P. medicae reared in different hosts. The appearances of live and freshly killed P. imitatrix and Polynema sp. d are quite similar; however, when closely compared, the scape, pedicel, petiole, and leg segments of P. imitatrix are straw-yellow while the same structures in Polynema sp. d are slightly darker, honey-yellow.

Polynema imitatrix was described as an egg parasitoid of the threecornered alfalfa hopper (Gahan 1918). In our laboratory, we found that the eggs of the threecornered alfalfa hopper were also readily accepted and parasitized by Polynema sp. d and we maintained that species in culture for 3 generations. However, these laboratory-reared progeny differed in appearance from their field-collected progenitors. The structures which has been honey colored in the parents were found to be a dark brown-black. This new color pattern was stable through 3 generations. Fecundity under laboratory conditions utilizing alfalfa hopper eggs was not noticeably affected throughout the time which the culture was maintained.

Field-collected P. imitatrix maintain the straw-yellow color throughout the year, so were we operating with one or two species? One possibility is that this condition represents a color variation within one species. If this were the case, would the color remain stable throughout 3 generations? It is possible that the color of Polynema sp. d was affected by laboratory rearing, or color change due

to the laboratory host's not being the normal field host. Could the wasps be sibling species? The obvious test for this is hybridization; however, only Polynema sp. d was cultured.

Polynema sp. d was not frequently identified from D-Vac samples of alfalfa. This could be due to difficulty in detecting the differences in color of the 2 species of wasps, also the low numbers could also occur if the wasps normally occurred on a plant other than alfalfa and the weeds sampled, occasionally going to alfalfa as adults.

Biological Studies

Host determination

A marked size differential in field-collected individuals that appeared to be the same species led to the search for other host species which might be causing the differential. Multiple host utilization leading to a differential in parasitoid size has been demonstrated in mymarids (Moratorio 1977). Polynema medicae, P. sp. c and P. sp. d, which had been determined to be egg parasitoids of the threecornered alfalfa hopper, were presented with eggs of the leafhoppers which occurred in abundance in alfalfa. The eggs of Aceratagallia were readily accepted and successfully parasitized by Polynema medicae and Polynema sp. c. Logistic problems prevented P. imitatrix from being tested for acceptance of this second host. At

the time of tests, the field-collected hosts were experiencing their normal winter decline in numbers, so an insufficient supply of eggs was available. Within this paper, the usage of "large", in reference to a field-collected alfalfa hopper parasitoid, will refer to wasps assumed to have been reared on alfalfa hopper eggs. Conversely, the term "small" will refer to these same field-collected species assumed to have emerged from leafhopper eggs. In order to make these size distinctions, field-collected wasps were grouped by comparison with wasps reared on known hosts in the laboratory.

Some species of Polynema were collected from weeds and rarely, if at all, found in alfalfa samples. One was Polynema sp. b. The host for this species was determined to be the leafhopper, Ollarianus strictus, Ball which was frequently recovered as adults from the field samples of alfalfa. Nielson and Currie (1962) reported O. strictus to be the second most common leafhopper (10%) in alfalfa in the Salt River Valley area of Arizona. When attempts were made to establish a culture of this leafhopper on alfalfa plants, little if any oviposition occurred and no nymphs emerged. When careless-weed was presented to the leafhoppers, a vigorous culture was established, eventually causing partial defoliation of the plants. The low number of Polynema sp. b collected from alfalfa may be due to

O. strictus reproducing on weeds but moving to alfalfa to feed as adults.

Polynema sp. e, collected from white horse-nettle (Solanum elaeagnifoliumCar.), was readily reared in the laboratory on the eggs of Aceratagallia spp. which had been collected from alfalfa; however, the wasp was infrequently collected from alfalfa. The field host on white horse-nettle was determined to be Aceratagallia obscura Omar, a species not found on alfalfa by Nielson and Currie (1962). No host preference tests were performed on Polynema sp. e, but it is possible that the wasp is host specific under field conditions. Another possibility is that the wasp host-search pattern is primarily directed to host plants of A. obscura and that alfalfa is not a preferred search site.

Polynema sp. d was infrequently identified from field-collected material; however, under laboratory conditions, it was easily cultured on threecornered alfalfa hopper eggs. Possible reasons for the low number of field-collected Polynema sp. d are discussed in the section on the Polynema sp. d - Polynema imitatrix identification problem.

Potential fecundity

Egg production within the genus Polynema is comparable to what is found in other mymarid genera. Table 4 shows the number of ovarian eggs counted in 5 species.

Females of P. medicae reared on two different hosts were examined. The number of eggs per female was found to be significantly greater ($P=.01$) in the wasps which developed in the larger host eggs (threecornered alfalfa hopper) than in the smaller eggs (Aceratagallia spp.). There was no significant difference ($P=.01$) in the ovarian egg counts between 1- and 17-day-old P. boreum which were not exposed to host eggs. D. Jackson (1966) reported smaller incompletely developed ovarian eggs along with mature eggs in Caraphractus cinctus, an occurrence which I observed in 1-day-old Polynema boreum; however, the incompletely developed eggs were counted. Sahad (1982 a & b) reported that the number of ovarian eggs in Gonatocerus cincticipitis was substantially smaller (mean=28, range =23-36) than the total number of eggs laid throughout the lifetime (mean=49, range=28-77). He provided no explanation for this difference; however, it seems plausible that there could be additional egg production. Lakin et al. (1984) found no significant difference between the ovarian egg count of P. boreum and its lifetime adult progeny production, indicating that P. boreum may have no additional egg production once its original complement is depleted. It should be noted that the techniques for ovarian egg count used by Sahad (1982 b) were more precise than the technique used by Lakin et al. (1984). Also, Sahad considered total egg

Table 4. Ovarian egg number for Polynema species from southern Arizona, reared at 26° C.

<u>Polynema</u> <u>species</u>	Mean No. ovarian eggs	Sample Size	Age of Female	Host Species
<u>medicae</u>	44.0+/-6.0	13	1 day	alfalfa hopper
<u>medicae</u>	35.6+/-6.1	7	1 day	<u>Aceratagallia</u>
<u>boreum</u>	20.8+/-2.8	19	1 day	<u>Nabis</u> spp.
<u>boreum</u>	20.5+/-3.1	12	17 days	<u>Nabis</u> spp.
sp. b	29.4+/-5.7	20	1 day	<u>Ollarianus</u> <u>strictus</u>
sp. c	41.8+/-5.0	8	1 day	alfalfa hopper
sp. d	37.0+/-5.8	12	1 day	alfalfa hopper

egg production including those that did not develop while Lakin et al. reported lifetime adult progeny production.

Longevity

The adult lifespan of mymarids is rather short, usually ranging from 1 to 3 weeks. Longevities of Polynema species from southern Arizona are shown in Table 5. In all cases except for Polynema sp. c reared in Aceratagallia spp., the longevity of males was significantly shorter than that of females. The exception may have been due to the small sample size (n=26). Sahad (1982 a) reported a greater female longevity for G. cincticipitis under 4 temperature regimes when both food and water were available. When only water was provided, male longevity was slightly greater. Sahad speculated that the females required more nutrients throughout their lives than the males. No statistical evaluation of his data was provided.

The greatest difference of longevity within a species was for P. boreum where the females, on the average, lived twice as long as the males. The female P. boreum lifespan was also ca. twice as long as that of females of most other Polynema species. This difference may have been due to the special reproductive needs of this wasp. It parasitizes the eggs of predaceous damsel bugs (Nabis spp.), a non-gregarious host. The host eggs are

Table 5. Longevity of Polynema species from southern Arizona, reared at 26° C.

<u>Polynema</u> species	Longevity Mean (da.)	Sample Size	Host Species
<u>medicae</u> ^{a/} (m)	11.1+/-3.6	33	alf. hopper > alf. hopper
<u>medicae</u> **	15.5+/-4.7	46	alf. hopper > alf. hopper
<u>medicae</u> ^{b/} (m)	6.2+/-0.9	10	alf. hopper > leafhopper
<u>medicae</u> **	8.2+/-1.3	28	alf. hopper > leafhopper
<u>medicae</u> (m)	6.2+/-1.9	13	leafhopper > leafhopper
<u>medicae</u> **	7.9+/-2.0	52	leafhopper > leafhopper
<u>medicae</u> (m)	7.0+/-1.0	3	leafhopper > alf. hopper
<u>medicae</u> **	11.3+/-2.1	21	leafhopper > alf. hopper
<u>boreum</u> (m)	14.9+/-4.5	48	<u>Nabis</u> spp.
<u>boreum</u> **	29.7+/-9.9	52	<u>Nabis</u> spp.
sp. b (m)	11.3+/-5.2	45	<u>Ollarianus strictus</u>
sp. b **	15.5+/-6.6	72	<u>Ollarianus strictus</u>
sp. c (m)	11.4+/-1.9	50	alfalfa hopper
sp. c **	15.5+/-2.9	57	alfalfa hopper
sp. c (m)	8.4+/-1.8	10	leafhopper
sp. c	9.2+/-1.8	16	leafhopper
sp. d (m)	12.9+/-1.5	8	alfalfa hopper
sp. d **	18.3+/-4.6	10	alfalfa hopper

a/ (m) = males, blank = female

b/ ** = sex with significantly greater (p<.01) longevity than opposite sex

usually low in density, so that the female wasp requires more time to find and parasitize the host eggs.

Another interesting relationship was found with the parasitoid-host relationships of P. medicae. As might be expected, the longevity of these wasps reared in the larger host (threecornered alfalfa hopper) was significantly greater than that of the wasps were reared in a much smaller host (Aceratagallia spp.). Differences in longevity due to differences in host species have been reported for Trichogramma by Boldt and Marston, 1974. These authors attributed the differences to the greater quantity of food available in the larger host egg. When I reared P. medicae in the eggs of the leafhopper, Aceratagallia spp., then reared the next generation in the eggs of the threecornered alfalfa hopper, the lifespan was significantly shorter than when the wasps were maintained in culture on alfalfa hopper eggs. Smaller eggs produced by the leafhopper-reared wasps could explain the resulting smaller adult wasps emerging from the alfalfa hopper eggs and consequently shorter lifespan.

Developmental time

Developmental time was determined for most of the Polynema species collected in southern Arizona (Table 6). Differences related to sex within a species were analyzed by ANOVA and reported at the 1% level. Polynema boreum and

Table 6. Developmental time for Polynema species from southern Arizona, and reared at 26° C.

<u>Polynema</u> <u>species</u>		Mean Develop. Time (da.)	Sample Size	Host Species
<u>medicae</u>	m	15.4	25	alfalfa hopper
<u>medicae</u>	f	15.7	96	alfalfa hopper
<u>medicae</u>	m	13.6	20	<u>Aceratagallia</u>
<u>medicae</u>	f	15.1 **	32	<u>Aceratagallia</u>
		a/		
<u>boreum</u>	m	17.0		<u>Nabis alternatus</u>
<u>boreum</u>	f	17.6		<u>Nabis alternatus</u>
<u>boreum</u>	m	17.1		<u>Nabis americanoferus</u>
<u>boreum</u>	f	17.7		<u>Nabis americanoferus</u>
sp. b	m	13.6	37	<u>Ollarianus strictus</u>
sp. b	f	14.0	66	<u>Ollarianus strictus</u>
sp. c	m	20.3	162	alfalfa hopper
sp. c	f	21.4 **	152	alfalfa hopper
sp. c	m	17.5	99	alfalfa hopper
sp. c	f	18.4 **	94	alfalfa hopper

a/ ** = sex had significantly longer (p<.01) developmental time than opposite sex of a species-host combination.

Table 7. Sex ratios of southern Arizona field-collected Polynema species in 1983 and 1984, and ratios occurring in laboratory cultures.

<u>Polynema</u> species	Laboratory- Reared		Collected 1983		Collected 1984	
	m:f ratio	sample size	m:f ratio	sample size	m:f ratio	sample size
<u>medicae</u> large	1:3.7	118	1:0.3	302	1:0.8	2082
<u>medicae</u> small	1:1.6	52	1:0.4	315	1:0.6	919
<u>imitatrix</u> large	NA	NA	1:1.5	37	NA	NA
<u>imitatrix</u> small	NA	NA	1:1	17	NA	NA
sp. b	1:1.2	174	1:15	31	0:5	5
sp. c large	1:1	328	1:0.8	96	NA	NA
sp. c small	NA	NA	3:0	3	NA	NA
sp. d	1:1	194	0:1	1	1:1.8	86
sp. e	1:1.6	92	NA	NA	0:2	2

P. medicae were also evaluated for the effect of host species on the wasp's developmental time. For P. boreum there was no significant difference in developmental time between rearings in Nabis americanoferus or N. alternatus.

Utilization of alternate hosts (alfalfa hoppers vs. Aceratagallia) produced a two-day longer developmental time for P. medicae males in alfalfa hopper eggs. Yet with these same two hosts, the developmental time in females showed only a half-day difference between the means. Developmental time has been shown by Arthur and Wylie (1959) to be related to host size for an ichneumonid, Coccygomimus turionellae (L.). Sandlan (1982) investigated the relationships of C. turionellae development within 5 different host species. When he compensated for variation in host size, the developmental time differed significantly when rearing was in different hosts. Sandlan speculated that these differences could be due to host nutritional differences, or to some host defense system. New (1969) also reported that the developmental time of Alaptus pallidicornis was affected when it was reared in 3 separate species of psocids.

Developmental times between the sexes of the mymarid Anaphes diana was found to differ significantly ($P < 0.1$) in 4 out of 5 of the temperature regimes tested, with the males having a faster developmental rate (Leibee

et al. 1979). The only temperature at which there was no significant difference was 7.2° C where the developmental time was ca. 5 months.

Sex ratios

Polynema sp. c, P. medicae, and P. boreum all were cultured in the laboratory and found in sufficient numbers in D-Vac samples to compare the sex ratios (Table 7). Laboratory-reared P. boreum from N. alternatus had a sex ratio which was very similar to the ratio of those laboratory-reared from N. americanoferus; however, the ratio for those collected from alfalfa fields in 1983 and 1984 differed greatly from each other and from the ratio of the laboratory culture. Substantial differences in sex ratios between laboratory-reared and field-collected samples also occurred with both sizes of P. medicae. Even with large samples of field-collected wasps, sex ratios varied enough to make the author skeptical of the value of determining sex ratios from field samples. Too many variables are involved. Alfalfa hoppers oviposit primarily in the lower portions of alfalfa stems (Graham & Jackson 1982), even ovipositing into the stems at or below ground level (Meisch & Randolph 1965), so this is where one might expect to find the female wasps. Male mymarids seem to respond more rapidly to phototactic stimuli than do females, so they might be found more at the tops of plants. If this is the

reason that more P. medicae males are collected than females, how can one explain the collection of equal numbers of male and female Polynema sp. c, which also parasitize the eggs of the alfalfa hopper? This species may parasitize the alfalfa hopper eggs found in the upper portions of the plants and thus more female Polynema sp. c would be captured along with the males of their species.

Seasonal distribution

Four of the Polynema species collected from southern Arizona have been shown to parasitize the eggs of the threecornered alfalfa hopper. The other four parasitize the eggs of leafhoppers or nabids. Collection records in alfalfa for this second group are sparse for all species other than P. boreum. The Polynema species collected from Astragalus sp. was not found in alfalfa.

Polynema sp. b, a parasitoid of the eggs of the leafhopper Ollarianus strictus, was collected almost exclusively from weeds, with high densities found in careless-weed. This is in spite of the report by Nielson and Currie (1962) that this species was the second most commonly collected as adults from alfalfa. When I attempted to rear O. strictus on alfalfa plants, no nymphs appeared. However, the plants were not checked for leafhopper eggs. It seems probable that the adult leafhoppers fed on alfalfa

and oviposited elsewhere. Careless-weed was found to serve as an excellent host plant for O. strictus.

Polynema sp. e was field-collected from white horse-nettle in conjunction with a leafhopper tentatively identified as Aceratagallia obscura, not reported by Nielson and Currie (1962) as being present in alfalfa fields. Laboratory rearings of Polynema sp. e were obtained by using Aceratagallia spp. collected from alfalfa. Therefore, one might speculate that either this wasp does not frequent alfalfa plants or those species of Aceratagallia found on alfalfa are not natural hosts, despite their acceptance as laboratory hosts.

Polynema boreum population density is correlated with the reproductive periods of nabids. Winter population sizes are small since most of the hosts have gone into reproductive diapause; however, a small portion of nabids still oviposit during the Arizona winter. This along with the relatively long lifespan of P. boreum females, accounts for the presence of small numbers of the wasps in field samples during the winter. Spring populations of parasitoids rose rapidly, probably in response to the rapid rise in nabids which feed heavily on the flourishing aphid populations. As summer temperatures rise, a decrease in the population sizes occurs for aphids, nabids, and P. boreum.

The section on host determination deals with the discovery that some Polynema species are capable of

successfully parasitizing both the eggs of the three-cornered alfalfa hopper and the eggs of leafhoppers in the genus Aceratagallia. I was unable to establish a pure culture of the individual leafhopper host species. Therefore, all laboratory biological work involved the use of field-collected individuals. Due to the difficulty of separating species of leafhoppers while they are alive, a combination of species within the genus Aceratagallia was used. Host preference of these mymarids among the species of leafhoppers was not determined. From laboratory data, we inferred that some of the Polynema species seemed to do equally well in alfalfa hopper eggs and leafhopper eggs; however, no preference tests were made between these two egg types.

The seasonal distribution of P. imitatrix was determined just for the year 1983, due to the difficulty in distinguishing it from Polynema sp. c. It can be separated from Polynema sp. c only when specimens are cleared and mounted on microscope slides. The D-Vac sample from one week in 1984 included over 1,000 of these two wasp species.

Figure 10 shows the population peaks for the alfalfa hopper and for the alfalfa hopper egg parasitoid complex populations in 1983. Other than the normal insect population decline occurring in the fall, the catastrophic population declines in the field collections may be attributed to the cutting of alfalfa fields and to application of

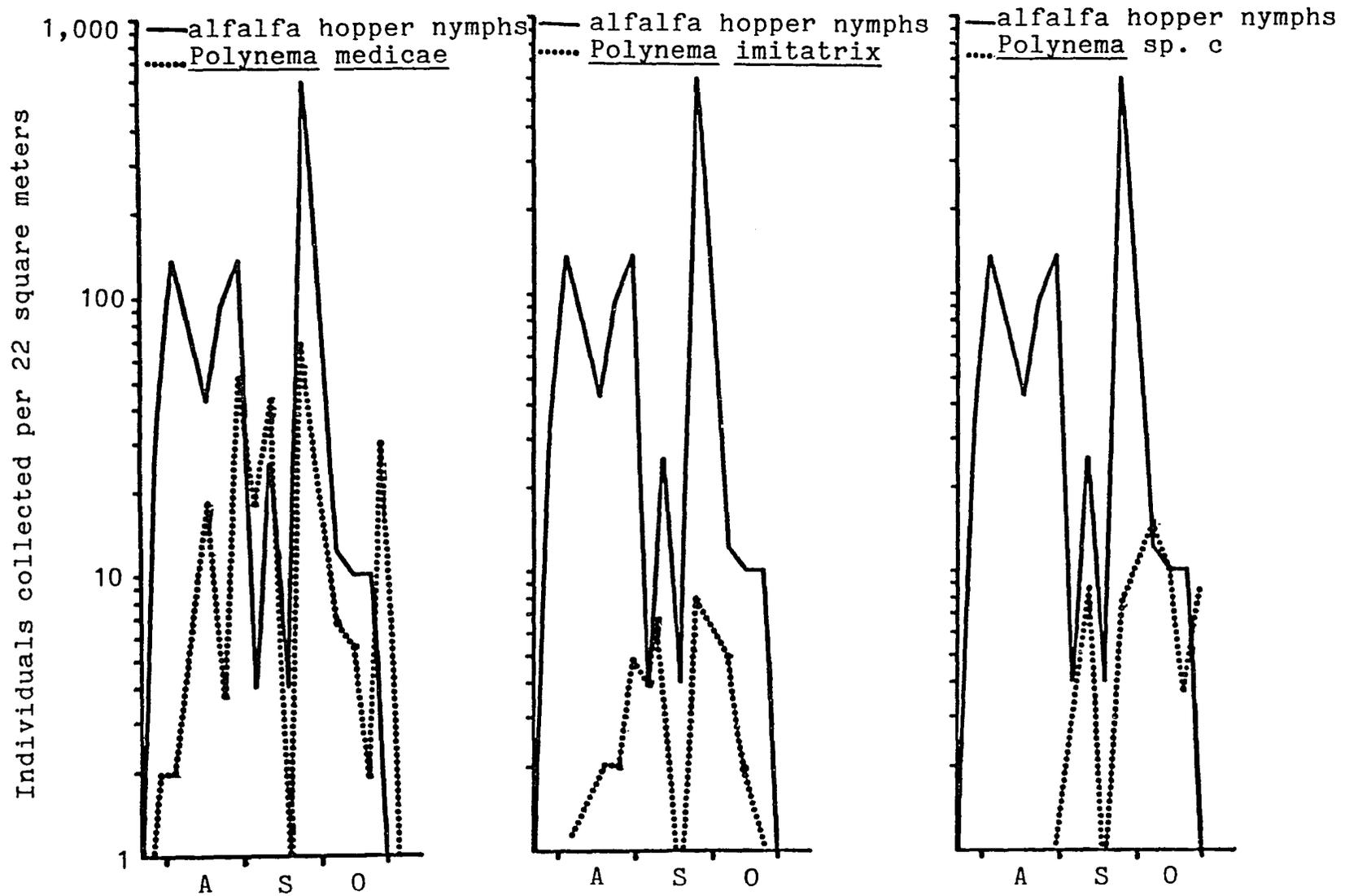


Figure 10. Threecornered alfalfa hopper nymphs and large Polynema spp. wasps collected by D-Vac from alfalfa fields near Tucson, Arizona. 1983.

insecticides. Alfalfa hoppers and the parasitoid complex were present throughout the rest of the year, although their numbers were much reduced. From the total D-Vac collection used to determine seasonal distribution for 1983, P. imitatrix had the lowest population level of these three species (43 vs. 99 for Polynema sp. c and 302 for P. medicae).

The population densities of the complex of alfalfa hopper parasitoids were somewhat reversed for 1984 (Figure 11). The number of P. medicae was larger for 1984 (1040) than for 1983 (302), but decreased in comparison with the combined numbers for Polynema sp. c and P. imitatrix, 2299 for 1984 and 142 for 1983. Preliminary indications for 1984 show that Polynema sp. c had a population density 2-3 X as great as that for P. imitatrix, so for this discussion the population increase for the two combined species will be attributed primarily to Polynema sp. c. This is graphically represented in Figures 10 and 11.

What factors may have contributed to this rapid reversal of the dominant parasitoid species? Figure 12 compares the mean temperatures (on a 5 day basis) during the months of July through October for 1983 and 1984 in an alfalfa field in Marana, Arizona. It should be noted that the temperature at which developmental times were determined was lower than the mean temperature throughout most of the time during which the alfalfa hopper parasitoids had

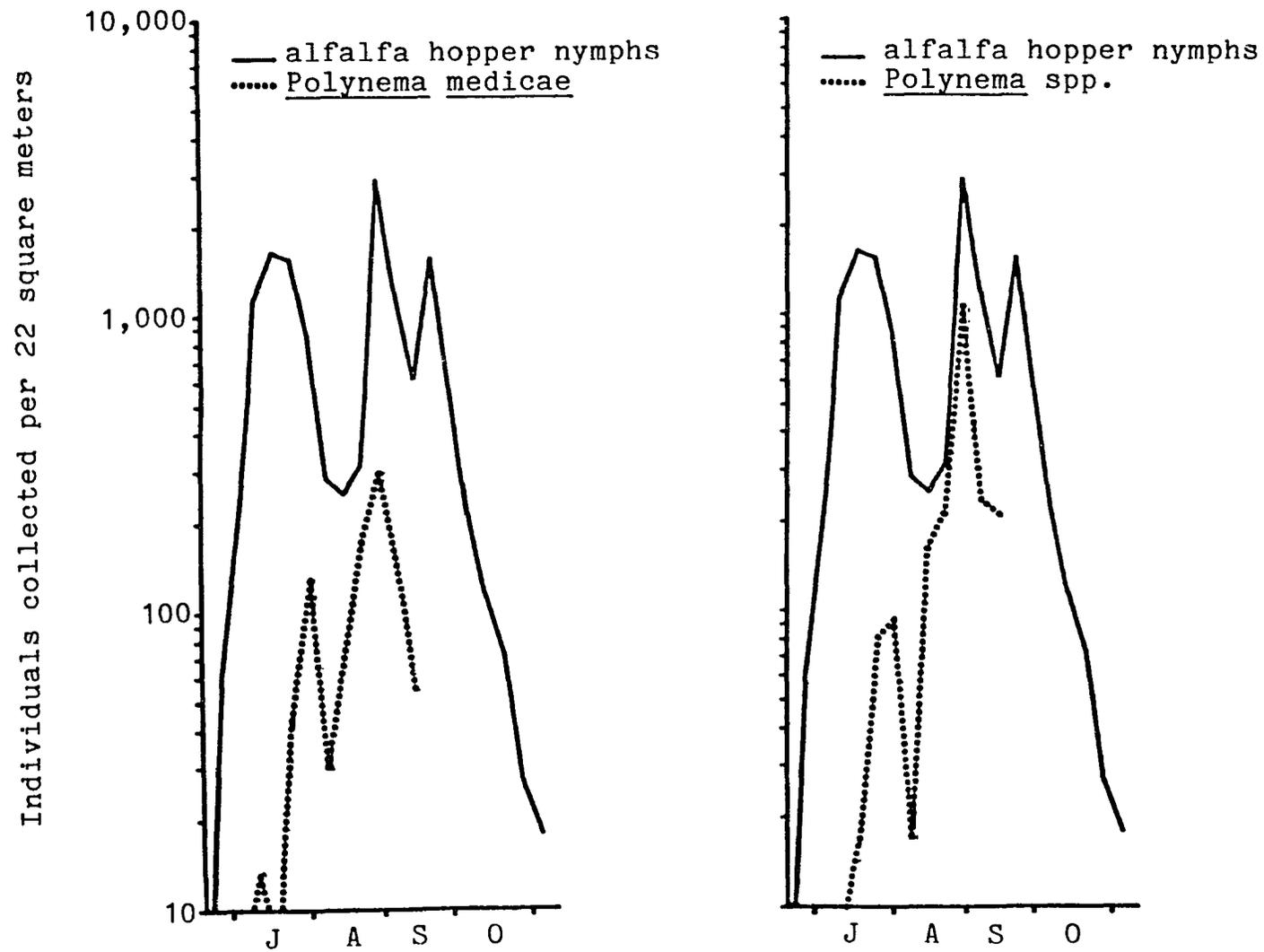


Figure 11. Threecornered alfalfa hopper nymphs and large Polynema spp. wasps collected by D-Vac from alfalfa fields near Tucson, Arizona. 1984

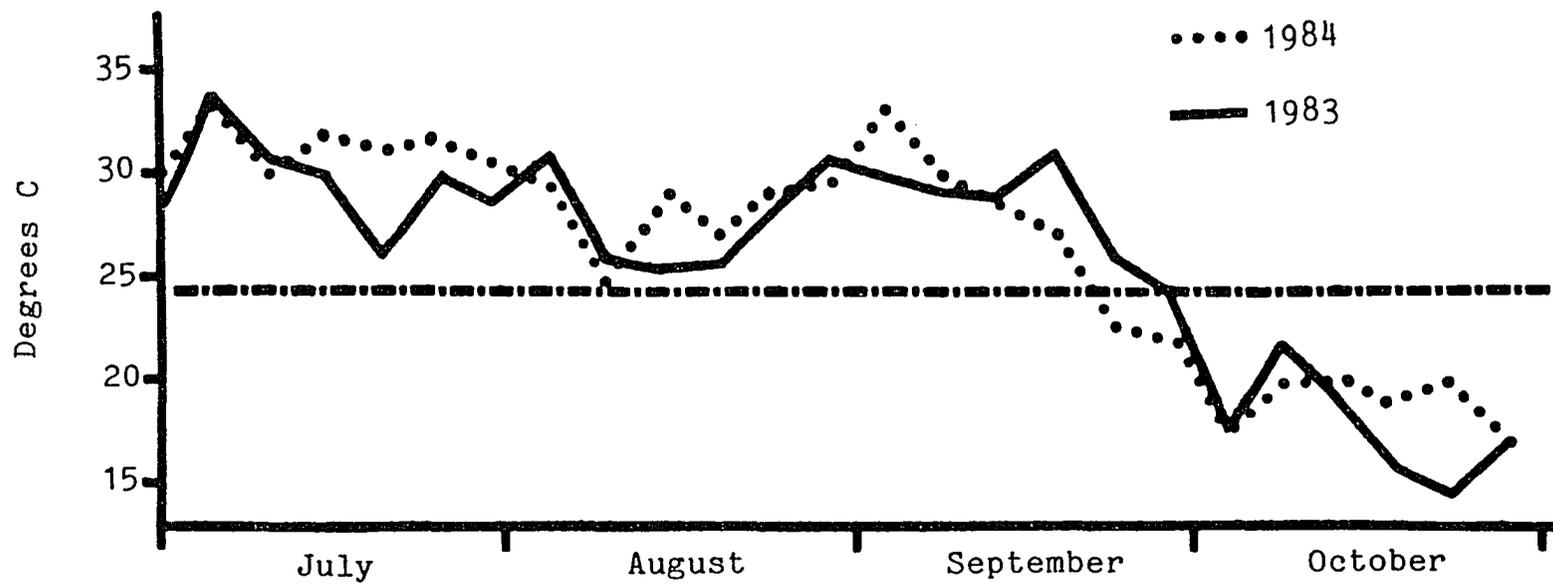


Figure 12. Daily mean temperatures from Glover's alfalfa field.

the highest population levels. C. G. Jackson (1982) reported that developmental time for Anaphes ovijentatus at a variable temperature with a range of (23.3 - 46.6° C) was about twice the time reported by Stoner and Surber (1971) for the same species at a constant 30° C. It is possible that one parasitoid might have had a greater developmental rate than another at the higher temperature extremes which occurred under field conditions. Anderson and Paschke (1970) found that cultures of Anaphes flavipes from 5 different European locations exhibited different developmental rates at different temperatures. Comparing the field temperature means on a 5-day basis throughout 1983 and 1984, there was no great and consistent difference during the time of rapid population increase that might indicate a temperature involvement.

Comparison of the mean humidity for these two years from field data reveals a definite and consistent difference (Figure 13). The earlier and prolonged high humidity for 1984 closely parallels the 1984 alfalfa hopper population increase, which was earlier and went higher in density than in 1983. Since this field was irrigated, the humidity differences between the two years was probably due to factors other than standard irrigation practices. Figure 14 reveals that the summer rainfall in 1984 began weeks earlier than in 1983. During some of the peak population periods, the alfalfa was lodged, a condition which might

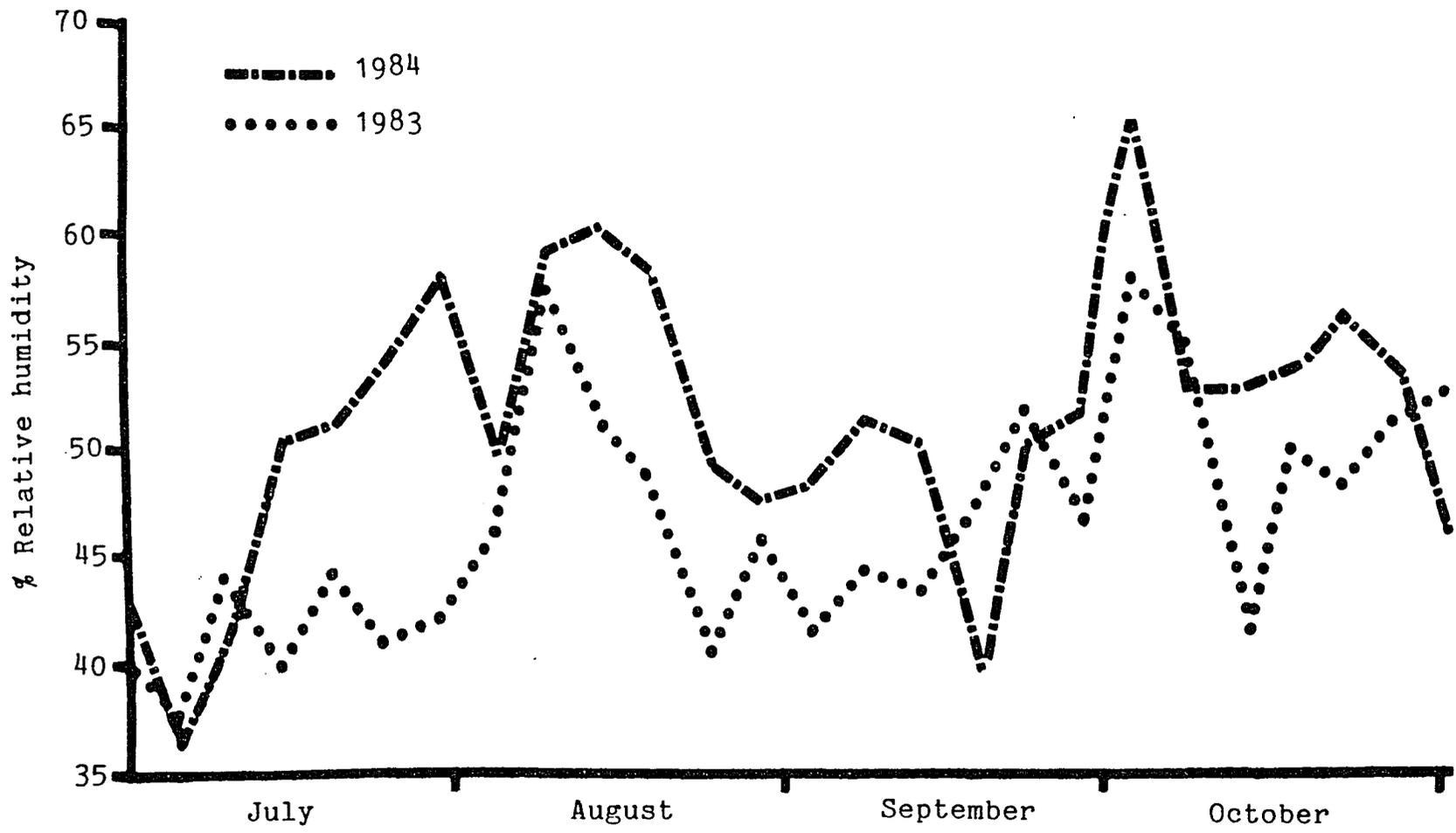


Figure 13. Daily mean humidity from Glover's alfalfa field, Marana, Arizona, for 1983 and 1984.

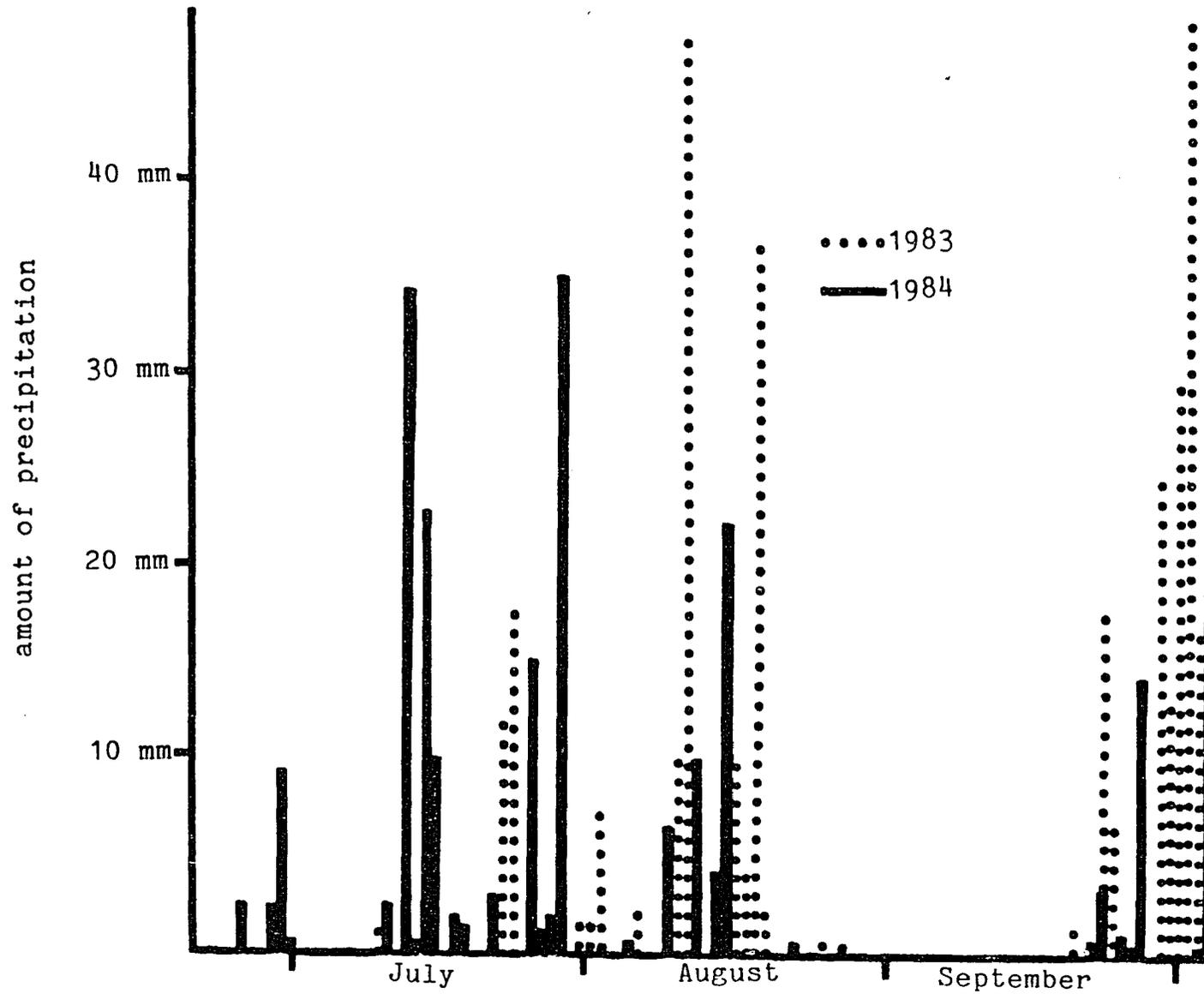


Figure 14. Precipitation at Glover's alfalfa fields in the years 1983 & 1984.

hinder collection and might favor the recovery of one species over another. However, in these samples, the dominant species collected from lodged alfalfa was the same as the dominant species for the entire year.

Another factor which should be considered is the microclimate within the alfalfa field itself. The environmental conditions reported were recorded at ca. 1.5 m above ground level, environmental conditions different from those experienced by the developing wasp larvae which were in host eggs imbedded in plant tissue. Pinter et al. (1975) reported that temperatures in a pre-irrigation cut alfalfa field reached ca. 43° C at 10 cm above the soil surface. Host eggs would be somewhat protected by the surrounding plant material; however, they would still experience rather high temperatures. On the other hand, the cooling provided by shading and evapotranspiration found in a stand of alfalfa just prior to cutting, could keep a midday temperatures at 35° C while air temperatures at 1 m reached 44° C.

A comparison of the biological factors (longevity, ovarian egg count, and developmental time) showed significant difference only in developmental time between Polynema medicae and P. sp. c. The developmental time at 26° C, for P. medicae was ca. 5 days shorter than for Polynema sp. c. All other factors being equal, one would expect P. medicae to recover faster from catastrophies and

to maintain higher population levels. This was not the case in 1984. Field-collected data contain too many variables to permit prediction of parasitoid population fluctuations. Laboratory studies of developmental rates throughout a range of temperatures and humidity regimes would help to clarify the situation when utilized in conjunction with a microecological interpretation of field conditions.

Factors contributing to seasonal distribution of the smaller-sized parasitoids from the alfalfa hopper parasitoid complex are even more involved than those for the large-sized parasitoids. In addition to temperature and humidity being affected by evapotranspiration, shading, and irrigation practices, we find that more than one potential leafhopper host is present. Nielson and Currie (1962) found 4 species of Aceratagallia in Arizona alfalfa fields, but only one species was among the 6 most commonly collected species of leafhoppers and accounted for 77% of these 6 species. In 1983 Polynema medicae was the most common species of small parasitoids collected (316) while only 17 P. imitatrix and 3 Polynema sp. c were collected. There were no records kept for the numbers and species of leafhoppers collected in 1983. As with the larger members of this complex, the small Polynema sp. c and the small Polynema imitatrix collected in 1984 have not been separately identified. These two species combined (303 for

1984) increased greatly in comparison with the number of P. medicae collected in 1984 (485) as compared to 1983. This corresponds to the increase in populations on the larger members of these species, which were probably parasitizing alfalfa hoppers. Figure 15 shows the 1984 seasonal distribution of these small Polynema species. It should be noted that the leafhoppers represent a combination of species. Also, since the numbers shown are of adult leafhoppers, parasitization rates for the eggs cannot be approximated from these graphs.

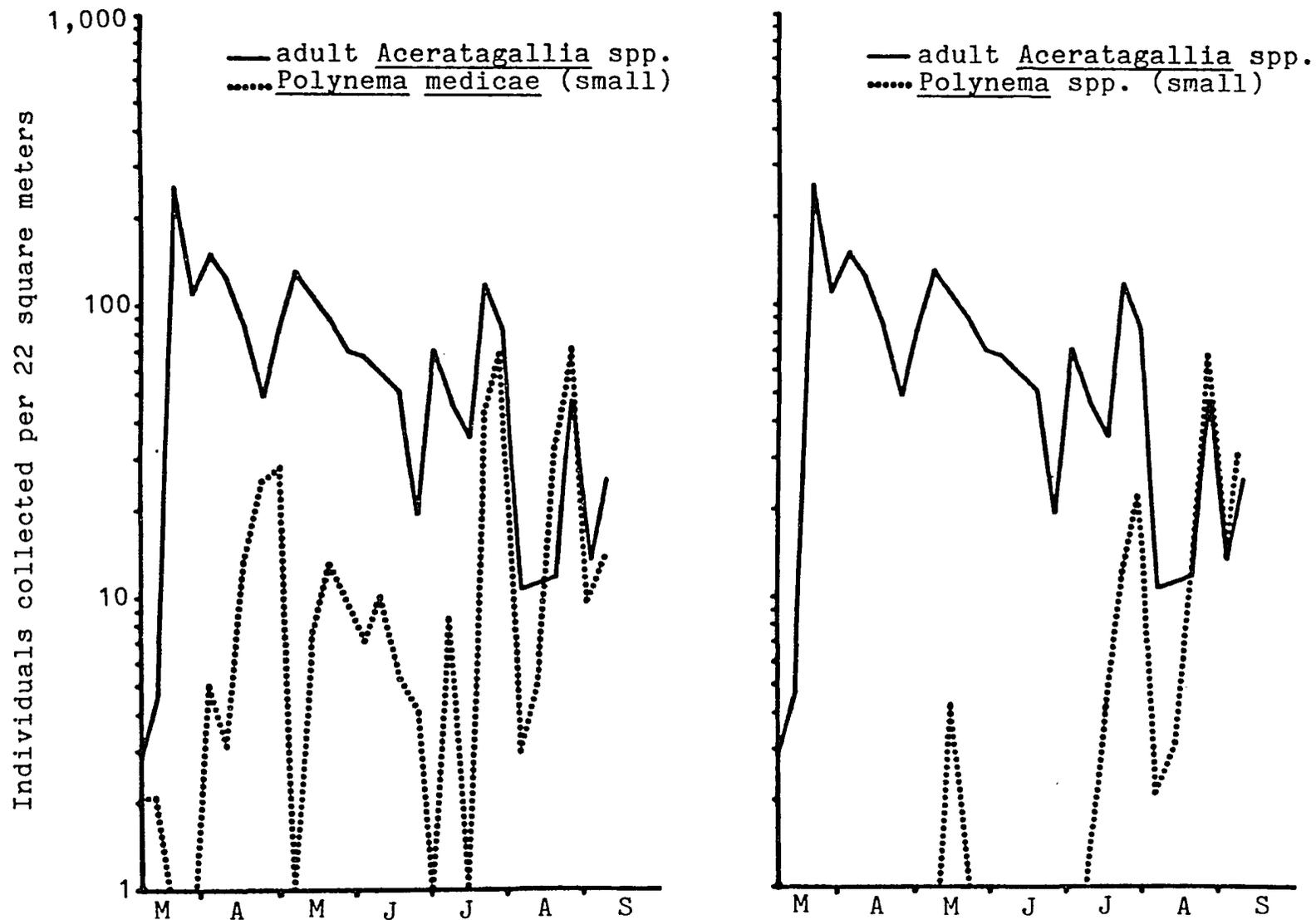


Figure 15. Adult Aceratagallia spp. leafhoppers and small adult Polynema spp. wasps collected by D-Vac from alfalfa fields near Tucson, Arizona. 1984

CONCLUSIONS

The hymenopterous family Mymaridae is well represented in southern Arizona crop-associated area, including at least 7 species in the genus Polynema. Identification of individual species within this group is difficult due to the size of the individuals and their morphological similarities. Girault's 1929 key lists 18 of the 25 species which are currently described for the U.S.; however, major characters which he uses to separate species are either subjective (number of rows of wing cilia) or may vary with seasonal change (color). Soyka's 1956 key for European Polynema relies heavily on measurements of individual wasps. I found that the ratios among the antennal segment lengths, and wing measurement ratios can differ significantly when individuals of the same species develop within different host species. New taxonomic characters for use with Polynema are discussed, and a key to members of the genus Polynema collected from southern Arizona is presented.

Host species have been reported in the literature for P. boreum and P. imitatrix as Nabis americanoferus and Spissistilus festinus, respectively. Laboratory determination of host species showed that P. medicae and P. species c and d all readily parasitizes S. festinus eggs. Also in

the laboratory, P. medicae and P. species c and e readily parasitized the eggs of leafhoppers of the genus Aceratagallia. When P. medicae and sp. c are alternated from one host species to another, a marked change in size occurs. Comparable size differentials of these two species, as well as P. imitatrix occur within the same field collections. These data lead to the inference that host alternation under sympatric conditions is a normal condition for these species. Eggs of another leafhopper, Ollarianus strictus were determined to be the host for P. sp. b.

Mean ovarian egg counts were determined for 5 Polynema species. When unexposed to host eggs, there was no significant difference between ovarian egg numbers of 1-day- and 17-day-old P. boreum females. Difference in host selection by P. medicae was shown to produce a significant difference in the mean number of ovarian eggs.

A significantly greater longevity occurred when a parasitoid was reared in a larger host, alfalfa hopper eggs, than when reared in a small host, leafhopper eggs. In most cases the longevity of the female was significantly longer than that of the male of the same species.

When developmental times between sexes within a species were analyzed by ANOVA, females exhibited a greater developmental time in 3 out of 7 of the cases. Host

differences produced significant differences in developmental time for P. medicae males but not for females.

Seasonal distribution of Polynema species in alfalfa fields was determined. The population growth curves for the threecornered alfalfa hopper egg parasitoid complex were analyzed for the effects of environmental factors such as mean temperature, humidity and rainfall, but no direct correlations could be made.

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