# THE BIONOMICS OF DIAPARSIS N. SP. (Hymenoptera: Ichneumonidae) A LARVAL PARASITOID OF THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.) (Coleoptera: Chrysomelidae)

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ABSTRACT

THE BIONOMICS OF DIAPARSIS N.SP. (Hymenoptera: Ichneumonidae) A LARVAL PARASITOID OF THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.) (Coleoptera: Chrysomelidae)

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Laboratory and/or field investigations of the biology and ecology of *Diaparsis* n.sp. (newly established in the U.S.) and *Diaparsis carinifer* (Thomson) (not established in the U.S.), parasitoids of the cereal leaf beetle, were carried out in Michigan and Yugoslavia from 1973-1975. Distinct differences in oviposition behavior between the two species were found. Longevity, fecundity, development, and identification characters of the immature parasitoids were determined. Field investigations in Michigan showed overwinter survival of *Diaparsis* n.sp. to be good. Emergence began in late May and continued for two to three weeks. Synchrony with the host was relatively good and parasitism rates were as high as 40%. Although some multiparasitism occurred, *Diaparsis* n.sp. appeared to mesh well between generations of *Tetrastichus julis* (Walker), a eulophid parasitoid of the cereal leaf beetle. Some indication was found that the presence of wild flowers as a source of food for adult parasitoids may be important in maximizing parasitization.

Field observations in Europe and the U.S. showed behavioral differences between European and U.S. populations of the cereal leaf beetle. The possible significance of this difference is discussed.

A number of possible strategies for management of *Diaparsis* n.sp. in an agricultural situation are suggested and discussed.

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# INTRODUCTION

The cereal leaf beetle, *Oulema melanopus* (L.), (Coleoptera: Chrysomelidae) is a native of Europe and Asia, ranging from Scandinavia to northern Africa and from the Atlantic coast and England to central Asia (Dysart, et. al., 1973). It is apparently an accidental introduction to the U.S. and was first identified in Berrien Co., Michigan in 1962 (Castro, et. al., 1965). Over most of its native range it is not of pest status or only sporadically a pest with the exception of the Balkan states where it is more frequently economically damaging. However, farmers in the U.S. were spraying for control of this pest as early as 1959 (Castro, et. al., 1965).

In 1963 a biological control program was begun by the U.S. Department of Agriculture with the initiation of field surveys in Europe. These were conducted by the European Parasite Laboratory to determine the presence and abundance of the CLB (=Cereal Leaf Beetle) and its natural enemies in Europe. Contracts were also made with Michigan State University and Purdue University to study the pest in the U.S., and two PL-480 projects were funded in Europe, one in Poland and one in Yugoslavia. In 1966 the Cereal Leaf

Beetle Parasite Rearing Station was established at Niles, Michigan for rearing and dissemination of CLB parasitoids in the U.S. (Dysart, et. al., 1973).

The result of the European work was the introduction of five parasitoids of the CLB into the U.S. One of these, Anaphes flavipes (Foerster), was a mymarid egg parasitoid, and the other four were larval parasitoids, Lemophagus curtus Townes, Tetrastichus julis (Walker), Diaparsis carinifer (Thomson), and Diaparsis n.sp. They were first released in southern Michigan and northern Indiana (Annon, 1972) and except for Diaparsis carinifer, all have been recorded as established /Stehr, (1970), Maltby, et. al., (1971), Stehr and Haynes, (1972), and Stehr, et. al., (1973)/.

Little other than host and general life cycle was known about any of these parasitoids prior to their establishment. Since then, Gage (1974) has studied the biology and population dynamics of T. julis which was the first larval parasitoid to build up a considerable population.

Diaparsis n.sp. populations subsequently began to increase also, which resulted in the decision to more thoroughly study the biology and bionomics of this parasitoid.

This research was part of a much larger study of the cereal leaf beetle, its dynamics, its behavior, and its parasitoids. The overall objective of the total research effort was to develop a management program for this pest.

The objective of this research was twofold; 1). to elucidate characteristics of the biology and bionomics of Diaparsis spp., 2). to provide data useful to the CLB management program.

The European work and much of the U.S. work was supported by two cooperative agreements.*

* Ent. Res. Div., Agr. Res. Serv., USDA cooperative agreements 12-14-100-10, 905 (33) and 12-14-1001-23.

# LITERATURE REVIEW

Various authors (Hodson, 1929, Hilterhaus, 1965, Venturi, 1942) have described the biology and behavior of Oulema melanopus in Europe. The natural history in Michigan has been described by Castro, et. al., (1965). Since the cereal leaf beetle has become a serious pest of small grains in the U.S., considerable study has been directed toward elucidation of its behavior, biology, and population dynamics. Wellso, et. al., (1970) have published a major bibliography on the CLB. Since then a number of additional papers dealing with various aspects of CLB behavior, biology, and dynamics have been published. They are listed in Table 1.

To simplify understanding the parasitoid behavior and relationship to the CLB, a review of the life cycle follows. The CLB is univoltine and the overwintering stage is the adult beetle. These emerge in the spring with peak abundance from mid-April to mid-May. Early populations are in wheat, but oats is attacked as soon as it is available. Immature stages consist of egg, four larval instars, prepupa and pupa. Pupation occurs in the soil and the adults emerge from mid-June to mid-July.

Table 1. Recent publications dealing with the cereal leaf beetle, Oulema melanopus.

| Topic | Author |
|---|---|
| Age specific mortality | Helgesen and Haynes, 1972 |
| Behavior and survival | Casagrande, 1975 |
| Host resistance | Gallun, et. al., 1973 |
| Host resistance | Webster, et. al., 1973 |
| Host resistance | Casagrande and Haynes, 1976 |
| Interactions with the host | Gage, 1972 |
| Interactions with the host | Jackman, 1976 |
| Laboratory oviposition studies | Wellso, et. al., 1973 & 1975 |
| Laboratory oviposition studies | Wellso and Cress, 1973 |
| Laboratory oviposition studies | Wellso, 1976 |
| Parasitoid relations | Gage, 1974 |
| Population management | Haynes, 1973 |
| Population management | Tummala, et. al., 1975 |
| Population monitoring | Ruesink and Haynes, 1973 |
| Population monitoring | Fulton, 1975 |

*Diaparsis* spp. are mentioned only briefly in the literature by European authors who were investigating the cereal leaf beetle (Venturi 1942, Hilterhaus 1965). Venturi (1942) reared a parasitoid he called *Thersilochus moderator* (L.) from *O. melanopus* in Italy and gave a brief description of the life cycle and host preference. Knechtel and Manolache (1936) also reared a *Thersilochus* sp. from *O. melanopus* in Rumania. Hilterhaus (1965) referred to a parasitoid reared from the same species by the name *Thersilochus carinifer* Thomson.

A description of the occurance and activity of these parasitoids in Europe under the name *D. carinifer*, was given by Dysart, et. al. (1973). Also Bjegović (1972, 1973) gave a similar report on these parasitoids in Yugoslavia, and Miczulski (1973) did the same for Poland.

A recent publication referring to *Diaparsis* spp. (Montomery and DeWitt, 1975) deals with the taxonomic separation of the larvae of parasitoids attacking the CLB.

Dysart, et al. (1973) outlined the collection of CLB parasitoids in Europe and their subsequent shipment to the U.S. Releases have been made in Michigan, Indiana, Ohio, W. Virginia, Virginia, New York, and Pennsylvania.

Through 1975, recoveries of *Diaparsis* n.sp. have been made in four counties in Michigan, three in Ohio, two in Indiana, and one each in W. Virginia, Pennsylvania, and New York. In several Michigan counties recoveries have been made at more than one site.

The primary host of these two *Diaparsis* species is *Oulema melanopus*. However, in Poland Miczulski (1973) reported finding a single dead male *Diaparsis carinifer* in a pupal cell of *Lema cyanella* (L.). He also reported having two *D. carinifer* emerge from host cells during the same season as they were collected. One of these emerged from *O. melanopus* and the other from *O. gallaeciana*.

At the time of European collection and subsequent release, it was not known that two species of *Diaparsis* were involved. Consequently all of these were released as *Diaparsis carinifer* and Stehr and Haynes (1972) reported the establishment of this species. Further work has shown that it was actually a new species, *Diaparsis* n.sp., which was established, and *D. carinifer* has not been recovered as yet.

## STUDY AREA

The research was primarily conducted in three locations from 1973-1975. The laboratory work was done at Michigan State University, and the field work was done near Niles, Michigan. Three months were spent in Europe in 1974, primarily in Yugoslavia, for the collection and rearing of parasitized CLB larvae in order to obtain material for laboratory work. Various other investigations including parasitoid flight activity, parasitoid and host density, and observations on the behavior of parasitoid and host were also conducted.

The work at Niles was done at the CLB field insectary in cooperation with the USDA APHIS CLB Parasite Rearing Station there. The field insectary is located west of Niles near Galien, Michigan and is managed for maximum production of CLB parasitoids. The management scheme is essentially the same as that outlined for the MSU, Gull Lake Research Station (Gage, 1974).

The insectary is divided into halves, with one half tilled and planted to crops one year and the other used the following year. This leaves the crop stubble undisturbed for one full year to allow maximum parasitoid survival and emergence.

In order to have an extended season for CLB oviposition and development, two crops are planted each year. The first is winter wheat which is planted in a single strip about five acres in size. The second crop is spring oats which is planted at three dates beginning as early as possible and continued at weekly intervals with each planting comprising about two acres. (Figure 1). Hereafter the oat crops will be refered to as Oats-1, Oats-2, or Oats-3 from the earliest to the latest respectively.

Since it is believed that most emigration of parasitoids is along the north edge of the insectary, an additional strip of oats (trap crop) is planted here to help retain the parasitoids in the insectary.

The topography varies considerably with most of the area being rolling, well drained loamy soil. Approximately the southern one third, however, is flat and low lying with poorly drained muck soil.

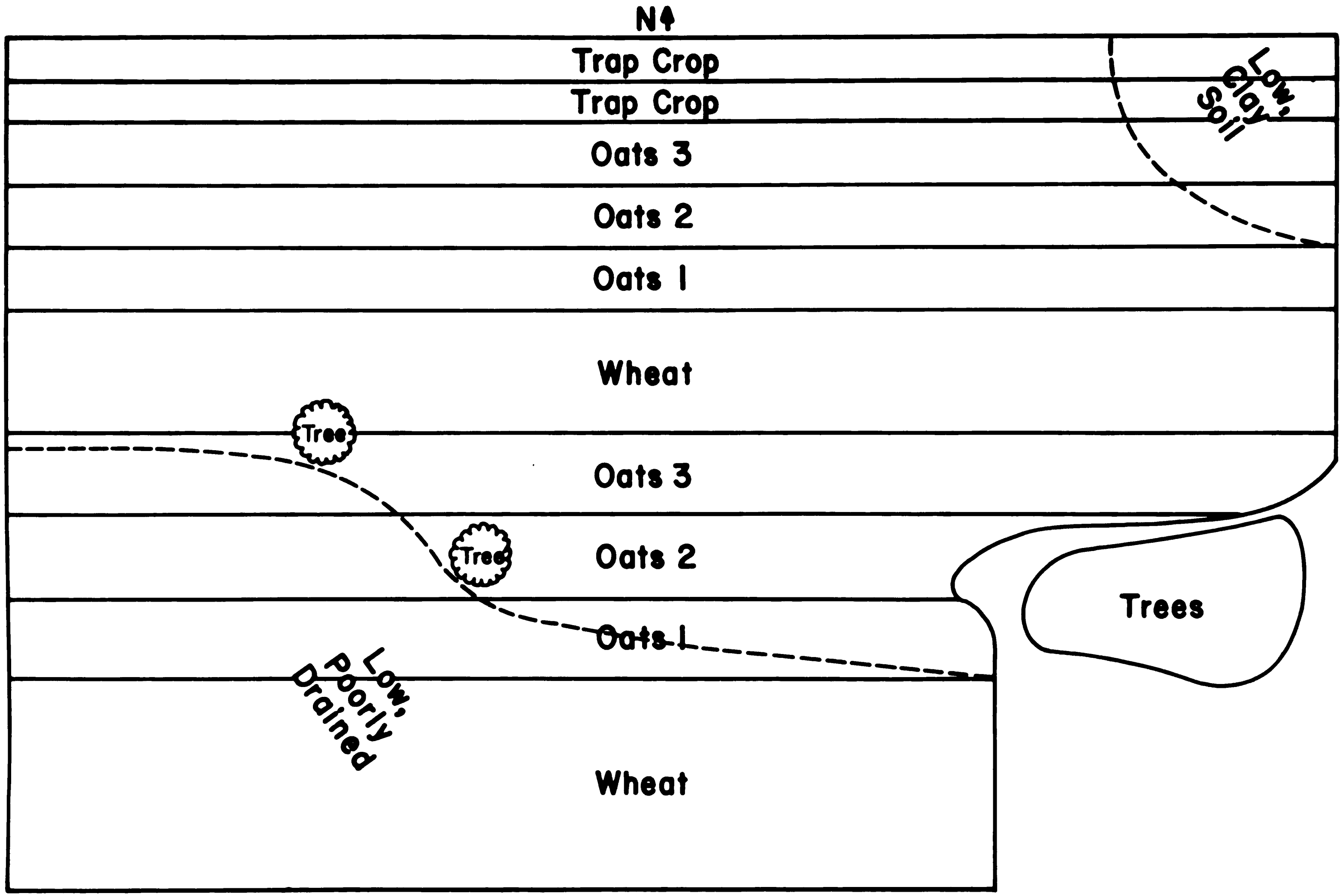



Figure 1. Diagram of the Niles insectary showing crop locations and topography.

## TAXONOMY

The parasitoids investigated were originally placed in the genus *Thersilochus*. However, in a revision of the European Tersilochinae, Horstman (1971) placed them in the genus *Diaparsis*.

Initially the imported specimens were thought to all be *D. carinifer*. Further examination by the USDA Systematics Entomology Laboratory in Washington, D. C., and consultation with Dr. Horstmann of the Institute für Angewandte Zoologie, Würzburg, Germany, revealed that two species of parasitoids were involved. One was *Diaparsis carinifer* (Thomson) and the other a new species, referred to hereafter as *Diaparsis* n.sp.

Also from these specimens, R. W. Carlson was able to establish a tentative distribution of the two species in Europe. In a letter (1971) to Horstmann he indicated that the distribution of *Diaparsis* n.sp. appears to be limited to lower Austria, France and Yugoslavia. *D. carinifer*, however, apparently occurs over all of Europe.

Failure to recover *D. carinifer* in the U.S. is noteworthy, particularly since it is probable that both species were released. According to reports from the USDA

(Anon, 1972) more than 60,000 Diaparsis spp. adults from Yugoslavia were released in the U.S. Since both species are known to occur in Yugoslavia, both species should have been included in the U.S. releases.

Both species have typical ichneumonid, parasitoid life cycles consisting of egg, several instars, prepupa, pupa and adult. They are univoltine and overwinter as prepupae in cocoons within the CLB cells in the soil.

The two species are nearly impossible to separate in the field except perhaps by oviposition behavior. In the laboratory, however, it is relatively easy to distinguish them since several characteristics are distinctly different. In an unpublished key for recognizing Diaparsis spp. parasitic on Oulema melanopus, Carlson (1972) described the characters in the following couplet.

> "Lower portion of the occipital rim (the outer margin of the occiput, usually and inappropriately referred to as a carina) scarcely visible in lateral view. Temples polished and distinctly punctate, if somewhat shagreened in males, then still with a distinct luster. Abdominal tergites occasionally mostly furruginous, but often largely black (more so in males)
>
> Diaparsis n.sp."

"Lower portion of occipital rim easily visible in lateral view. Temples strongly shagreened, dull and only obscurely punctate. Abdominal tergites usually not extensively darkened except at the abdominal apex of some males.

*Diaparsis carinifer*"

In addition to the characters outlined above (Figures 2a and 2b) a consistent difference in the ovipositors was observed (Figure 3). The ovipositor of *Diaparsis* n.sp. is slender and definitely curved upward whereas *D. carinifer* has a somewhat stouter ovipositor, slightly broader, and with considerably less curvature. This difference was found to be reliable for separation of live females of the two species in the laboratory. Since the ovipositor of *D. carinifer* has less curvature, it projects slightly farther beyond the abdomen. *Diaparsis* n.sp. also tends to have more black coloration on the dorsal aspect of the abdomen than *D. carinifer*.

Since the laboratory work required identification of the parasitoids prior to their use, it was necessary to find a way to positively identify the live material. To do this the parasitoid and a small piece of cotton were placed in the lower half of a 5 cm plastic petri dish which was upside down on a note card. Since the parasitoid tended to walk upside down on the inside of the petri dish, it was carefully picked up and placed over the cotton.

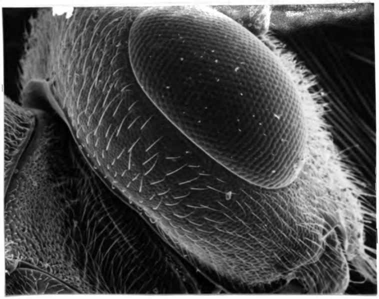
This held the parasitoid tightly enough to allow examination of the key characters under a dissecting microscope.

Diaparsis n.sp.
50X

Diaparsis carinifer
50X

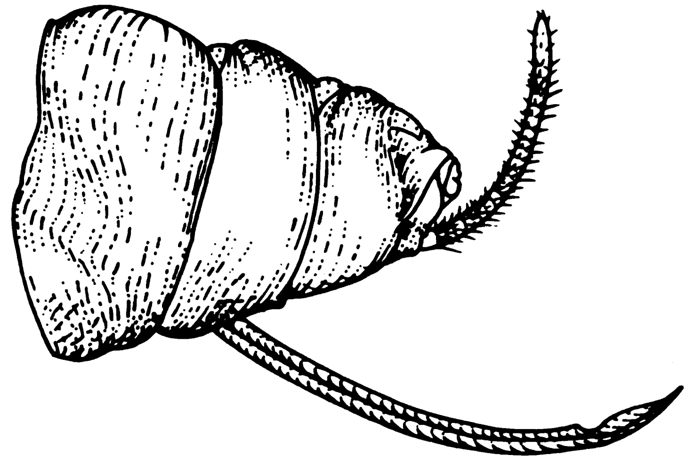
Figure 2a. Scanning electron microscope photographs showing the difference in the occipital rim between *Diaparsis* n.sp. and *D. carinifer*.

Diaparsis n.sp.
100X

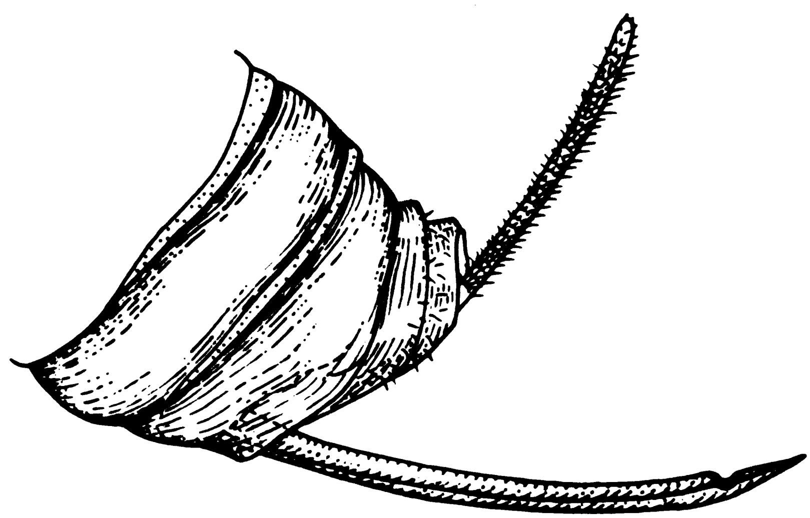


Diaparsis carinifer
100X

Figure 2b. Scanning electron microscope photographs showing the difference in the texture of the temples between Diaparsis n.sp. and Diaparsis carinifer.



Diaparsis n.sp.



Diaparsis carinifer

Figure 3. Terminal abdominal segments and ovipositors of Diaparsis n.sp. and D. carinifer. (Note curvature of n.sp.)

# LABORATORY BIOLOGY

## Oviposition behavior

### Methods

Oviposition behavior was observed in the laboratory by placing a single parasitoid in the lower half of a plastic 5 cm petri dish which was upside down on a note card under a dissecting microscope.

Host larvae were introduced on leaves of greenhouse grown barley seedlings. A leaf with a larva on it was broken off and slipped under the edge of the petri dish. The larvae were of various sizes; however, mostly second and third instars were used and generally only one larva was presented at a time. To avoid disturbing the parasitoids a piece of heavy paper was taped around the dish to shield hand movements.

### Results and Discussion

Observation of oviposition revealed a number of characteristics of *Diaparsis* spp. In the situation described above, the parasitoid appeared to walk randomly around the dish until it contacted the leaf. It then began to search along the leaf, tapping the surface constantly with the antennae. If the leaf was contacted in the middle and the initial search for the host was in the wrong direction, the parasitoid turned and searched the entire length of the leaf until the host was located.

If an area of host feeding was contacted, the parasitoid became more excited and investigated the area thoroughly with its antennae.

It was found that the plant juices occuring where the leaf was broken off elicited a more intense searching behavior from the parasitoid. In several cases it elicited a brief oviposition response, with the ovipositor being exserted and a few probes being made.

Stimulation to oviposit was received primarily from the host larval fecal coat. Contact with this caused the parasitoid to exsert the ovipositor and probe repeatedly for the host body even when the fecal coat was not on the larva.

Several larvae with little or no fecal coat were presented to the parasitoids which were slow to accept them or rejected them altogether. In one case where D. carinifer rejected such a host, some fecal material was added from another host and it was stung immediately.

Most larvae were accepted immediately, regardless of size; however, some were refused by the parasitoid for no apparent reason. In one case six larvae were rejected by a D. carinifer, but when they were cleaned of their fecal coat and dabbed with fecal material from other larvae they were accepted immediately.

Since Diaparsis spp. are solitary parasitoids, only one egg was normally deposited each time the ovipositor was inserted. In a few instances two eggs were deposited in the same puncture, and in several cases the eggs passed down the ovipositor simultaneously.

Generally larvae were parasitized only once, but occasionally a female oviposited twice in one host. If the parasitoid left the leaf after oviposition, and subsequently relocated it, she did not search along it. In cases where two or more larvae were close together, normally only one was stung.

Only the very tip of the ovipositor was inserted and this apparently stimulated release of the egg. Once this was accomplished, oviposition was completed regardless of circumstances unless the puncture was made for feeding purposes. During one oviposition attempt, a parasitoid accidentally placed its mesotarsus in the mandibles of the larva which immediately fastened on it. Following oviposition, the parasitoid struggled to free itself. In struggling it pushed with its ovipositor and accidentally punctured the host integument. Struggling ceased immediately and an egg was deposited. This occurred twice before she was able to free herself.

Observation of oviposition led to the discovery that sometimes Diaparsis spp. feed on host larval fluid. This was observed in _Diaparsis_ n.sp. more frequently than in _D. carinifer_. Parasitoids were observed to insert the ovipositor tip, generally into the abdomen, and wiggle it back and forth laterally until a drop of haemolymph oozed out. They then lowered their mouthparts to it and ingested for several minutes. This often occurred with one of the first larvae presented and was then followed by a period of oviposition.

Several significant differences in oviposition behavior between the two species were noted. One of these was the location of oviposition. _Diaparsis_ n.sp. nearly always oviposited in the cervical or gular region of the larva. This was generally accomplished by orienting parallel to the larva and probing along it with the ovipositor until the head capsule was located, whereupon oviposition took place immediately behind the head capsule. The role of the host head capsule became apparent when a parasitoid was observed probing normally along a host which had a head capsule and larval skin from the previous instar stuck to its side. The ovipositor happened to contact this and oviposition occurred immediately at this point. This was observed on two separate occasions.

In contrast, _D. carinifer_ was much less specific about the location of oviposition. When the parasitoid located a larva it exserted the ovipositor and attacked rapidly with a violent, stabbing ovipositor motion. No particular location was selected for oviposition; it occurred where the ovipositor first was inserted. Consequently it occurred most often in the abdominal region.

In order to analyze this difference more closely, records were kept of the location of a number of ovipositions of each species. The host larvae were divided into three regions - cervical, thoracic, and abdominal. The results are shown in Table 2.

The time required for oviposition also appeared to differ in the two species, so the ovipositions of a number of individuals of each species were timed with a stop watch to see how much difference existed. The timed period was from the time the parasitoid antennae contacted the host until the ovipositor was withdrawn (Table 3).

The data resulting from this test were analyzed with a two-level nested analysis of variance for unequal sample sizes. (Sokal and Rohlf, 1969). This analysis tested for differences among wasps within a species as well as for a difference between the species. Since the wasps used did not all oviposit the same number of times it was necessary to use a test allowing for unequal sample sizes.

Table 2. Location of oviposition in *Oulema melanopus* larvae by *Diaparsis* n.sp. and *D. carinifer*.

| | Total | Cervical | Thoracic | Abdominal |
|---|---|---|---|---|
| | | *Diaparsis* n.sp. | | |
| Number | 185 | 155 | 25 | 5 |
| Percent | | 84 | 14 | 2 |
| | | *Diaparsis carinifer* | | |
| Number | 151 | 2 | 50 | 99 |
| Percent | | 1 | 33 | 66 |

Table 3. Time of oviposition in seconds of <u>Diaparsis</u> spp. from point of antennal contact until ovipositor withdrawal.

| Parasite # | Time for each larva |
|---|---|
| | <u>Diaparsis</u> n.sp. |
| 1 | 30 45 61 32 28 |
| 2 | 26 25 26 80 30 20 30 24 27 42 57 40 28 58 28 |
| 3 | 29 27 27 30 27 48 43 29 37 |
| 4 | 60 22 30 55 37 31 55 27 32 40 |
| 5 | 51 54 59 34 45 33 47 54 40 88 54 35 40 49 42 57 49 43 40 |
| 6 | 26 20 31 34 29 34 23 26 28 |
| 7 | 31 31 30 32 44 56 32 51 57 40 38 63 54 33 |
| 8 | 20 25 21 41 30 24 |
| 9 | 62 42 31 35 37 59 19 22 17 23 37 17 31 19 29 16 72 23 19 23 39 17 15 26 38 |
| | 30 19 35 19 39 39 58 36 21 42 51 21 22 |
| | <u>Diaparsis carinifer</u> |
| 1 | 23 12 16 37 20 13 13 15 12 13 13 13 10 16 15 13 17 12 11 17 17 14 19 12 9 |
| | 11 11 14 12 18 17 9 |
| 2 | 20 15 18 16 17 18 15 18 14 17 16 20 18 |
| 3 | 33 23 20 26 17 18 18 14 17 17 18 17 |
| 4 | 20 28 31 24 22 23 25 28 28 22 33 |
| 5 | 18 18 13 22 15 25 15 18 21 |
| 6 | 17 16 15 17 15 19 20 19 18 |
| 7 | 17 28 20 19 27 45 24 |

The results of this analysis* indicate that a significant difference in oviposition time exists at the .01 level both within each species and between the species.

The difference in ovipositor morphology between the two species (Figure 3) may be related to the variation in oviposition behavior discussed above, D. carinifer being more adapted to thrusting quickly and Diaparsis n.sp. more adapted to careful selection of a particular location on the larva.

Post oviposition behavior was the same in both species. When the ovipositor was withdrawn, the head was lowered toward the surface of the larva with the mandibles widely spread. It was held there briefly, sometimes just barely touching the fecal coat but usually slightly above it. Following this the parasitoid walked a short distance away and cleaned itself thoroughly.

The host larva usually exhibited some reaction to the parasitoid. As soon as the antennae tapped the larva it raised its head from the leaf surface bending back considerably. This occurred more frequently in third and fourth larval instars than in first and second. Gage (1974) reported that the reaction of CLB larvae to approach

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*ANOVA Table

| Source of variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Species | 1 | 18,410.53 | 18,410.53 | 21.07** |
| Among Wasps | 14 | 6,484.87 | 463.21 | 3.92** |
| Within Wasps | 202 | 23,896.67 | 118.3 | |
| Total | 217 | 48,792.07 | | |

by Tetrastichus julis is to raise the abdomen. This was never observed to occur with the contact of Diaparsis spp.

In the field it was noted that this defense reaction resulted in the larva falling from the leaf surface. This occurred almost invariably in larger larvae - fourth instar and late third - and the result was avoidance of parasitization.

## Prediapause Development

### Methods

To examine development of Diaparsis n.sp. from egg to prepupa, laboratory-parasitized host larvae were placed on barley seedlings grown in pots and held at room temperature. Dissections were then made at daily intervals and parasitoid eggs and larvae were preserved in 70% ETOH.

### Results and Discussion

The results showed that development of prediapause Diaparsis n.sp. larvae was quite variable making it difficult to determine the duration of each developmental stage. Figure 4 shows an approximation of the frequency of several stages and their duration.

Diaparsis spp. lay two types of eggs. One is a smooth, typical, ichneumonid egg and the other more common type has a lateral projection on one side which is referred to as a knob. D. carinifer lays only knobbed eggs whereas Diaparsis n.sp. lays both types. In the latter species the

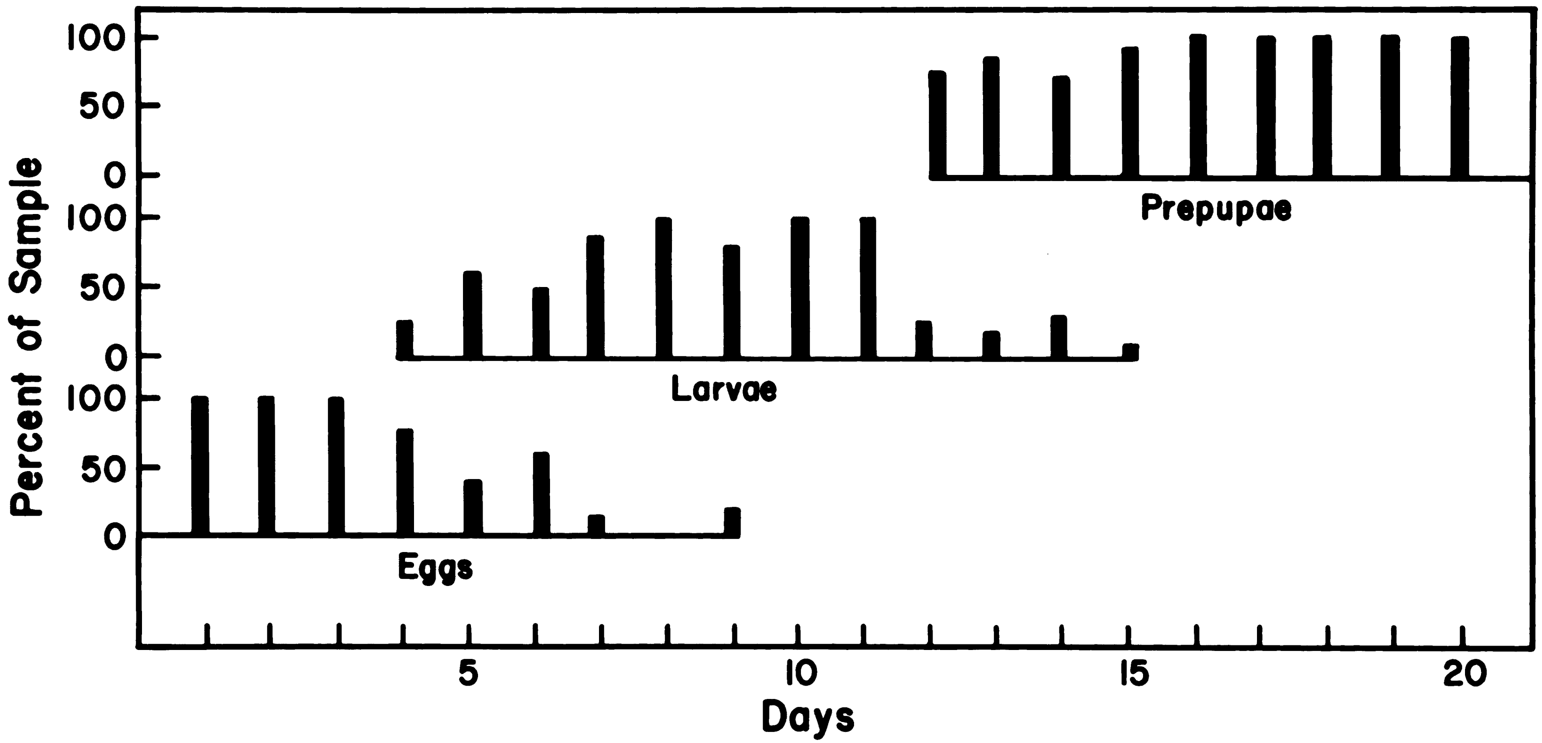



Figure 4. Duration and frequency of prediapause life stages of Diaparsis n.sp. under laboratory conditions.

knobs are at times only partially developed (Montgomery and DeWitt, 1975).

The knob, which is flattened on top, apparently functions to attach the egg to host tissue. Most often this is the inside of the host integument, but occasionally it may attach to other tissues.

The adhesive nature of this structure became evident in dissections of female parasitoids which had been preserved and dissected in alcohol. In dissection of eggs from the ovaries, it was found that lightly touching the flat side of the knob with the dissecting needle caused it to adhere firmly to the needle.

The eggs hatch in from four to seven days with two changes becoming visible prior to hatching. The first change is the appearance of a highly visible, dense, very white spot in the middle of the egg, and the second change is the appearance of the larva within the egg.

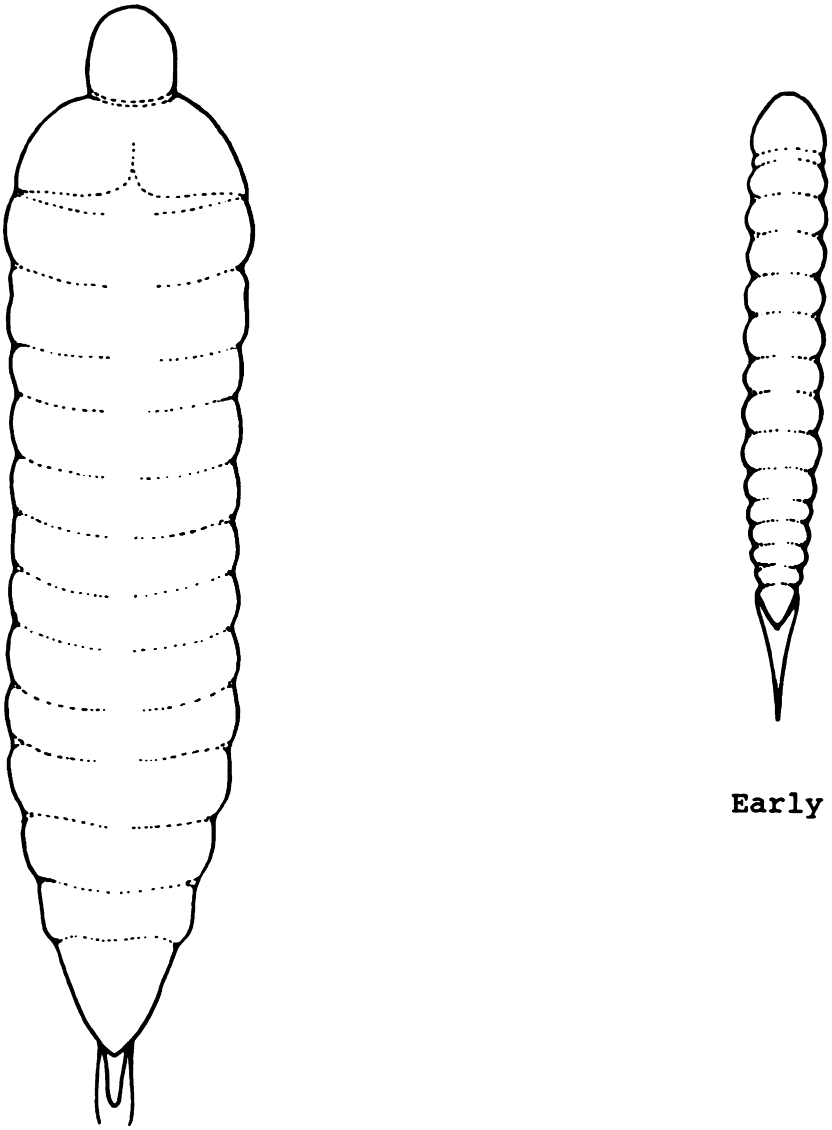
The larvae pass through several instars in their development. The first and last instars are recognizable by a sclerotized head capsule and well defined mouthparts, but the intermediate instars lack these making it very difficult to separate them. The changes in appearance are discussed by Montgomery and DeWitt (1975).

One developmental characteristic of Diaparsis n.sp. was that it goes through only one instar before the host larva spins its cocoon. This became apparent when only first instar parasitoid larvae were found in field dissections. Late first instars may reach considerable size before molting, whereas early first instars are relatively slender, the body being the same diameter as the head capsule (Figure 5).

This characteristic, coupled with the fact that *Diaparsis* n.sp. females parasitize any instar host larva, seems to account for the variation in development of individual larvae. Since development is not completed until after the host spins its cocoon, an egg deposited in a first instar host obviously takes longer to develop to the prepupal stage than one which is deposited in a third or fourth instar.

When the parasitoid reaches the prepupal stage, it exits from the host skin and spins its cocoon inside the host cocoon. This is a three-layered structure with the outer layer of fibrous brown silk, the middle layer a smooth, tough material, and the inner a thin, transparent membrane. A cream colored band encircles the middle of the center layer and is visible externally. One end of the outer layer has more extraneous silk and is fuzzy in appearance (Figure 6). Inside the cocoon the head of the larva is always oriented in this direction.

The development of *D. carinifer* is assumed to be the same as that of *Diaparsis* n.sp. The same study outlined above was also attempted with *D. carinifer*, but only a few parasitoid larvae were obtained. Of 81 dissections of CLB



Late

Early

Figure 5. Drawings showing relative size of early and late first instar Diaparsis n.sp. larvae.

Figure 6. Diaparsis n.sp. cocoons.

larvae known to be stung by *D. carinifer*, only seven (8.6%) were found to contain parasitoids and five of these were encapsulated.

The reason for the lack of parasitoids in hosts which were known to be stung by *D. carinifer* is not clear but two hypotheses are suggested, both of which likely contribute to these findings.

The first is that encapsulation may interfere with locating the parasitoids in dissections. As pointed out above, five of seven found were encapsulated which indicated that encapsulation was frequent. If the eggs were encapsulated while they are attached to the epidermis, it would have been easy to miss them in the dissections, particularly if they were melanized or reduced in size.

Examination of cast host skins showed that some eggs were lost at the time of molting, remaining attached during the molting process. Whether or not this occurs depends on the stage of host development at the time of oviposition. If the egg is placed into the haemocoele of the host, as is normal, it would not be lost at molt since it would be inside the epidermis. However, if the host has secreted a new cuticle from which the old cuticle has separated, it may be that the ovipositor does not penetrate the epidermis. The result would be deposition of the egg between the old and new cuticle which is possible with either *Diaparsis* species since they normally insert only the very tip of the ovipositor.

The first point discussed above, encapsulation, probably is the main cause of unsuccessful parasitism by D. carinifer and also probably accounts for the lack of establishment of this species in the U.S.

## Post-diapause Development

### Methods

Post-diapause development of Diaparsis n.sp. was also studied in the laboratory using cocoons obtained by rearing field collected CLB larvae. Following emergence of adult beetles, the remaining cells were held at room temperature for 12 weeks and were then placed in 40$^{\circ}$F for an additional 12 weeks. They were then held at room temperature for parasitoid development. During this entire time the cells were sprayed with water periodically to prevent dehydration.

Development was examined by dissection of cocoons at daily intervals after their removal from diapause temperatures.

### Results

Development could essentially be divided into eight easily recognizable stages. These were 1) thoracic constriction, 2) appearance of eyespots, 3) voiding of the meconium, 4) pupation, 5) eyes dark, 6) thorax dark, 7) abdomen dark, and 8) adult emergence. These various stages grade into one another to some extent except for pupation and emergence which are more definite stages in development.

When the eyes first appear they are pale red, but they become progressively darker, and following pupation they become very deep red. The body of the parasitoid also goes through several color changes. The prepupal larvae are a grey color mottled with white from fat granules showing through the integument. As they near pupation they become cream colored. After pupation they remain this color until about the time the eyes darken. The rest of the body then becomes a semi-translucent white, which gradually darkens until it is the adult color. Figure 7 shows several of the stages of development.

In addition to the development stages, Figure 7 also illustrates the variable development rate of *Diaparsis* n.sp. The three specimens shown were all subjected to the same conditions. Although the bulk of the population develops at the same rate, there is a great deal of individual variation. Records received from the Newark, Delaware USDA lab on emergence of European-collected *Diaparsis* n.sp. to be used in laboratory studies, also showed great variability. The earliest emergence was a male which emerged in 14 days and the latest was two females which emerged in 55 days.

With such great variation, it is difficult to characterize a population and conclude that a given stage of development covers a definite period of time. Figure 8 shows an approximation of the duration and frequency of several of the stages discussed above.

Figure 7. Post-diapause development of Diaparsis n.sp. All three specimens were out of cold temperatures for 14 days.

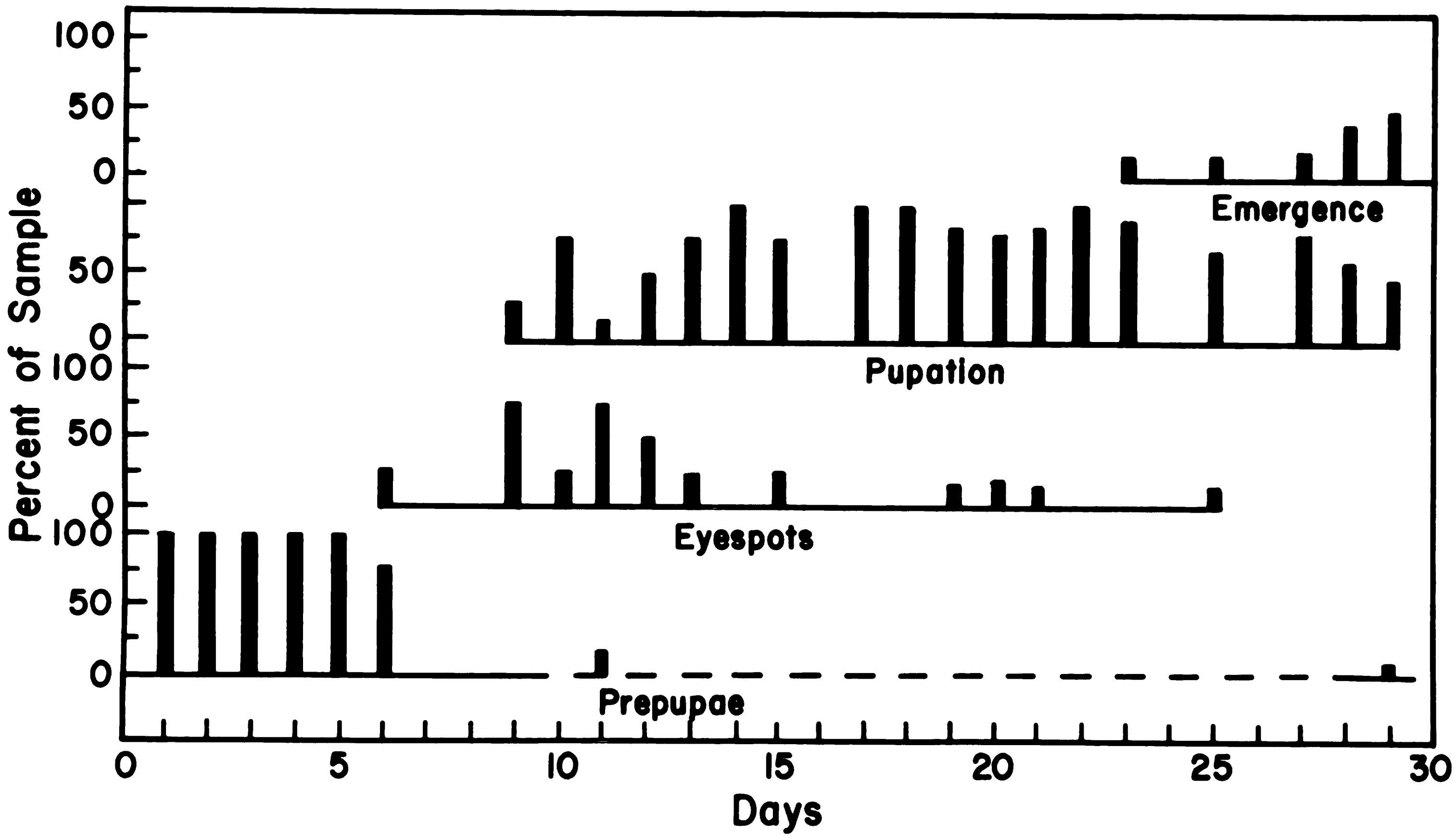



Figure 8. Duration and frequency of several post-diapause development stages of *Diaparsis* n.sp. under laboratory conditions.

In some of the dissections late in the development period, larvae were found which had apparently not undergone any development but were still viable. It has recently been found (Montgomery, pers. comm.) that some *Diaparsis* n.sp. go through two seasons of diapause prior to emergence. This apparently is the explanation for the lack of development found in some of the dissections. For this reason the prepupal stage in Figure 8 is represented as open ended.

It was also found that prepupal larvae can be sexed. This was reported by Wilson and Ridgeway (1975) for *Campoletis sonorensis*, an inchneumonid parasitoid of *Heliothis virescens*. In *C. sonorensis* the sex is apparent in the fourth instar, the female being distinguished by having several ventral ovoid depressions in segments 10, 11 and 12, and the male having only one in segment 12.

However, in *Diaparsis* n.sp. sex differences cannot be distinguished prior to the prepupal stage at which time they appear in the terminal segments (Figure 9). The male structures are simply a pair of small round bodies. The female structures are rod-shaped, with four of them lying side-by-side, and with the anterior pair having the distal ends bent sharply laterally. These characters are most easily visible in preserved specimens and seem to be more

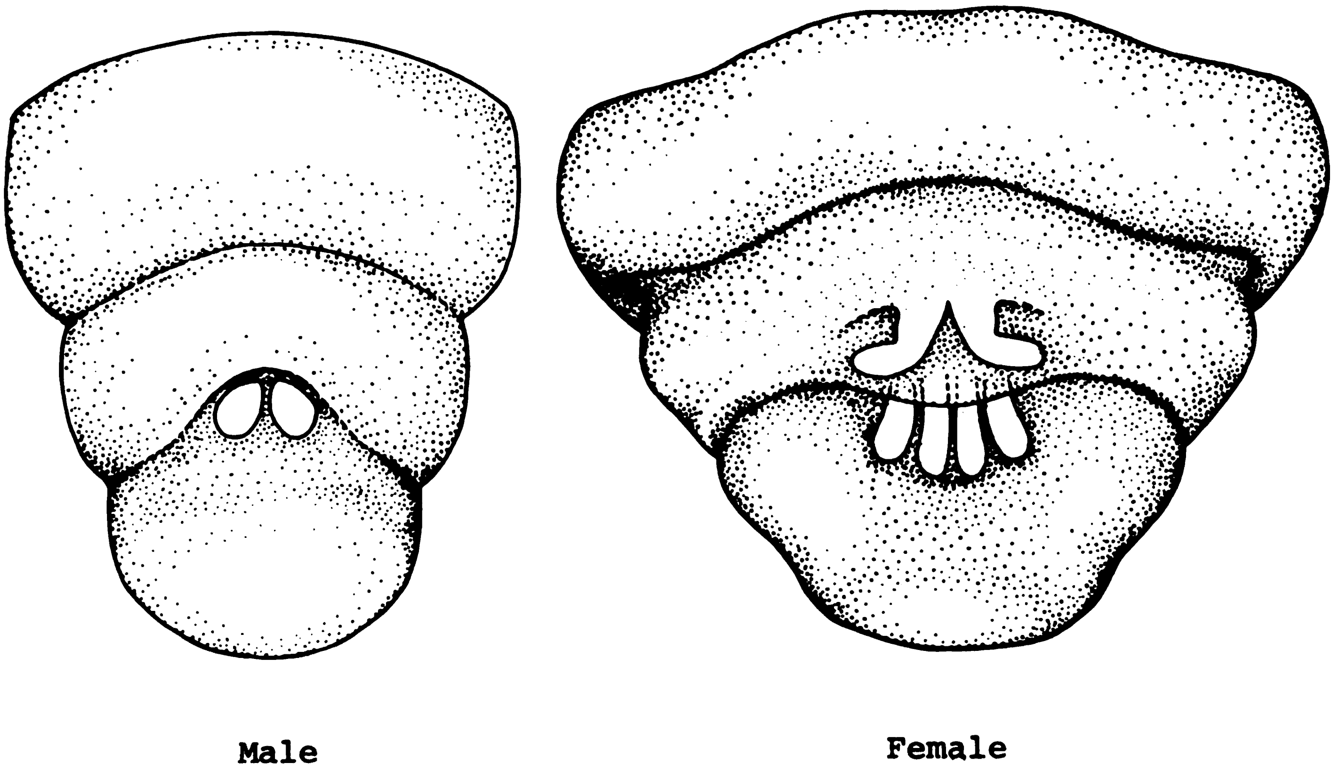



Figure 9. Terminal segments of prepupal male and female larvae of Diaparsis n.sp. showing primordia of external sex characters.

apparent in larvae preserved in FAA[+] than in those preserved in 70% ethyl alcohol.

## Longevity and Fecundity

### Methods

To examine longevity and fecundity a single female parasitoid and four host larvae on barley leaves were placed in small, covered, plastic dishes ca. 6 cm dia x 3 cm deep (Figure 10). A hole cut into the bottom of the dish and covered with fine mesh copper screen provided ventilation. A water drop was placed inside and honey was streaked on the screen. The dish was kept coverside down in a 16 hr. photoperiod.

Larvae were changed every 24 hours except in several instances when this was not feasible and they were left for 48 hours. The larvae removed from the cages were either dissected immediately or preserved in FAA for later dissection.

Since larval instars were estimated visually when they were placed in the cage, head capsules were measured at dissection to accurately determine the instars present. The number of eggs in each larva was counted, and the tests were continued until the parasitoids died. Both *Diaparsis* n.sp. and *D. carinifer* were tested.

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[+]FAA is a larval preservative with the following composition: 50 parts $H_2O$, 47 parts 95% ETOH, 2 parts formalin, 1 part glacial acetic acid.

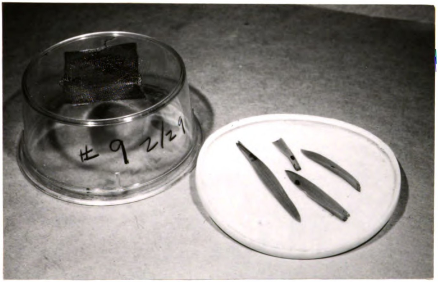



Figure 10. Chamber used for longevity and fecundity tests.

## Results and Discussion

### Longevity

Longevity of the parasitoids was calculated from the date of emergence until death. This was not the same as the number of days they were exposed to hosts since some parasitoids were held from one to several days before they were tested.

The tests on 14 females of each species showed that the two species live nearly the same length of time in the lab. The figures for Diaparsis n.sp. longevity are $\bar{x}$ = 11.4 ± 5.0 days, Range = 3-18 and for D. carinifer $\bar{x}$ = 11.3 ± 2.7 days, Range = 5-15. Figure 11 shows a frequency distribution of this data.

The field longevity of Diaparsis spp. may be longer. An approximation can be obtained by comparing the final emergence trap catch with the final malaise trap catch which will be discussed later. The last parasitoid caught in a malaise trap was 18 days after the last parasitoid emerged in an emergence trap. This, of course, can not be construed to be an accurate measure of longevity, however, it is probably considerably closer than the laboratory data. The physical constraints of a small unnatural environment and being handled daily may have affected the length of life in the laboratory.

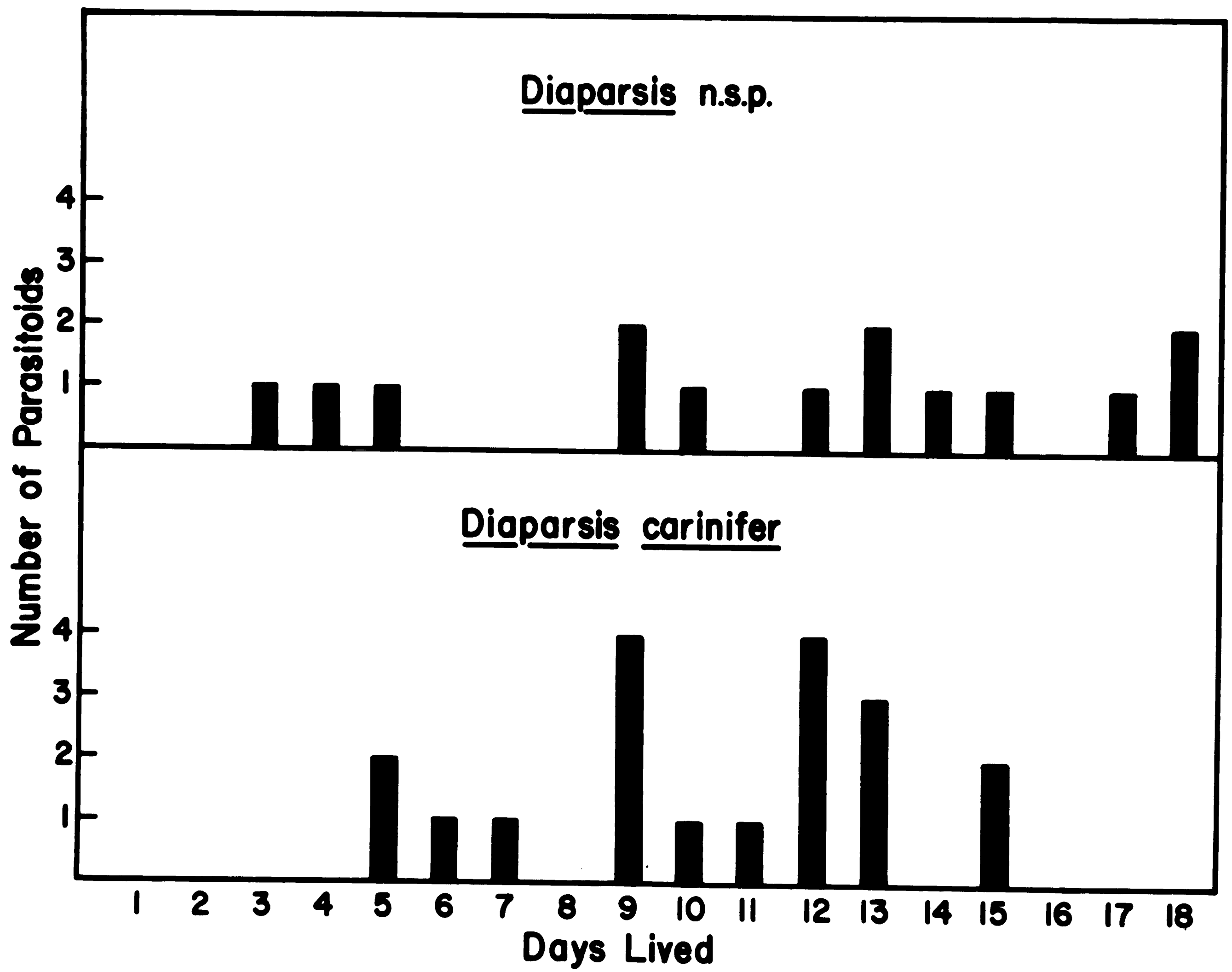



Figure 11. Frequency distribution of longevity of _Diaparsis_ n.sp. and _D. carinifer_ under laboratory conditions.

## Fecundity

Fecundity of the two species was also obtained from these tests. Although the results obtained for *D. carinifer* appeared to be reasonably accurate, those for *Diaparsis* n.sp. appeared much lower than they should have been. Consequently ten females of each species were dissected and the eggs in the ovaries were counted. These females had been fed water and honey but had not had access to hosts and only the apparently mature eggs in the ovaries were counted. The results are given in Table 4.

In *D. carinifer* the mean from the dissections compares favorably with that found in the test. The mean for *Diaparsis* n.sp., however, was much higher in the dissections than in the test and is likely much nearer to the true fecundity.

The reason for the low numbers of eggs deposited by *Diaparsis* n.sp. in the lab test may be due to one or both of the following reasons. First, it may be more sensitive to the constraints of a small, unnatural environment which did not allow normal behavior. The second reason may be that it has a stronger defense against superparasitism than *D. carinifer*. Any defense the latter may have against this apparently broke down completely since in the host dissections it was not uncommon to find more than 20 eggs in a single larva. One first instar contained 26 eggs and a third instar contained 31. In contrast, most parasitized

Table 4. Fecundity of *Diaparsis* n.sp. and *D. carinifer* determined from laboratory tests and dissections.

| | | *D. carinifer* | *Diaparsis* n.sp. |
|---|---|---|---|
| | $\bar{x}$ eggs/female | 131.1 | 29.9 |
| Tests | SD | 76.8 | 25.3 |
| | Range | 24-291 | 4-70 |
| | N | 14 | 14 |
| | $\bar{x}$ eggs/female | 143.8 | 185.0 |
| Dissections | SD | 20.4 | 20.7 |
| | Range | 119-186 | 131-202 |
| | N | 10 | 10 |

larvae from the test with Diaparsis n.sp. contained only one or two eggs although one second instar contained 25. This, however, was an exception.

## Mating Behavior

Mating of Diaparsis spp. was observed a number of times in the laboratory. The specimens used for mating experiments were isolated prior to emergence so they were unmated prior to the observations. Both species were observed and no differences in behavior were noted.

Apparently a pheromone is operative in mating of these parasitoids, but it did not appear to be a strong attractant. Males became excited only after contacting a female or walking across a spot where the female had been. If the female had left the area, the male continued to search but did not seem to be able to orient toward the female.

When a male contacted a female he became obviously stimulated. Movement became more rapid, and the antennae were used to search for further contact with the female. The wings were held up over the back at an angle and buzzed in rapid, short bursts and the abdomen was lowered and curled forward. Coupling occured in a typical male-above position and lasted from 30-45 seconds.

Once a male released the female he did not mate immediately again, but if other males were present, they often mated with the female immediately.

If the female was receptive, she mated readily; however, some were found to be unreceptive. These struggled and usually succeeded in dislodging the male by kicking with the hind legs. Often the females struggled initially but then submitted to mating.

Since two closely related species were being studied, a number of attempts to cross mate them were made. *Diaparsis* n.sp. males and *D. carinifer* females mated readily, but it is not known if these matings were successful since progeny were not obtained due to encapsulation which has been discussed earlier.

The reciprocal cross, *Diaparsis* n.sp. females with *D. carinifer* males, did not take place. The males attempted to mate repeatedly, but the females would not submit. The females kicked with their back legs and also exserted the ovipositor to prevent coupling by the males. This latter behavior was never observed except in this cross mating attempt. The same females, when placed with *Diaparsis* n.sp. males, mated immediately.

## Identification of Immature Parasitoids

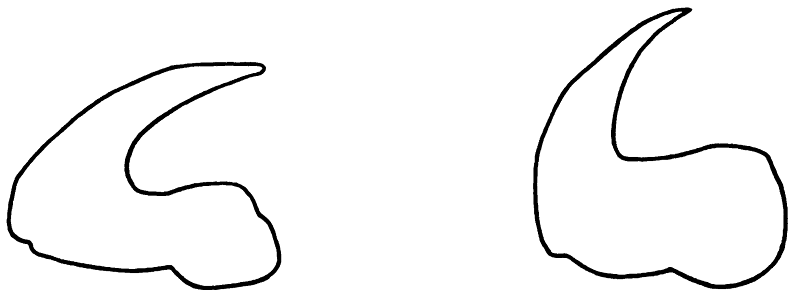
One of the objectives of this research was to find a way to distinguish between the two species of immature parasitoids in order to be able to identify the species present in field dissections. Since both *Diaparsis* n.sp. and *D. carinifer* remain as first instars until the host spins its cocoon, this is the most important stage to differentiate.

Therefore, first larval instars preserved in 70% alcohol, were cleared in 10% KOH for approximately one hour and then rinsed in distilled water and mounted in glycerin on microscope slides for examination.

A definitive character for their separation has not yet been found, however, it appears that the mandibles may supply this character. To examine the mandibles it is necessary to place pressure on the coverslip to help orient the mandibles in the proper plane, since they project somewhat ventrally from the head of the larva.

The two types of mandibles observed in this study are shown in Figure 12. Since only five positively identified _D. carinifer_ were available, the conclusions presented here are only tentative.

The mandibles of both species are sickle-shaped, and have a similar general appearance. However, one significant difference is the curvature on the inner aspect of the mandible. In _Diaparsis_ n.sp. this is a sharp curve which forms a nearly parallel sided U. In _D. carinifer_ the inner curve is much more open with the sides divergent.



<u>Diaparsis</u> n.sp. <u>Diaparsis</u> <u>carinifer</u>

Figure 12. Drawings showing difference between mandibles of first instar larvae of <u>Diaparsis</u> n.sp. and <u>D</u>. <u>carinifer</u>.

# FIELD BIOLOGY

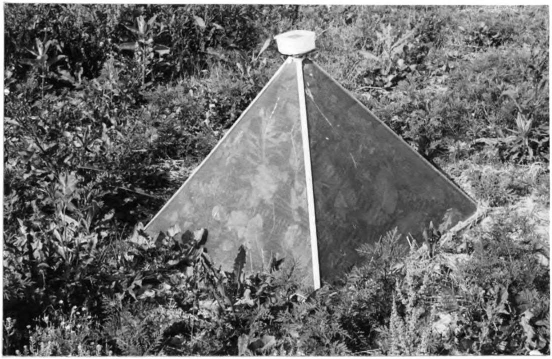
## Emergence

### Methods

Emergence of overwintering Diaparsis n.sp. was monitored by the use of emergence traps (Gage, 1974) covering one $yd^2$ at the base. (Figure 13). The bottom of the container at the top of the trap was filled with ethylene glycol which killed the trapped parasitoids and preserved them for removal and examination.

The traps were set up at a density of three per crop strip except in 1975 when 15 traps were placed in second oats and seven in each of the other strips (Figure 14). The 15 traps in second oats were placed adjacent to the ½ $yd^2$ plots where samples for the overwinter study had been taken. Thus the same traps served for evaluating normal spring emergence and overwinter mortality.

The extra four traps in each of the other strips were set up as a check on the trapping of previous years. However, no difference in the emergence curves for the three years was found which could be attributed to better data obtained from a greater trap density.



(a)

(b)

Figure 13. Photographs showing (a) emergence trap set up in stubble field and (b) part of catch in the container at the top of the trap.

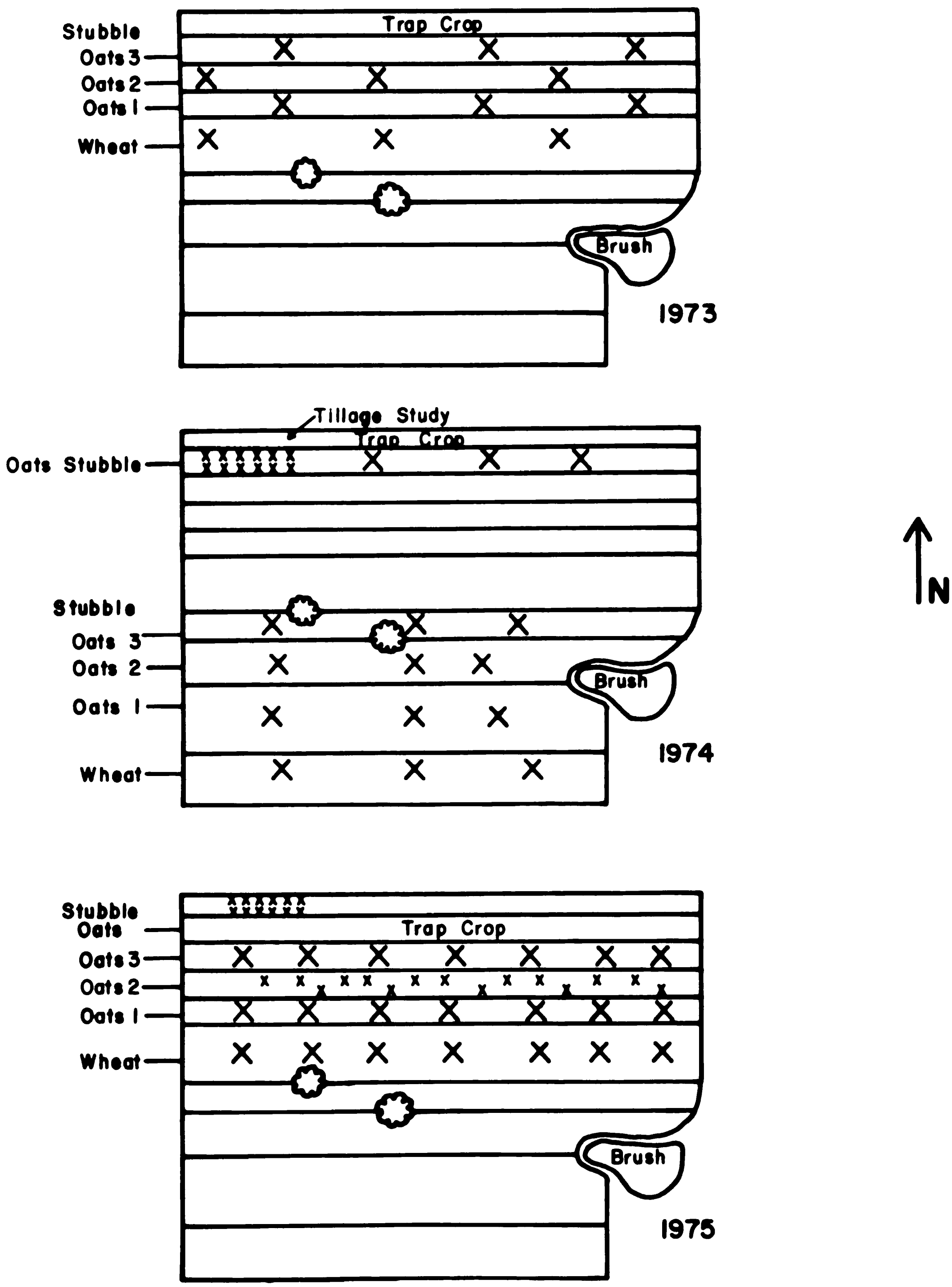



Figure 14. Diagram of Niles insectary showing layout of crops and location of emergence traps (1973-1975). X = emergence trap.

The traps were checked daily over the entire period of emergence and one week beyond emergence of the last parasitoid. Each day the parasitoids were removed from the traps and taken to the laboratory for positive identification.

To determine if Diaparsis n.sp. was able to survive and emerge from a field which was plowed and planted, a small section of trap crop stubble was plowed, tilled, and planted to oats in the spring of 1974 and 1975.

A series of six emergence cages was placed in the plowed strip immediately after planting and six control traps were placed in the untilled stubble parallel to the first six. These were monitored daily with the emergence traps in the rest of the insectary. However, the data from these traps were not used in calculation of the emergence curves.

Air temperatures were used in calculating the degree days for plotting the emergence curves. Although soil temperatures would be more accurate for predicting emergence, it was not feasible to record them.

Since there is no weather station at Niles which records temperature data, this was taken from two other stations, Dowagiac, which is 14 miles NNE of Niles, and South Bend, Ind. which is eight miles south of Niles. The daily maximum and minimum temperatures from each station were averaged and the means were used as the maximum and minimum temperatures for Niles.

The degree days were calculated using the formula

$$^{o}D = \frac{\text{max} + \text{min}}{2} - \text{Base Temp.}$$

for all days when the minimum temperature was above the base temperature. If the minimum temperature was below the base, the Baskerville-Emin (1969) sine curve method was used.

In a laboratory study Gage (1974) determined the developmental threshold temperature of Tetrastichus julis to be 50°F. Similar attempts to determine this temperature for Diaparsis n.sp. were unsuccessful. Consequently an alternate method was used to obtain an approximation. This was a comparison of the variances of heat accumulation for different degree day bases at the time of initial emergence (Figure 15). The fluctuation in variance is probably due to the fact that only three years data were available for analysis. Although 49°F was the point of least variance, 48° was used in the analysis in order to facilitate comparison with Gage's (1974) work on T. julis which uses 48° as a base temperature. Also the variances at the 48 and 49 degree day bases were not significantly different at the .05 level.

## Results and Discussion

Emergence trap catches are summarized by crop strip in Table 5. The data from this trapping program were analyzed using a two level analysis of variance for unequal

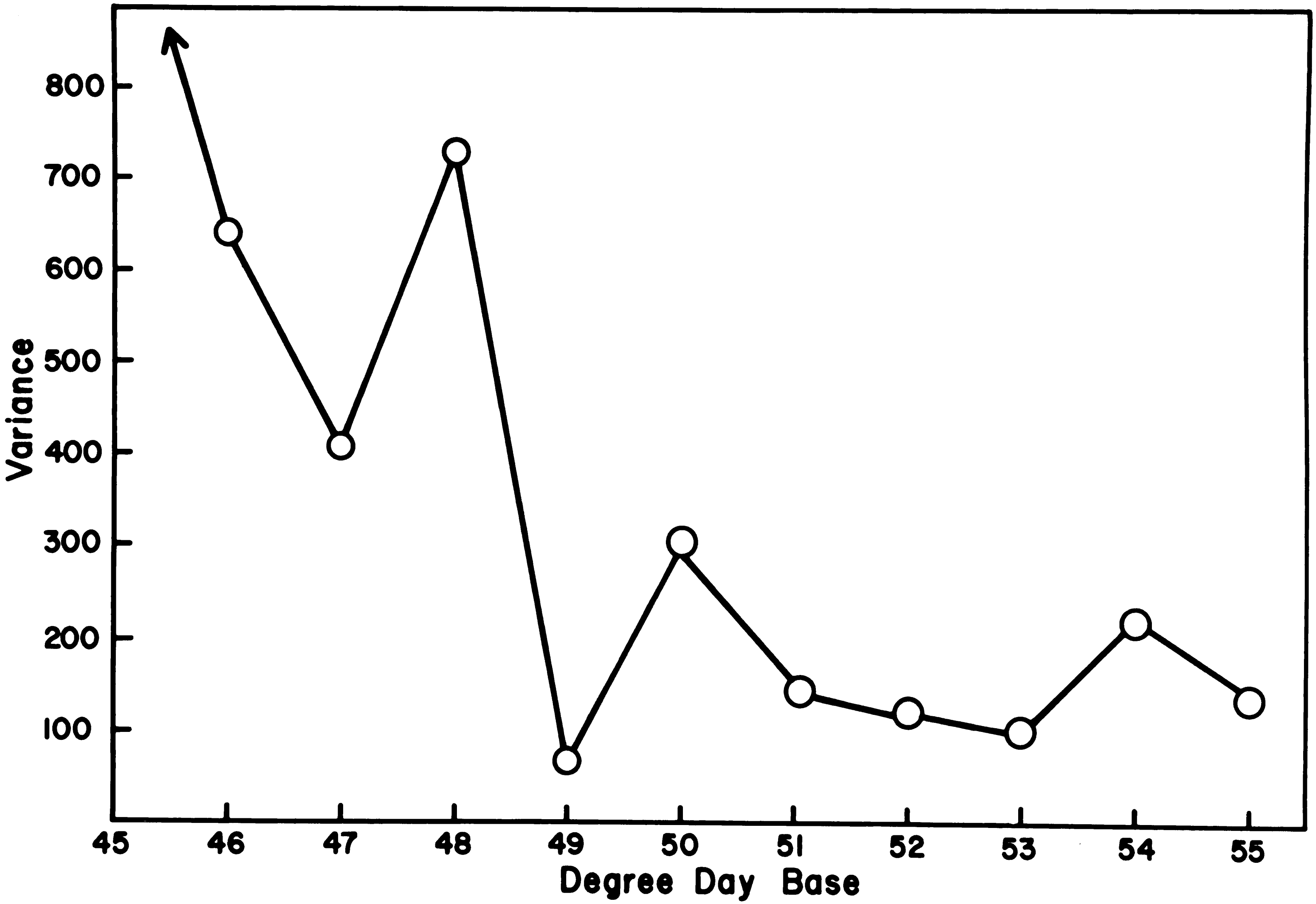



Figure 15. Variance of degree days accumulated at various day bases at initial emergence date of Diaparsis n.sp. at Niles, Michigan (1973-1975).

Table 5. Emergence trap catch of *Diaparsis* n.sp. at Niles, Michigan (1973-1975).

| Year | Wheat | Oats-1 | Oats-2 | Oats-3 | Trap Crop |
|---|---|---|---|---|---|
| <u>1973</u> | | | | | |
| Total | 1 | 9 | 28 | 26 | |
| $\bar{x}$/Trap | .33 | 3.0 | 9.33 | 8.67 | |
| <u>1974</u> | | | | | |
| Total | 25 | 15 | 8 | 12 | 44 |
| $\bar{x}$/Trap | 8.33 | 5.0 | 2.67 | 4.0 | 14.67 |
| <u>1975</u> | | | | | |
| Total | 2 | 118 | 408 | 68 | |
| $\bar{x}$/Trap | .29 | 16.86 | 27.20 | 9.71 | |

sample sizes (Sokal and Rohlf, 1969). The results* indicate that a significant difference in emergence at $F_{.05}$ occurred between the years and also between the strips. Initially a three level analysis was used considering parasitoid sex as the third level. However no differences were found so this was combined with the error term to increase the degrees of freedom.

The emergence curves shown in Figures 16-22 were fitted to the data using probit analysis. The probit equations for these curves are listed in Table 6.

The curves in Figures 16-18 show that males emerge consistently earlier than females. Although considerable overlap occurs, a given percent emergence occurs from three to five days earlier in males than in females.

Sex ratio for each year was analyzed using a Chi square analysis resulting in the values 1.503, .154, and 12.722 for 1973, 1974, and 1975 respectively. This indicates a significant difference in 1975 only, when the number of females was greatest.

According to Flanders (1939, 1946) and Clausen (1939, 1940) a number of factors influence egg fertilization and thus sex ratio in parasitoids. These include host size,

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*ANOVA Table

| Source | | df | SS | MS | $F_s$ |
|---|---|---|---|---|---|
| $\bar{Y}_a - \bar{\bar{Y}}$ | Years | 2 | 1,664.495 | 832.248 | 4.270* |
| $\bar{Y}_b - \bar{Y}_a$ | Strips | 10 | 4,330.372 | 433.037 | 4.997*** |
| $Y - \bar{Y}_b$ | Error | 113 | 9,793.117 | 86.665 | |
| $\bar{Y} - \bar{\bar{Y}}$ | Total | 125 | 15,787.984 | | |

Table 6. Probit equations for cumulative percent emergence of Diaparsis n.sp. plotted over $^{\circ}D_{48}$.

| Curve | Equation | Chi sq. | df |
|---|---|---|---|
| 1973 Males | Ep = -5.359 + .0192X | 1.10972 | 14 |
| 1973 Females | Ep = -3.002 + .0138X | .50029 | 17 |
| 1974 Males | Ep = -7.291 + .0231X | .09615 | 13 |
| 1974 Females | Ep = -5.124 + .0169X | .21977 | 17 |
| 1975 Males | Ep = -5.970 + .0216X | 5.46107 | 16 |
| 1975 Females | Ep = -6.795 + .0218X | 1.55005 | 12 |
| 1973 Total | Ep = -3.765 + .0158X | .7128 | 18 |
| 1974 Total | Ep = -4.006 + .0158X | .3617 | 17 |
| 1975 Total | Ep = -5.722 + .0202X | 12.8316 | 16 |

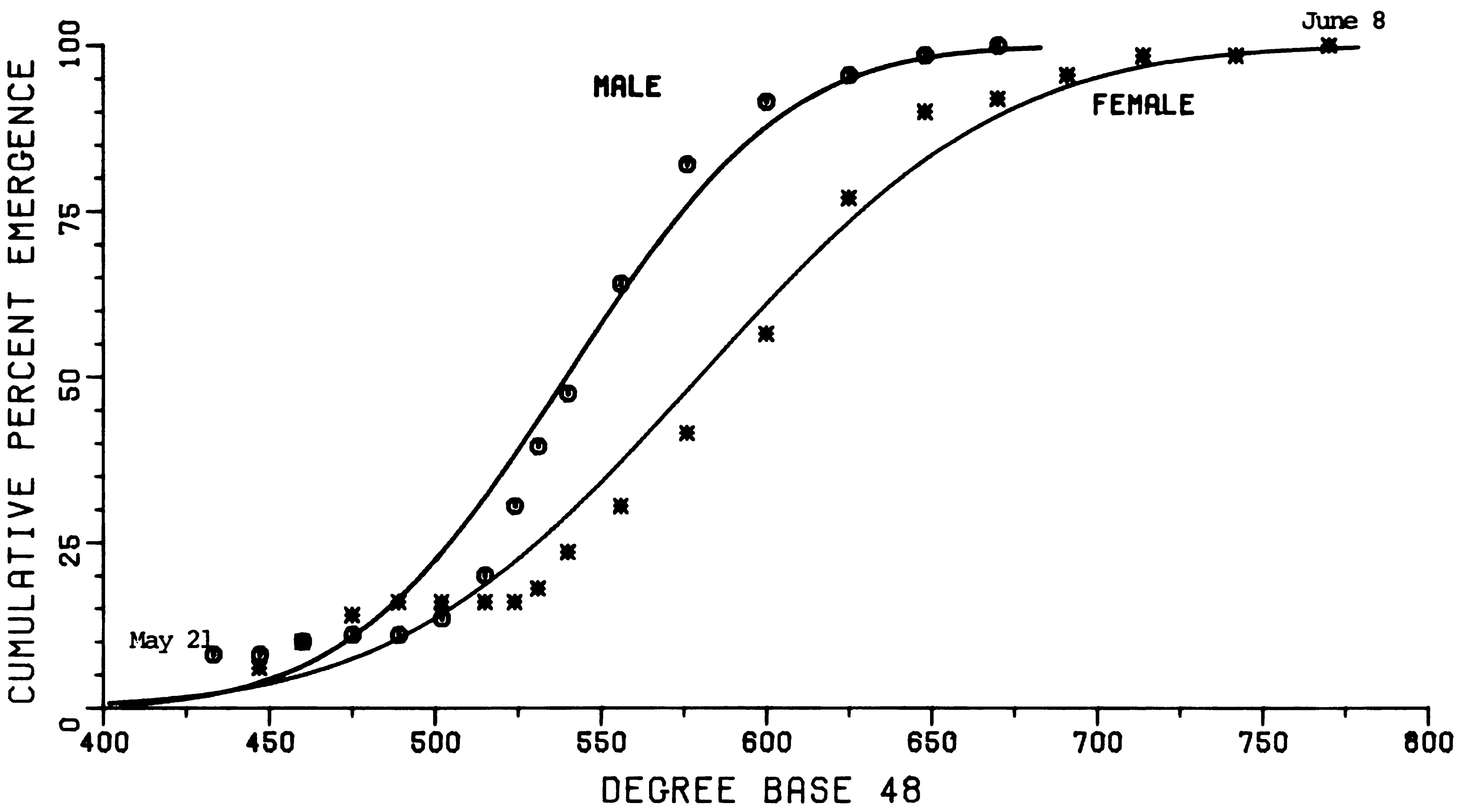



FIGURE 16. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPARSIS N.SP. AT NILES, MICHIGAN. 1973

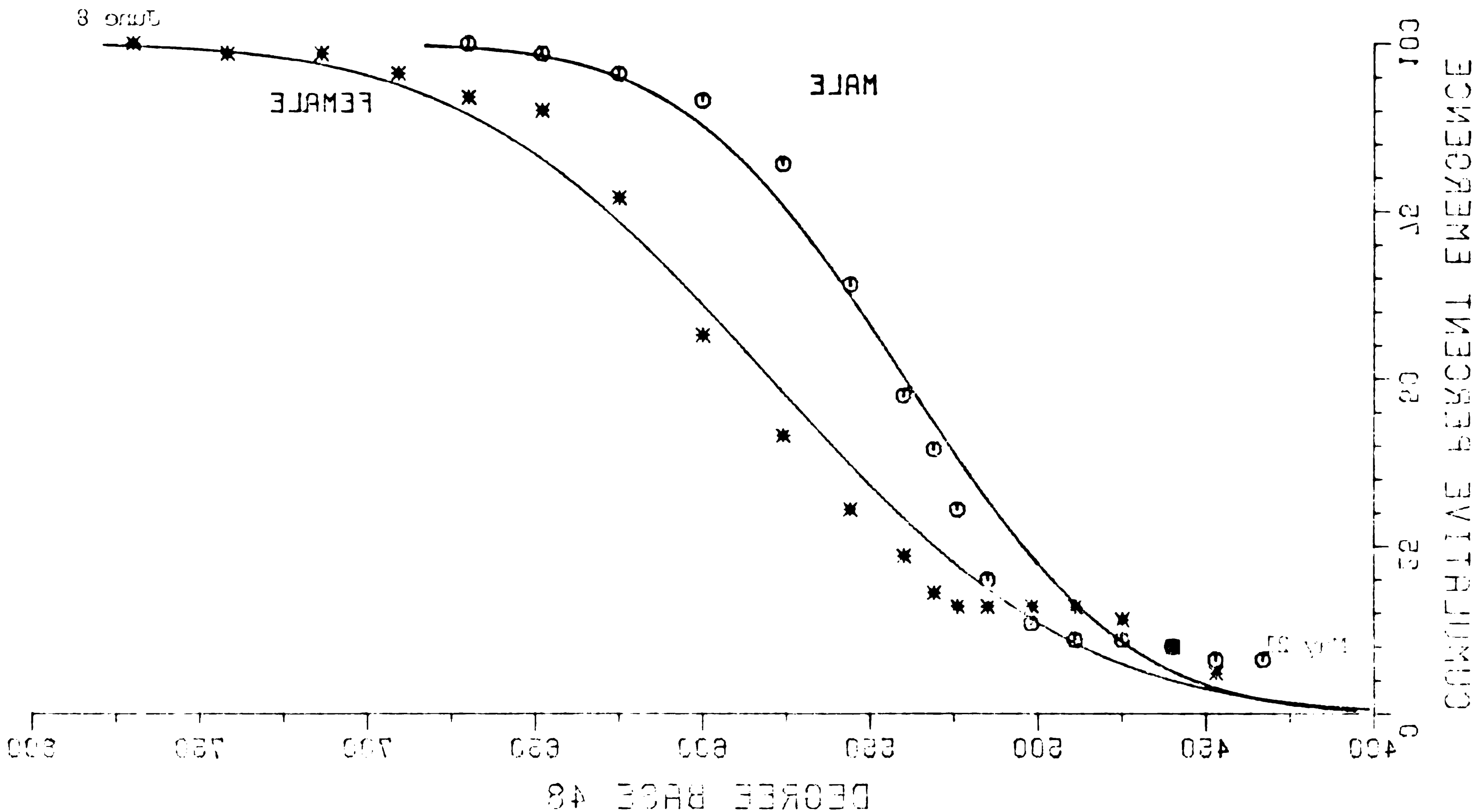



FIGURE 16. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPARSIS N.SP.
AT NILES, MICHIGAN. 1973

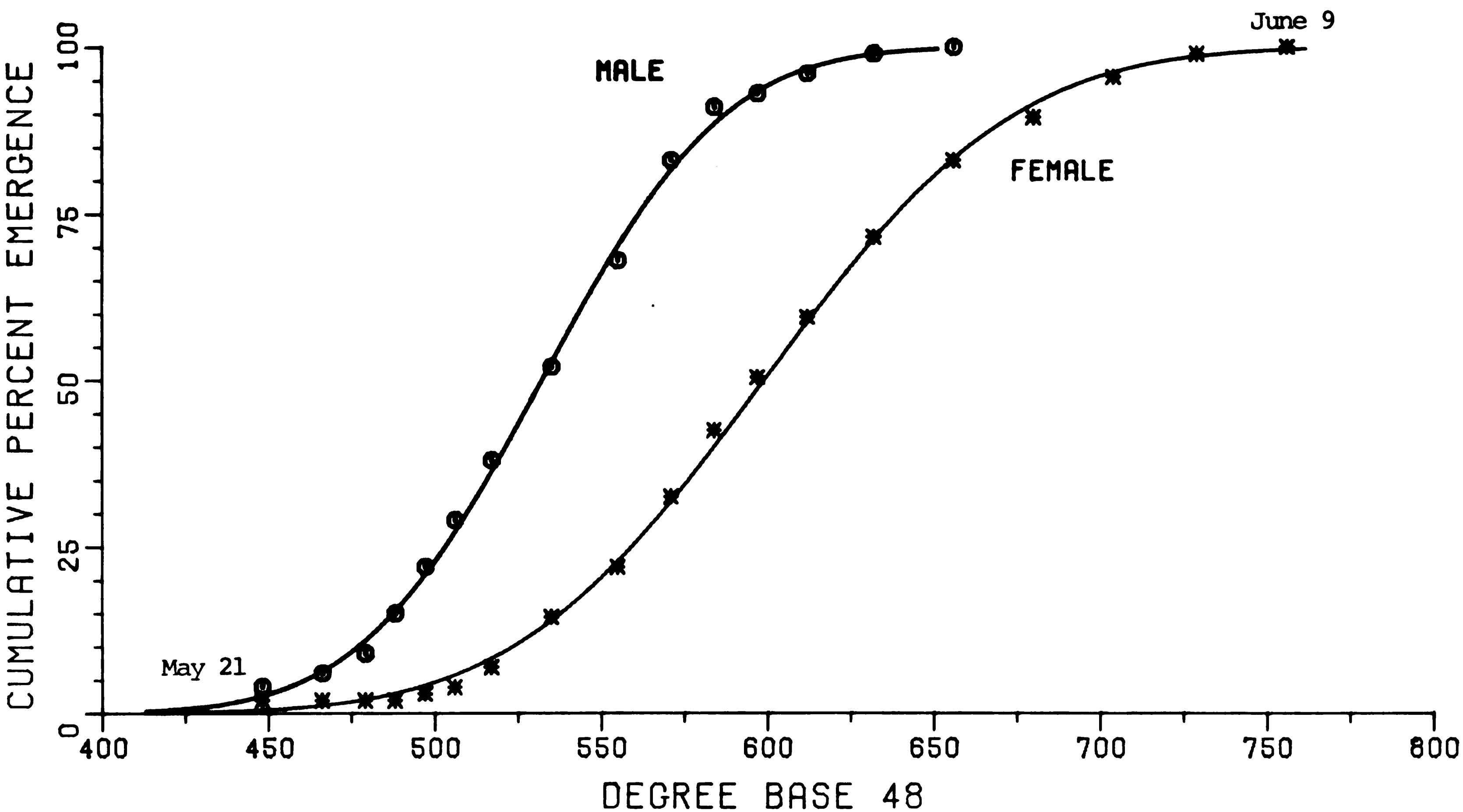



FIGURE 17. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPARSIS N. SP. AT NILES, MICHIGAN. 1974

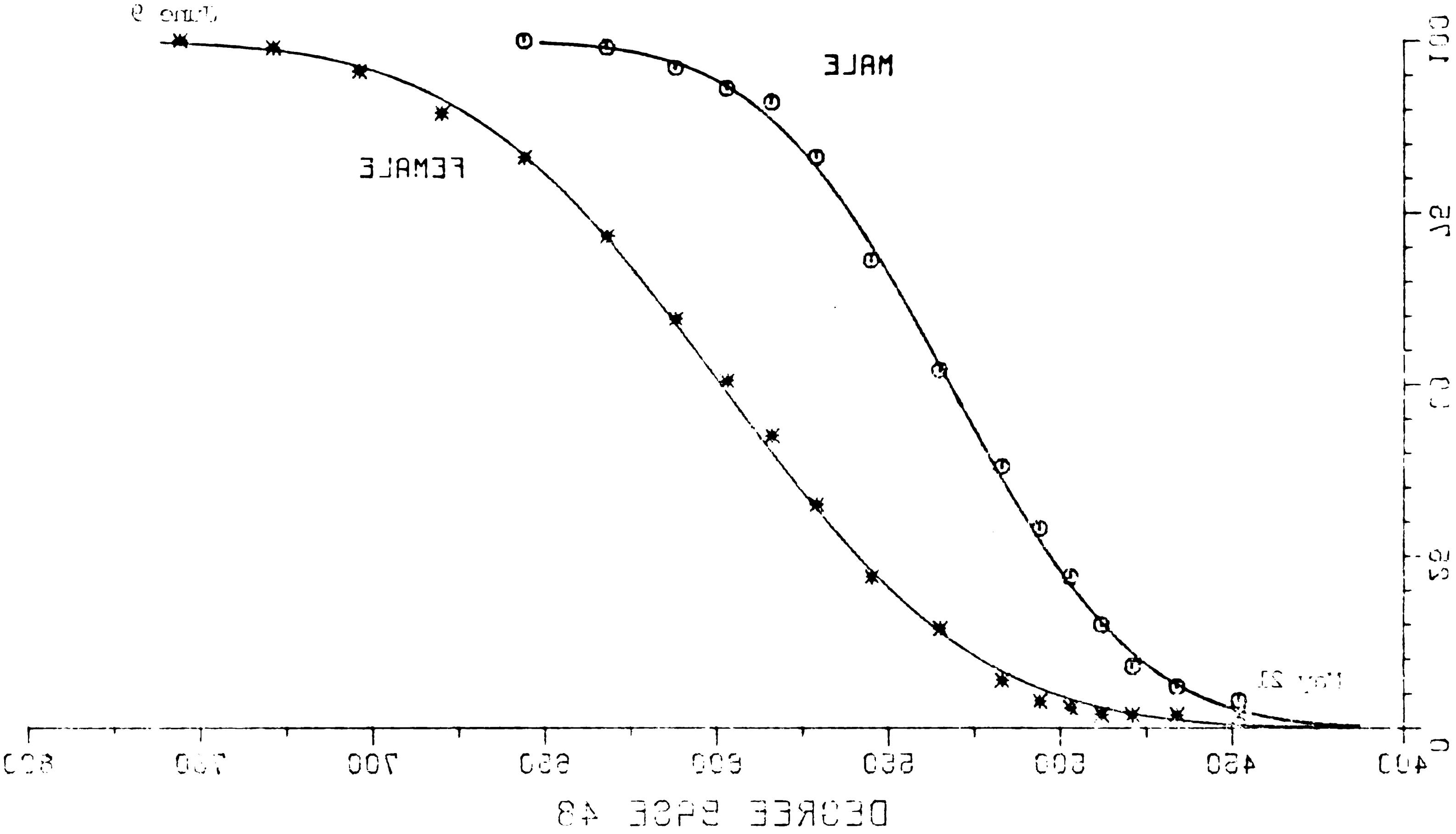



FIGURE 17. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPARSIS N. SP.
AT NILES, MICHIGAN. 1974

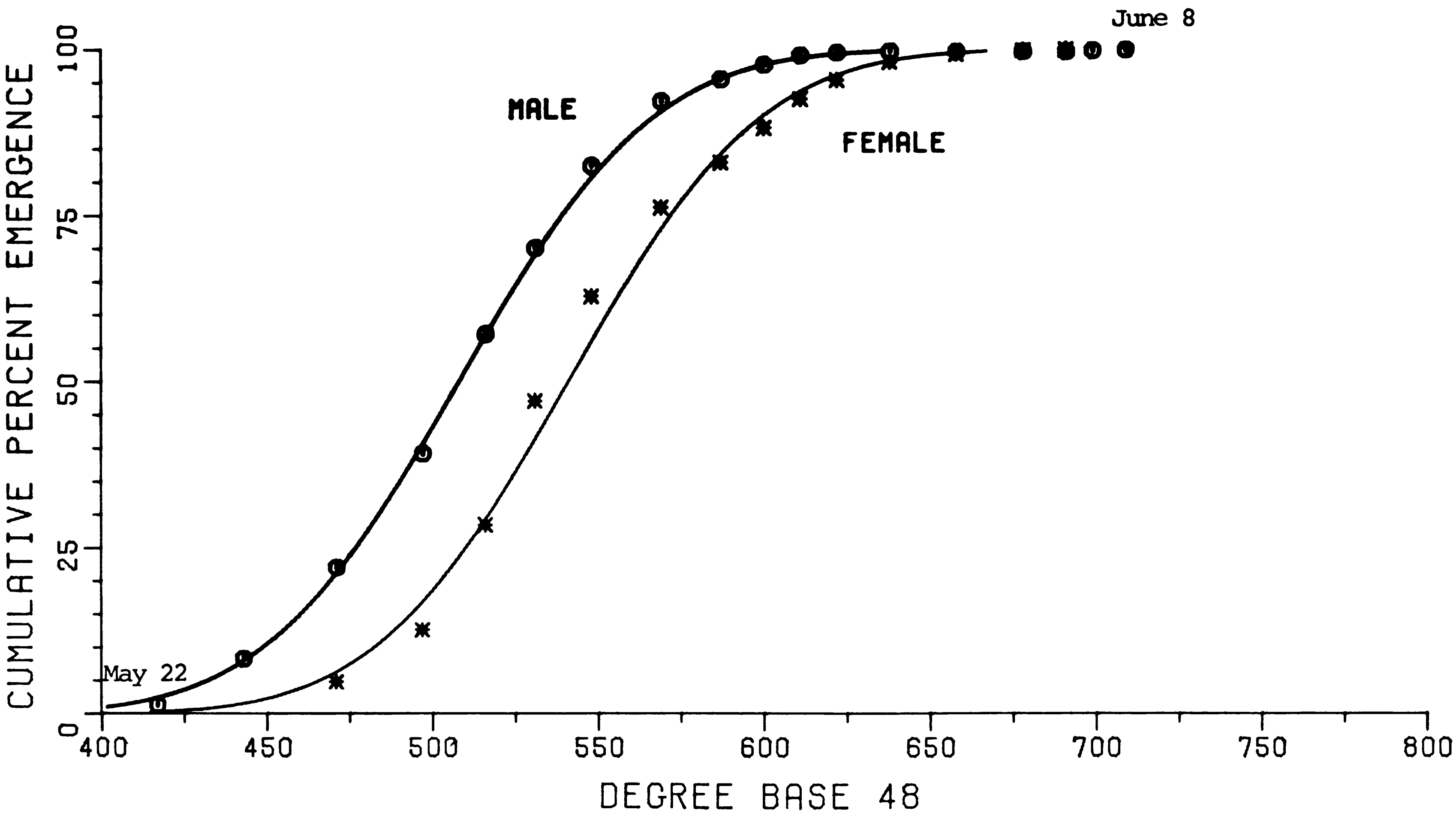



FIGURE 18. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPARSIS N.SP. AT NILES, MICHIGAN. 1975

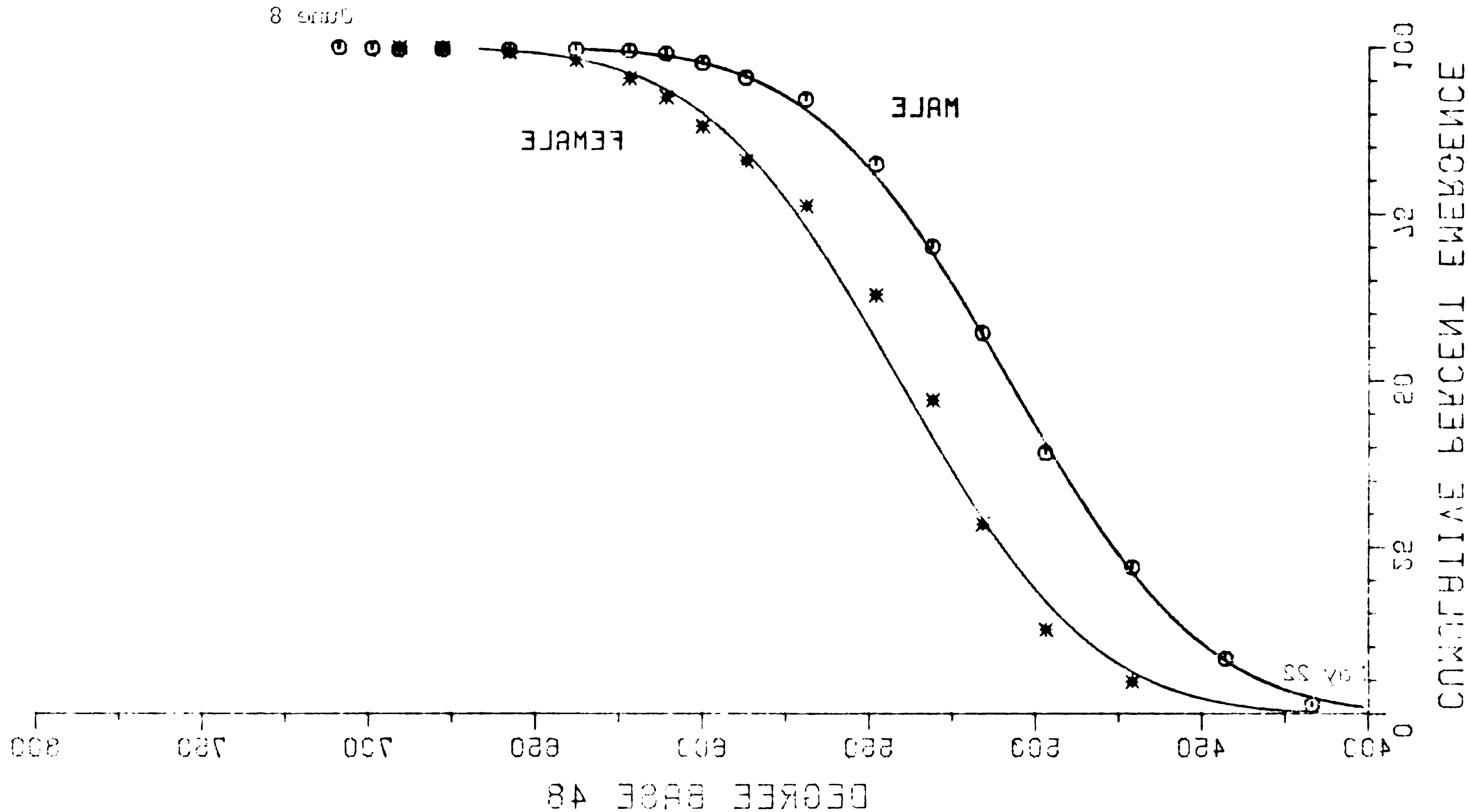



FIGURE 18. PROBIT CURVES OF EMERGENCE OF MALE AND FEMALE DIAPHASIS N.SP. AT NILES, MICHIGAN. 1975

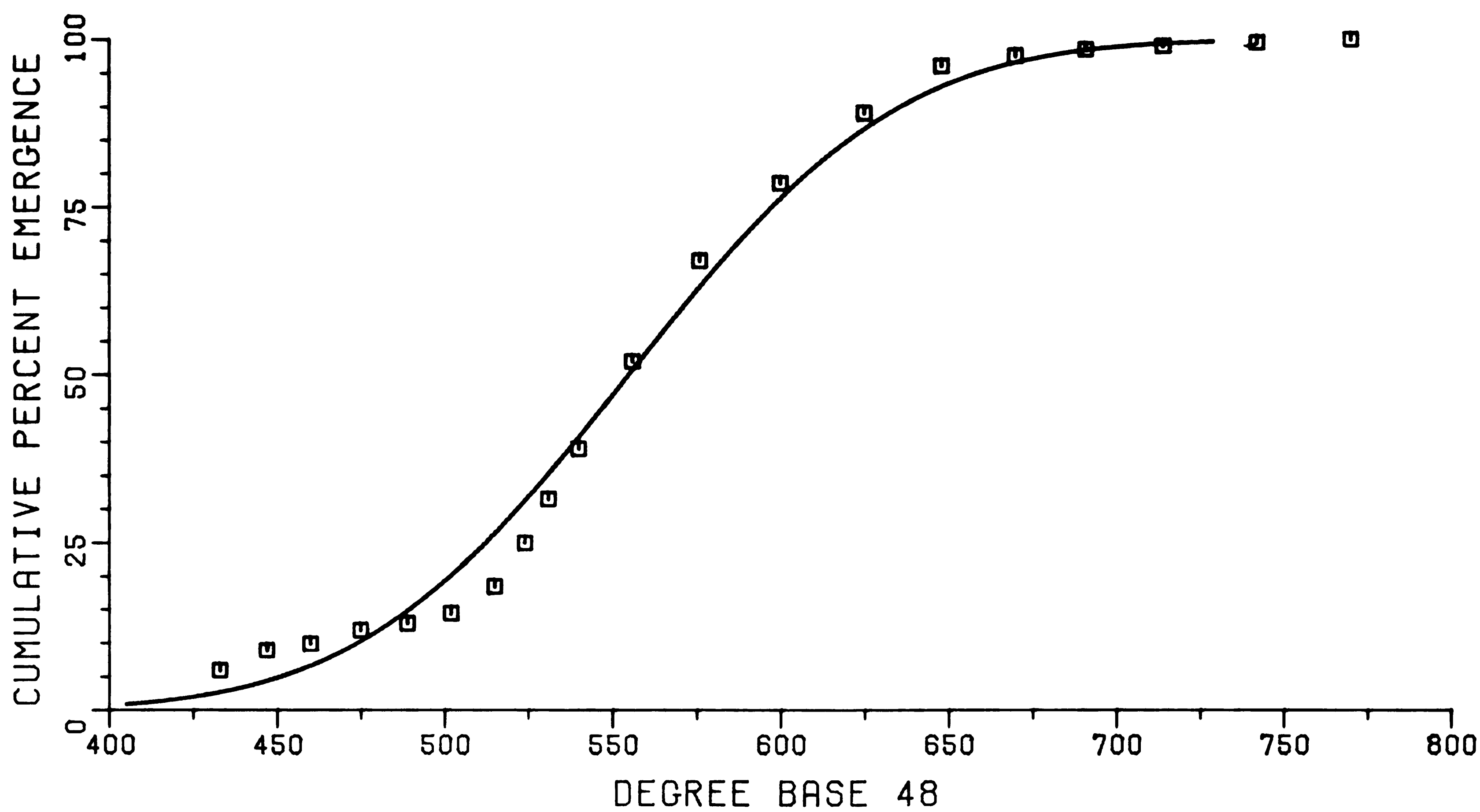



FIGURE 19. PROBIT CURVE OF EMERGENCE OF DIAPARSIS N.SP. AT NILES, MICHIGAN. 1973.

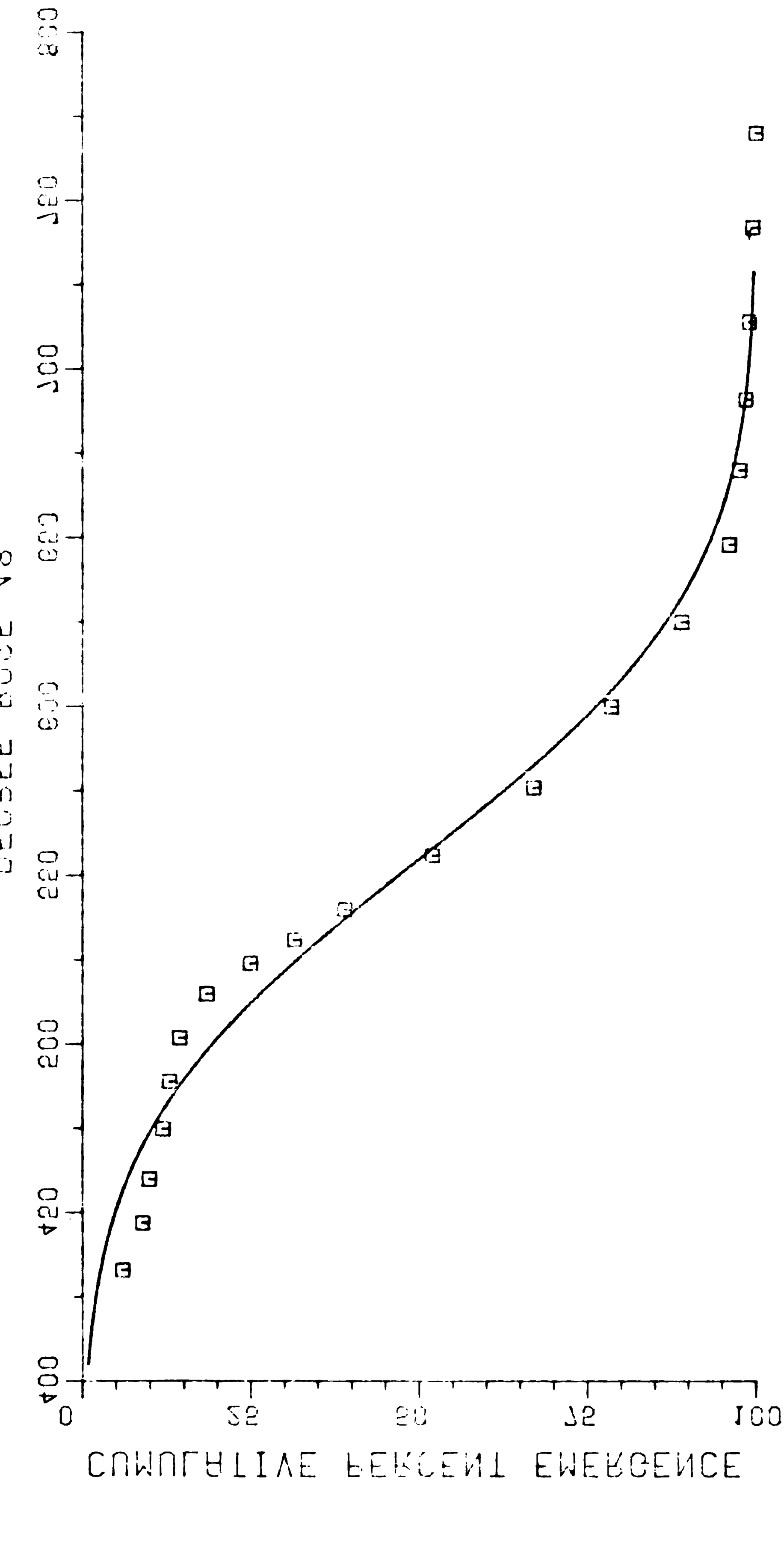



FIGURE 19. PROBIT CURVE OF EMERGENCE OF DIAPARSIS N.SP. AT NILES, MICHIGAN. 1973.

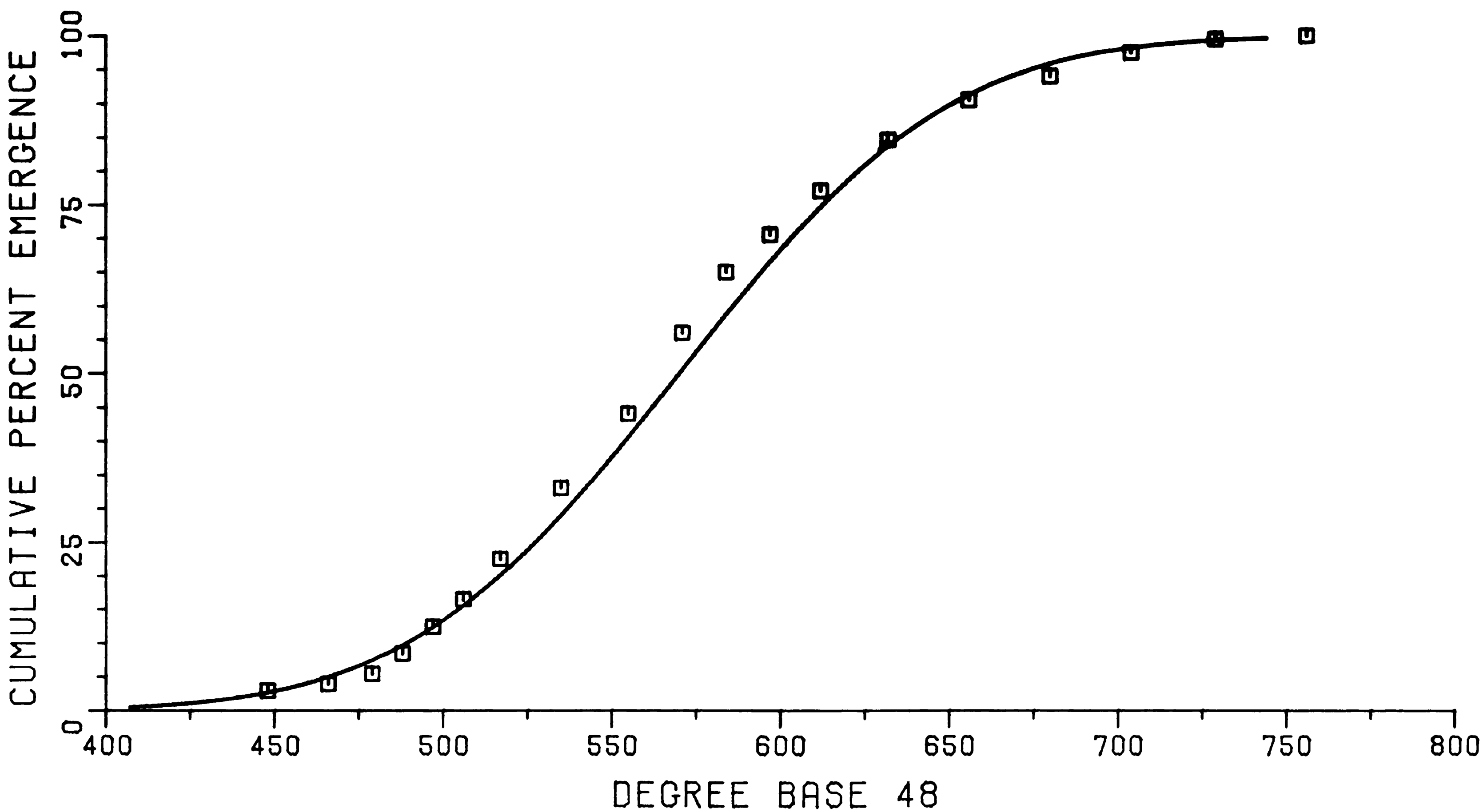



FIGURE 20. PROBIT CURVE OF EMERGENCE OF DIAPARSIS N.SP. AT NILES, MICHIGAN. 1974

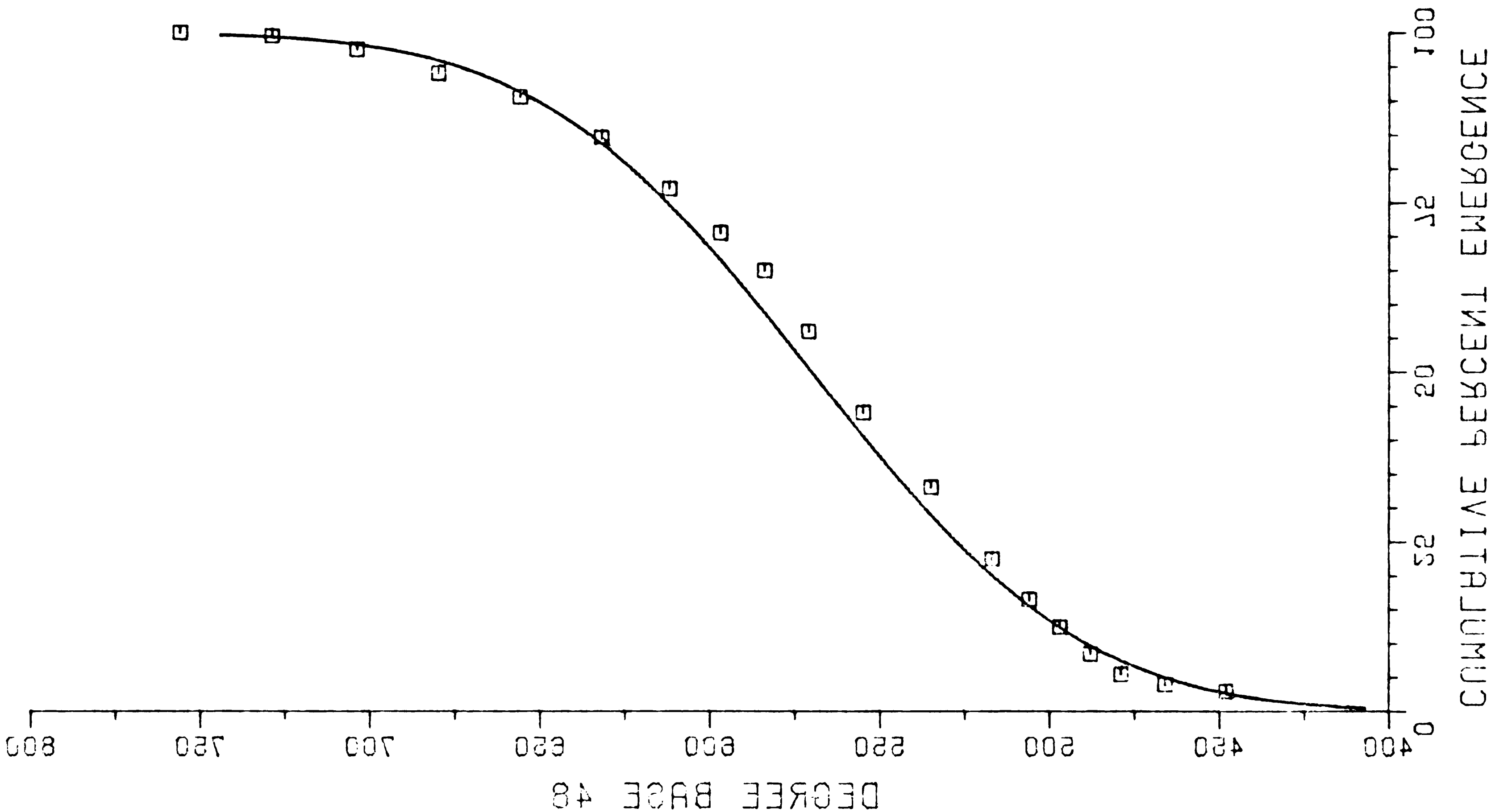



FIGURE 20. PROBIT CURVE OF EMERGENCE OF DIAPARSIS N.SP. AT NILES, MICHIGAN. 1974

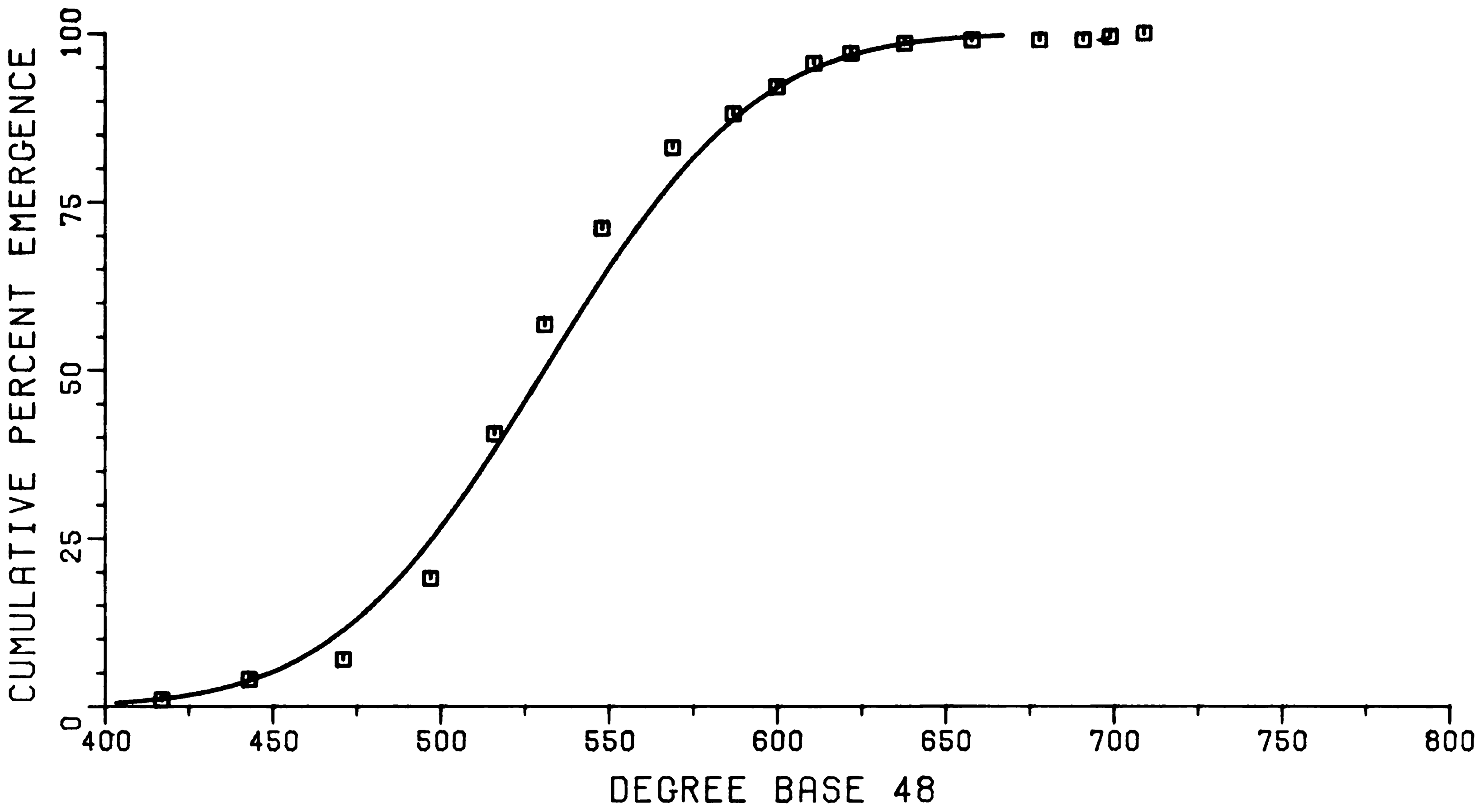
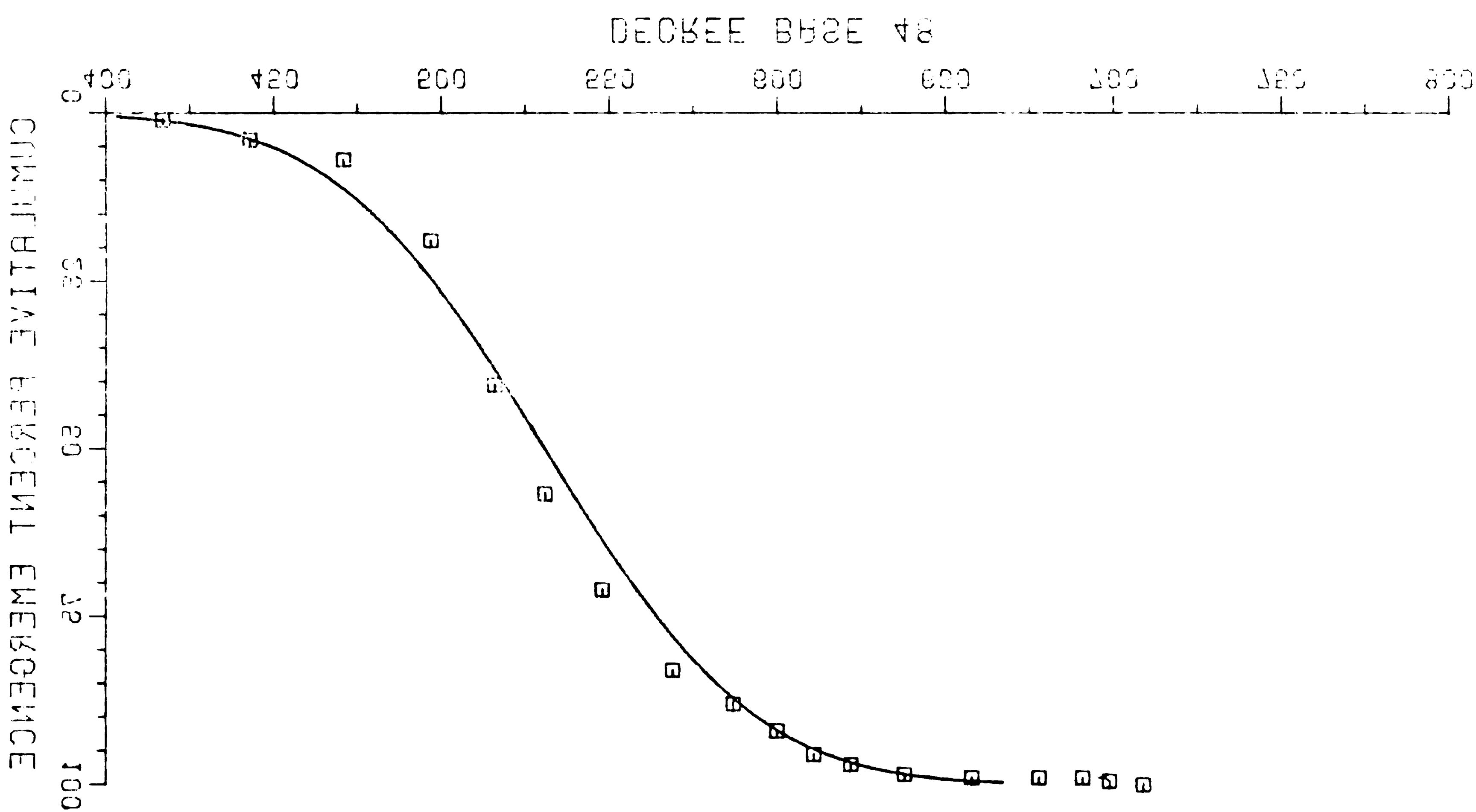



FIGURE 21. PROBIT CURVE OF EMERGENCE OF DIAPARSIS N.SP. AT NILES, MICHIGAN. 1975

FIGURE 21. PROBIT CURVE OF EMERGENCE OF CIR[illegible]SIS N.SP. AT NILES, MICHIGAN. 1979



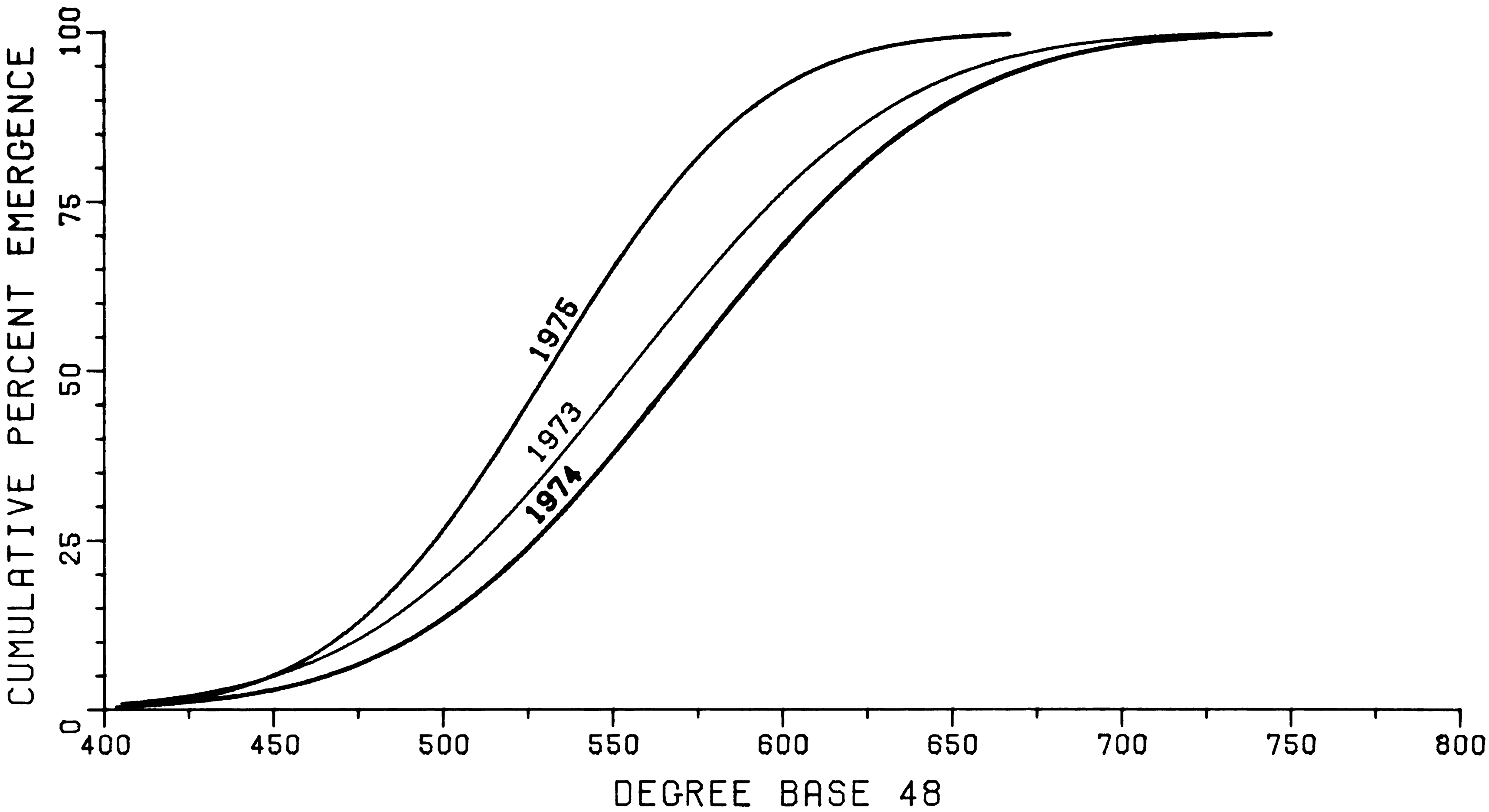



FIGURE 22. PROBIT CURVES OF DIAPARSIS N.SP. AT NILES, MICHIGAN. COMPARISON OF 1973, 1974, AND 1975.

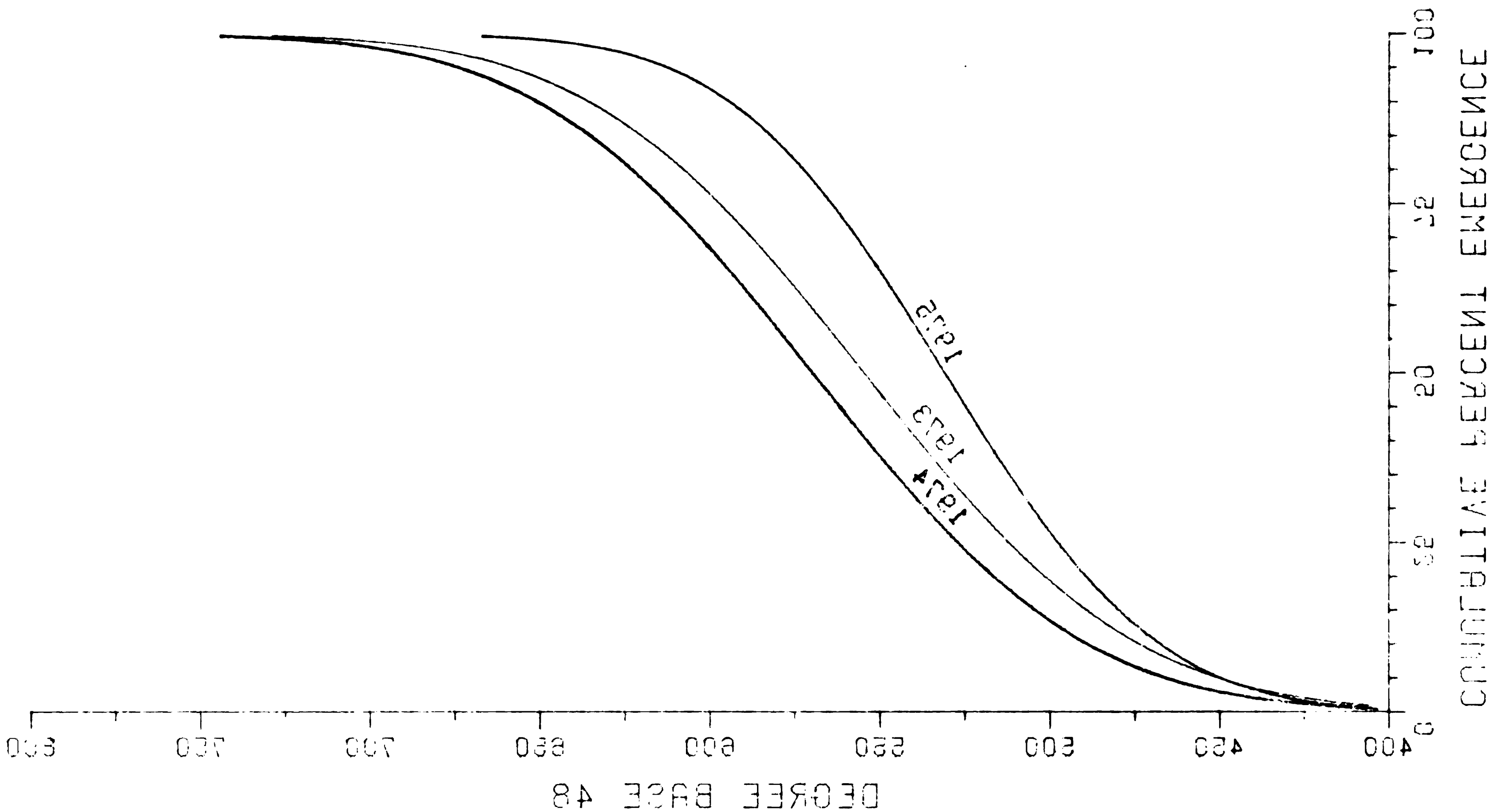



FIGURE 22. PROBIT CURVES OF DIAPARSIS N.SP. AT NILES, MICHIGAN.
COMPARISON OF 1973, 1974, AND 1975.

host suitability, oviposition rate, and host density. It is not known at this point which, if any of these are factors in determination of sex ratio in Diaparsis n.sp.

The emergence curves for the total parasitoid population are seen in Figures 19-21. These also show the data to which these curves were fitted. Figure 22 shows a comparison of the total emergence for the three years.

Although accumulated degree days during emergence did not vary greatly between the three years, emergence in 1975 was earlier by approximately $50^{o}D_{48}$ than in 1973, and in 1974 it was about $10^{o}D_{48}$ later than in 1973.

Variation in topography and soil type may be sufficient to explain the difference between emergence times of 1973 and 1974 since these were from different parts of the insectary. However, the emergence in 1975 was from the same area as in 1973 so topographic and edaphic conditions were the same.

It is hypothesized that variation in rainfall may have been the most important contributing factor in the differing emergence times. Rainfall cools soil temperatures directly when it reaches the ground and indirectly by evaporation (Geiger, 1950). During the month of May rainfall totaled 4.93, 5.48, and 2.46 inches respectively. Since May is the month of greatest heat accumulation in relation to pupal and larval development, it appears that greater rainfall in 1973 and 1974 slowed down emergence as compared to 1975.

Tillage Study

Table 7 and Figure 23 show the results from the investigation of the effects of tillage on emergence of Diaparsis n.sp.

The data from the tillage study were analyzed with a three level analysis of variance.* This analysis shows that tillage significantly reduces emergence, but the ratio of males to females is not significantly different from tilled and untilled.

No research was conducted to determine the actual cause of mortality, however, two hypotheses may be offered. One is that parasitoid cocoons were physically destroyed by plowing, harrowing, etc., but this was likely minimal. The more likely cause was that the cocoons were buried too deeply for the emerged parasitoids to dig their way to the soil surface.

These data also show that tillage prolongs emergence considerably (Figure 23). Degree day accumulations for the tilled vs. untilled areas in 1974 were 417 and 241 respectively, which equal 25 and 16 calendar days. The point at which 90% of both populations had emerged was

*ANOVA Table

| Source | | df | SS | MS | $F_s$ |
|---|---|---|---|---|---|
| $\bar{Y}_a - \bar{\bar{Y}}$ | Years | 1 | 363.0 | 363.0 | 1.549 ns |
| $\bar{Y}_b - \bar{Y}_a$ | Plots | 2 | 468.75 | 234.375 | 13.236* |
| $\bar{Y}_c - \bar{Y}_b$ | Sex | 4 | 70.833 | 17.708 | .532 ns |
| $Y - \bar{Y}_c$ | Error | 40 | 1332.667 | 33.317 | |
| $\bar{Y} - \bar{\bar{Y}}$ | Total | 47 | 2235.25 | | |

Table 7. Emergence trap catch of Diaparsis n.sp. from tilled and untilled areas.

| | Tilled | | | Untilled | | |
|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total |
| 1974 | 22 | 32 | 54 | 61 | 86 | 147 |
| 1975 | 2 | 7 | 9 | 35 | 25 | 60 |

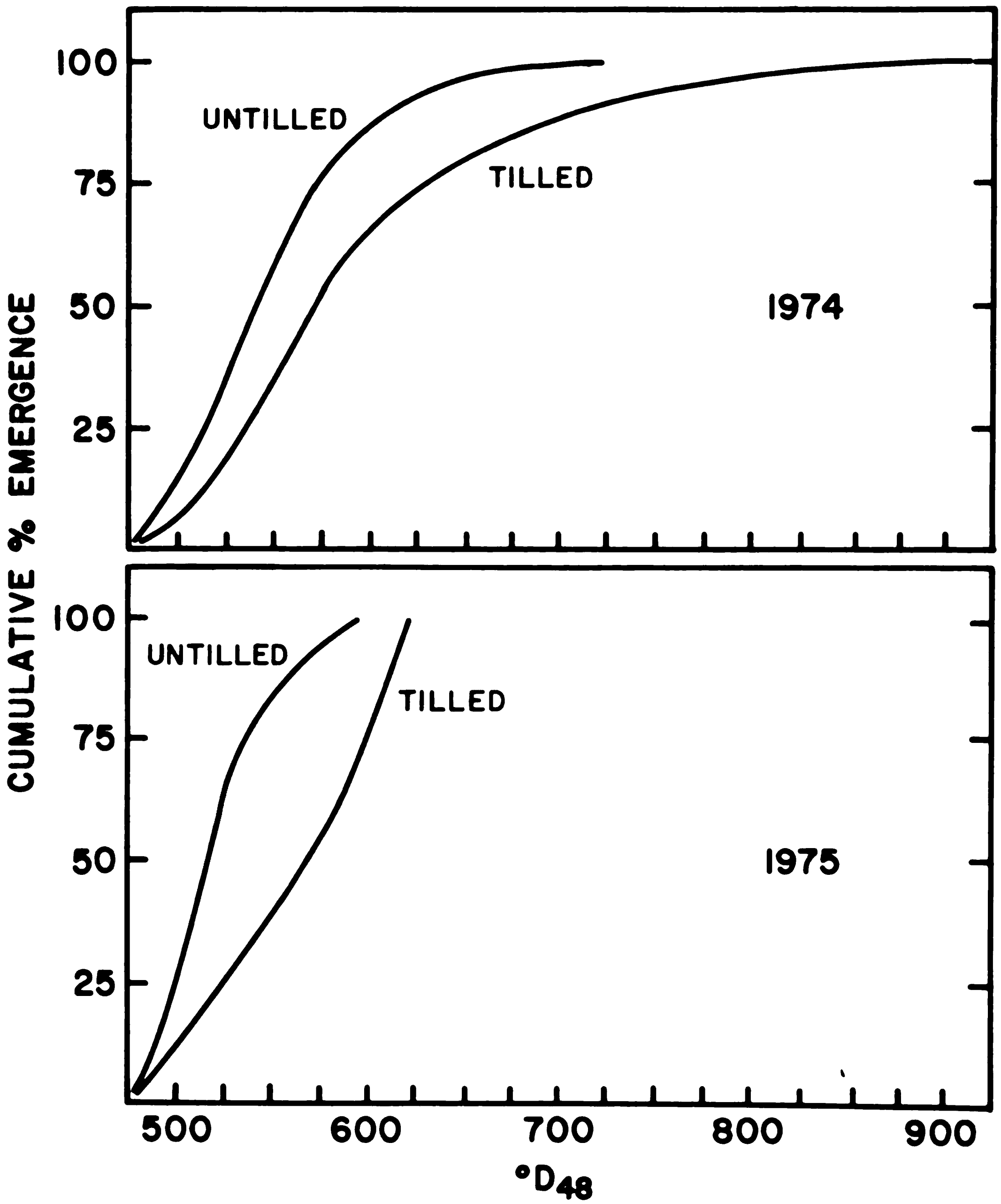



Figure 23. Emergence curves of Diaparsis n.sp. from spring planted oats and from untilled stubble (1974-1975).

separated by approximately 115 $^{o}D_{48}$. It appears, however, that some cocoons were not buried deeply by plowing since initial emergence was only 7 $^{o}D_{48}$ apart.

The extension of emergence time in the tilled strip is attributed primarily to the parasitoid cocoons being buried deeper in the soil. Shaw (1952) indicates that temperature variations between depths of three and six inches in bare soil may exceed three degrees. In addition, lack of cover in the tilled strip and the disturbed condition of the soil allow it to radiate accumulated heat more rapidly at night. Shaw (1952) also points out that organic mulch (untilled strip) insulates the soil and reduces the rate of heat exchange at the surface. This indicates that once the threshold temperature is reached in the untilled strip, it is maintained and the accumulation of heat units continues without interruption.

In the tilled strip, the soil temperature drops rapidly at night and the accumulation of heat units slows or stops completely and does not continue until temperatures again rise. Under normal spring conditions this may not occur every day. Therefore it is felt that tillage effectively reduces the rate of heat accumulation in the soil at lower depths.

Parasitism Rates

### Methods

Parasitism rates of *Diaparsis* n.sp. were monitored for three field seasons by sampling for parasitized CLB larvae twice weekly in each crop strip. Since row spacing in oat fields is 6 or 7 inches, 24 lineal inches of a single row were considered to be equivalent to one square ft, and therefore this unit was used in the density measurements.

Ten plots were selected randomly throughout each strip and all the CLB larvae were counted in each one. The total host larval density for a given crop strip on a sample date was expressed as the mean of these ten samples.

On the same day, 50 CLB larvae of various instars were collected randomly, placed in plastic boxes, and frozen in water to preserve them for later dissection to monitor parasitism. In a few instances early or late in the season, larvae were not present in sufficient numbers to collect 50 so the sample size consisted of 25 larvae.

In 1975 in second oats an attempt was made to determine if the presence of wild flowers in the field influenced the rate of parasitism. Sampling for parasitism was on a transect consisting of 12 evenly spaced collection points through the length of the field. Collections of 50 host larvae were made six times throughout the season at each collection site, and the parasitism rate for a given day was expressed as the mean parasitism of all the sites for that day.

In 1973 wheat was sampled as well as the three oats plantings, but, in 1974 and 1975 the CLB larval density in wheat dropped so low that it was no longer sampled; hence, the parasitism rates reported consider only the three plantings of oats.

The data resulting from these samples are shown graphically in Figures 24-26 in which the total number of larvae/$ft^2$ for a given crop in each season is plotted over $^{o}D_{48}$. (See Appendix V for raw data.) On the same graph the total density of parasitized host larvae per square foot is also plotted. This was determined by multiplying the percent parasitism for a sample day by the total number of larvae present.

This was further analyzed by graphical summation (Southwood, 1966) which makes it possible to determine a seasonal incidence by calculating the total area under each of the curves. Each total larval area is then divided by 283.8 to obtain a square foot larval density for the season. This figure, 283.8, is the total larval development time of the CLB in $^{o}D_{48}$ and was calculated from data reported by Wellso (1973). Parasitized larval incidence was then expressed as a percentage of the total larval incidence.

This method assumes equal development time for parasitized and unparasitized larvae which appears to be a

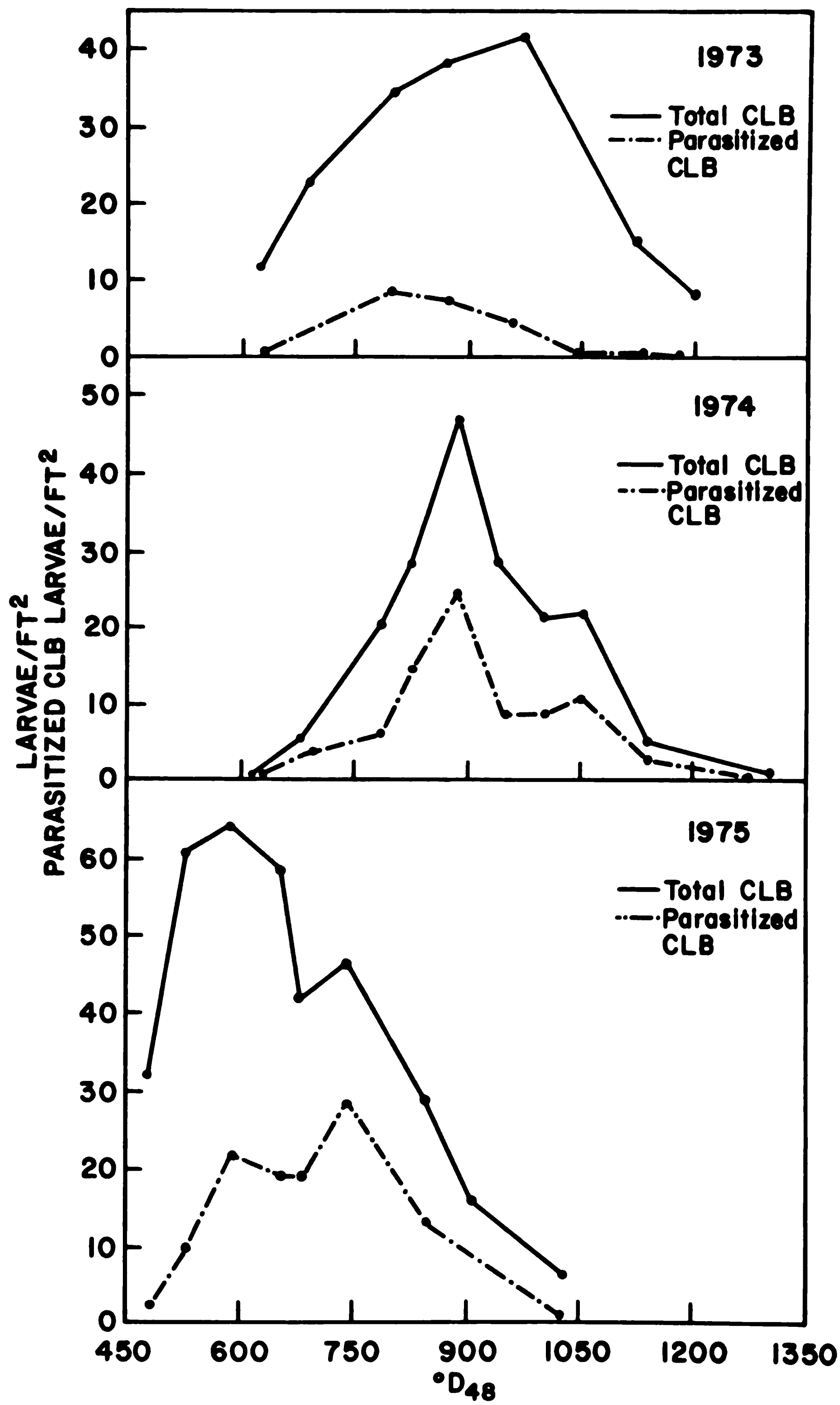



Figure 24. Total CLB larval incidence and parasitized larval incidence for Oats-1 at Niles, Michigan (1973-1975).

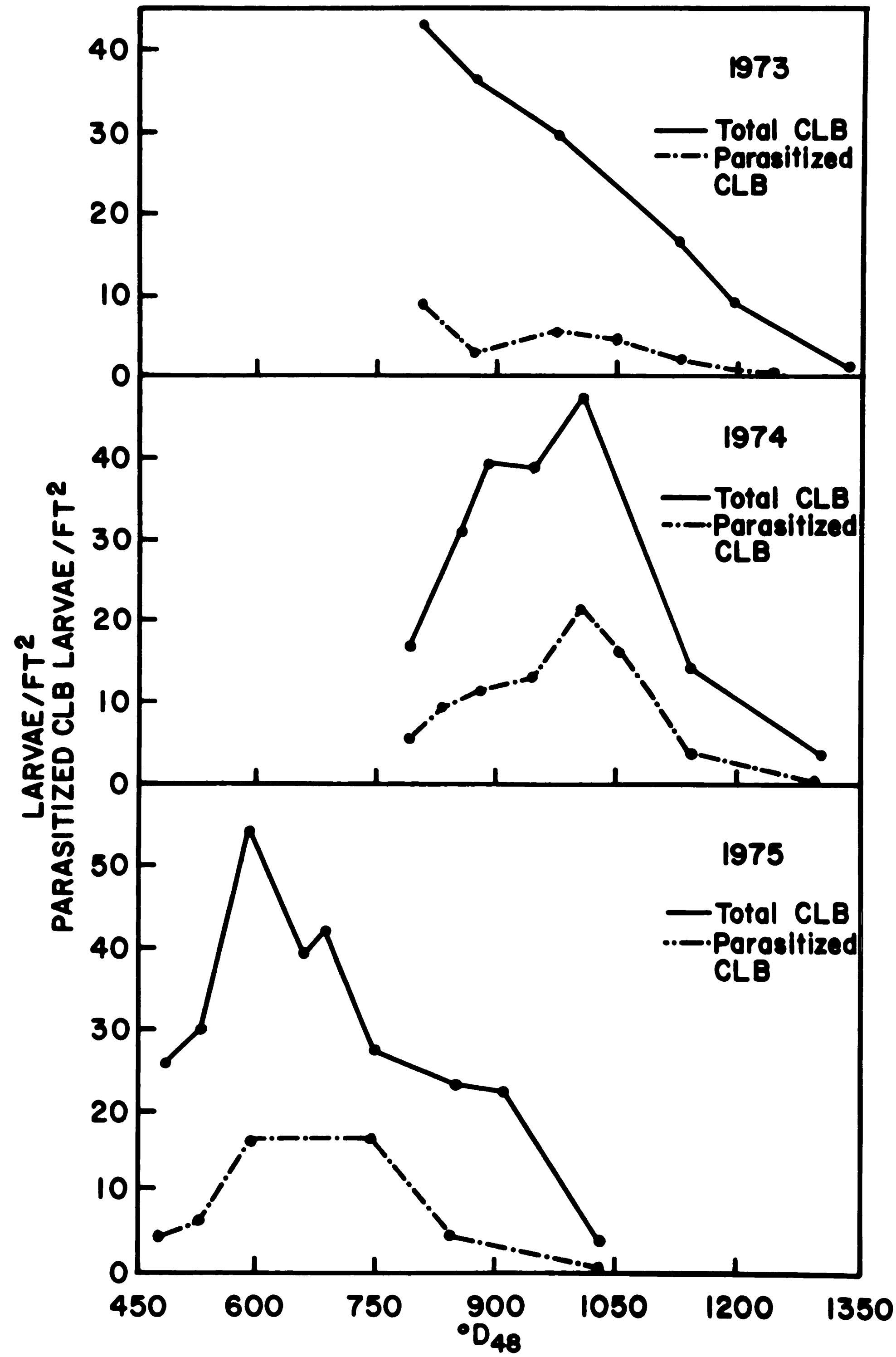



Figure 25. Total CLB larval incidence and parasitized larval incidence for Oats-2 at Niles, Michigan (1973-1975).

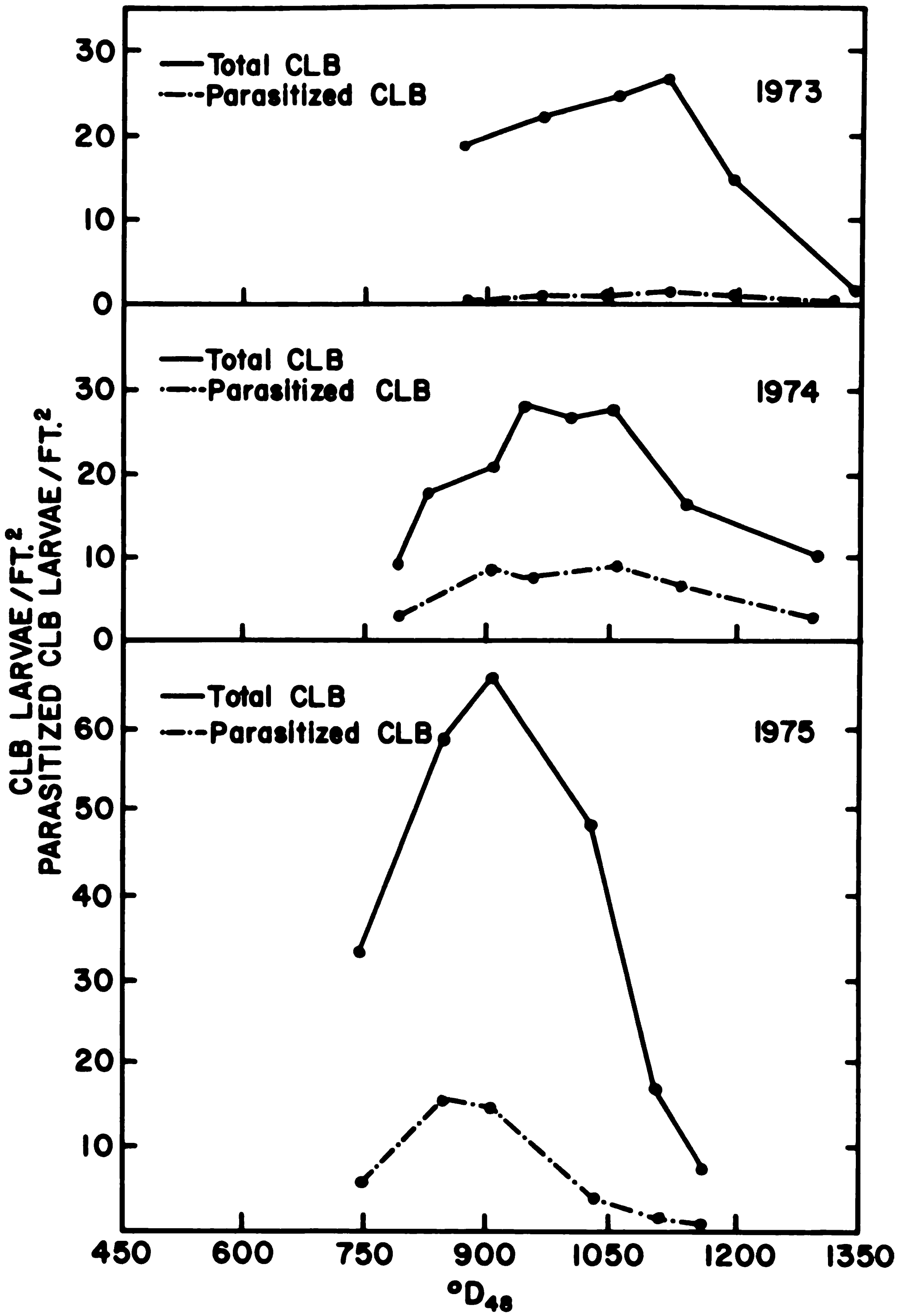



Figure 26. Total CLB larval incidence and parasitized larval incidence for Oats-3 at Niles, Michigan (1973-1975).

reasonable assumption for two reasons. One is that Gage (1974) reports that parasitization by _T. julis_ has no effect on development of CLB larvae. Also since _Diaparsis_ n.sp. does not develop to the second instar prior to CLB cocooning, it would seem that any effect on host development is likely insignificant.

## Results and Discussion

The larval incidence curves are shown in Figures 24-26, and the larval densities and parasitism rates are shown in Table 8.

These data show that parasitism percentages went from fairly low in 1973 to a high in 1974 and then dropped slightly in 1975. However in terms of parasitized larvae per $ft^2$ there was an increase each year.

Two factors affecting parasitism rates are parasitoid density and host density. These changed considerably over the three years covered by this study as can be seen in Tables 8 and 9.

In addition, it appears that weedy field conditions may also have lowered parasitism in 1975 below what would be expected with such a great increase in parasitoid density.

The transect sampling scheme in second oats, 1975, is shown in Figure 27 in which the numbers represent the sampling points, and the shaded areas around numbers four and ten are the areas where field cress (_Lepidium campestre_

Table 8. CLB larval densities, parasitized CLB larval densities, and <u>Diaparsis</u> n.sp. parasitism rates in three oat strips for three years at Niles, Michigan.

| Year | CLB Larvae/ft$^2$ | | Parasitized CLB Larvae/ft$^2$ | % Parasitism |
|---|---|---|---|---|
| <u>1973</u> | | | | |
| Oats-1 | 56.1 | | 7.7 | 13.7 |
| Oats-2 | 39.1 | $\bar{x}$=41.7 | 5.3 | 13.5 |
| Oats-3 | 30.0 | | .9 | 3.0 |
| <u>1974</u> | | | | |
| Oats-1 | 38.7 | | 17.0 | 43.9 |
| Oats-2 | 46.9 | $\bar{x}$=40.2 | 17.4 | 37.1 |
| Oats-3 | 34.9 | | 11.3 | 32.4 |
| <u>1975</u> | | | | |
| Oats-1 | 71.9 | | 27.9 | 38.8 |
| Oats-2 | 55.4 | $\bar{x}$=69.4 | 16.1 | 29.1 |
| Oats-3 | 81.0 | | 10.9 | 13.5 |

Table 9. Summary of emergence of *Diaparsis* n.sp. at Niles, Michigan (1973-1975).

| | Males | Females | Total | # of Traps | #/Trap | % Increase |
|---|---|---|---|---|---|---|
| 1973 | 37 | 27 | 64 | 12 | 5.3 | |
| 1974 | 50 | 54 | 104 | 15 | 6.9 | 30 |
| 1975 | 254 | 342 | 595 | 36 | 16.5 | 139 |

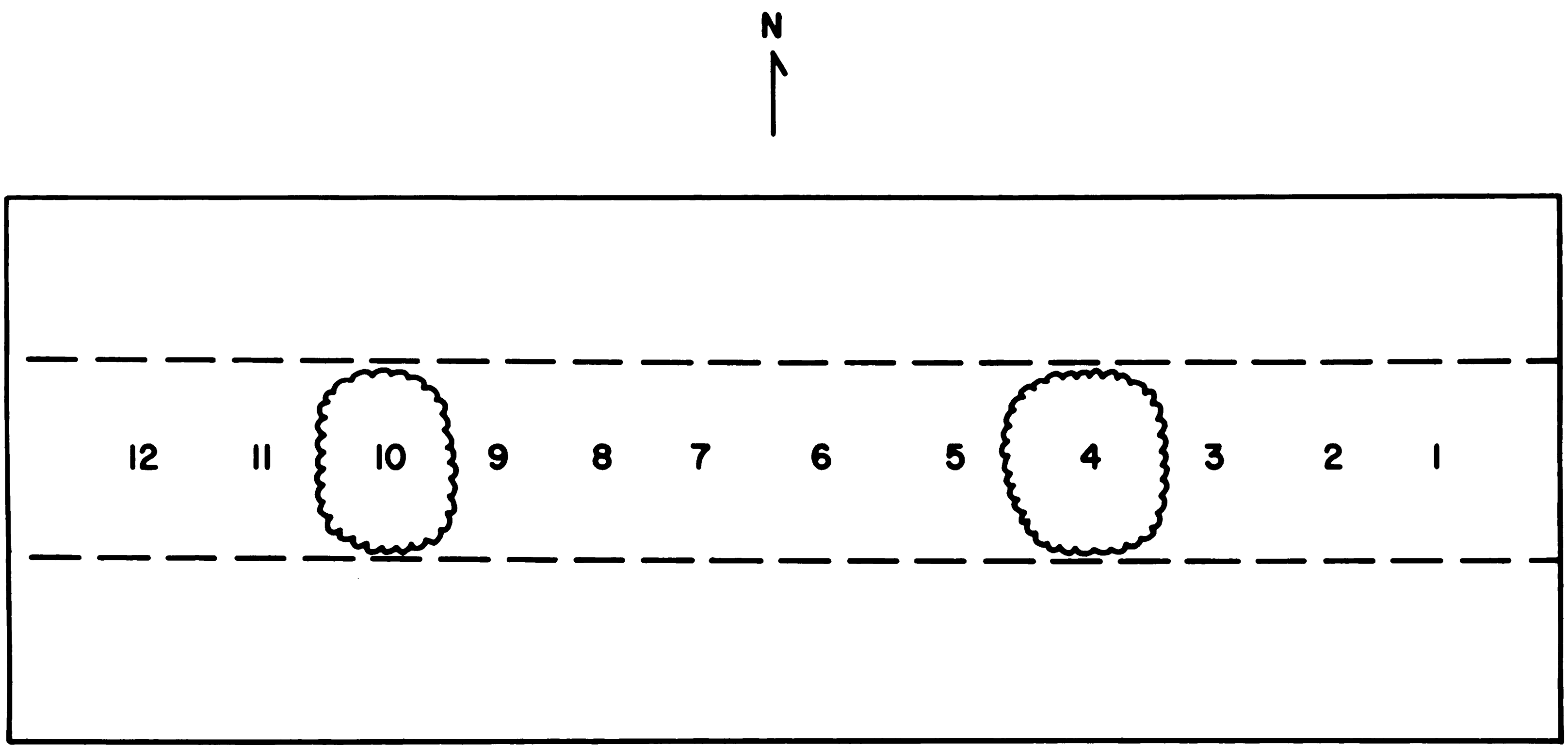



Figure 27. Diagram of the sampling scheme for parasitism by <u>Diaparsis</u> n.sp. in Oats-2, 1975. Numbers 1-12 represent the sampling sites (ca. 20 yds. apart). Field peppergrass was planted around sites four and ten.

L.) was planted as a source of food for the adult parasitoids. The dotted lines enclose the middle section of the field which was undisturbed by the USDA personnel and was reserved for this study.

Figure 28 shows the mean parasitism given for each sample site and the results of analysis using Duncan's multiple range test. This shows that the field was essentially divided in half with respect to parasitism. Explanation for this difference may lie in the difference in vegetation found at each end.

The east end of the field was high and well drained with light soil with the oats stand relatively pure with few weeds. Between the sixth and seventh collection sites on the transect, the field sloped down to the west end, which was primarily muck soil and quite flat with a high density of weeds, primarily thistle (Cirsium sp.), smartweed (Persicaria sp.), and nutsedge (Carex sp.). Carex sp., nutsedge, was by far the most abundant, being nearly as dense as the oats itself. This condition gave rise to a very dense canopy of vegetation. Since Diaparsis n.sp. searches for hosts by flying from plant to plant within the canopy, it is hypothesized that the density of vegetation lowered the movement and resultant oviposition of the parasitoid at this end of the field.

The results of the analysis shown in Figure 28 show that site three in the east end is statistically similar in

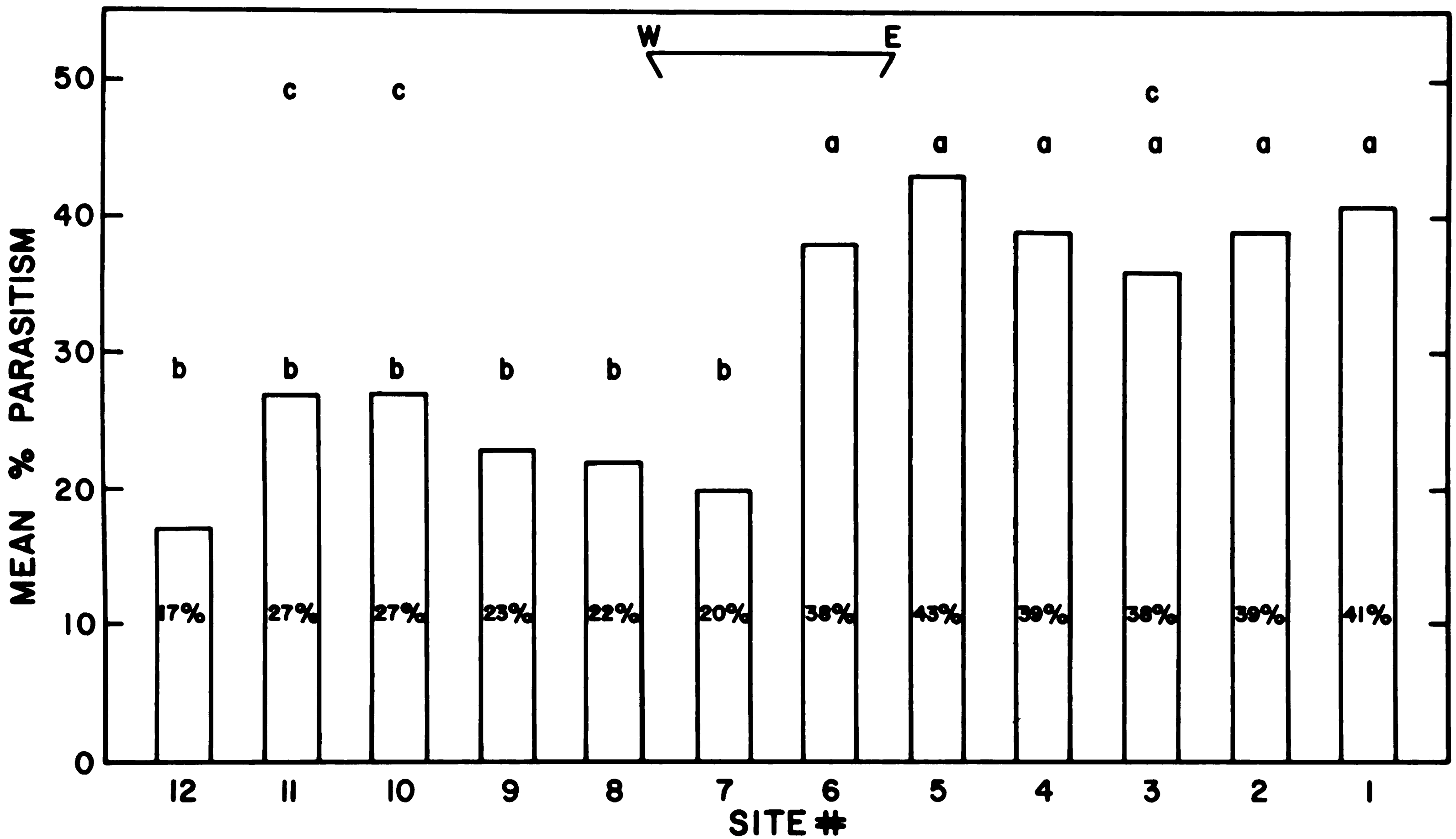



Figure 28. Mean parasitism rates in Oats-2 at each sample site and results of analysis with Duncan's multiple range test. Points with the same letters are statistically similar.

parasitism to sites 10 and 11 in the west end, but it is doubtful that this is of any biological significance.

The weedy condition described above in the west end of second oats also existed in the west end of first and third oats (Figure 1) and may help to explain the lower parasitism rate in 1975. The area of the insectary where the crop was grown in 1974 was nearly all high and well drained with few weeds.

## Synchrony

In order to determine how well *Diaparsis* n.sp. is synchronized with its host, larval densities and parasitized larval densities were converted to cumulative percentages (Tables 10 and 11) and compared using a distribution free test by Friedman (Hollander and Wolfe, 1973). The results showed significant differences at the .05 level in only three instances - oats two and three, 1973, and oats one, 1975. This indicates that *Diaparsis* n.sp. is generally well synchronized with its host.

## Encapsulation, Superparasitism, and Multiparasitism

Dissections of CLB larvae for monitoring of parasitism rates also yielded considerable data on encapsulation, superparasitism, and multiparasitism. These were recorded and are discussed below.

Table 10. Cumulative percentages of cereal leaf beetle larvae and parasitized cereal leaf beetle larvae at Niles, Michigan (1973-1974).

| | Oats-1 | | Oats-2 | | Oats-3 | |
|---|---|---|---|---|---|---|
| Date | CLB | Parasitized CLB | CLB | Parasitized CLB | CLB | Parasitized CLB |
| | | | 1973 | | | |
| June 4 | 5.7 | 0 | | | | |
| 7 | 17.2 | 14.8 | | | | |
| 11 | 34.6 | 48.0 | 27.1 | 36.6 | | |
| 14 | 53.6 | 78.4 | 50.0 | 48.9 | 17.2 | 0 |
| 18 | 74.4 | 95.2 | 68.8 | 71.4 | 37.7 | 23.1 |
| 21 | 88.5 | 97.6 | 83.5 | 89.3 | 59.9 | 35.9 |
| 25 | 95.9 | 100 | 94.2 | 97.8 | 84.4 | 64.1 |
| 28 | 100 | | 99.7 | 99.9 | 97.8 | 97.4 |
| July 5 | | | 100 | 100 | 100 | 100 |
| | | | 1974 | | | |
| June 3 | .5 | .4 | | | | |
| 6 | 3.6 | 4.5 | 7.6 | 7.0 | | |
| 10 | 15.2 | 13.1 | 19.0 | 17.9 | 5.9 | 5.3 |
| 13 | 31.1 | 30.4 | 36.8 | 32.2 | 17.1 | 15.3 |
| 17 | 57.4 | 61.6 | 54.3 | 48.1 | | |
| 18 | | | | | 30.4 | 32.2 |
| 20 | 73.1 | 72.4 | 75.8 | 74.5 | 48.5 | 47.2 |
| 24 | 84.8 | 83.2 | 91.9 | 94.3 | 65.7 | 63.7 |
| 27 | 97.1 | 97.0 | 98.2 | 99.3 | 83.2 | 81.6 |
| July 1 | 99.7 | 99.9 | 100 | 100 | 93.6 | 94.8 |
| 5 | 100 | 100 | | | 100 | 100 |

Table 11. Cumulative percentages of cereal leaf beetle larvae and parasitized cereal leaf beetle larvae at Niles, Michigan (1975).

| | Oats-1 | | Oats-2 | | Oats-3 | |
|---|---|---|---|---|---|---|
| Date | CLB | Parasitized CLB | CLB | Parasitized CLB | CLB | Parasitized CLB |
| May 26 | 9.2 | 2.1 | 11.7 | 6.9 | | |
| 29 | 26.6 | 11.0 | 25.1 | 16.5 | | |
| June 2 | 44.9 | 28.6 | | | | |
| 3 | | | 48.9 | 40.1 | | |
| 6 | 61.6 | 44.6 | 66.0 | 64.4 | | |
| 9 | 73.5 | 59.4 | | | | |
| 12 | 86.8 | 82.7 | 78.1 | 88.1 | 14.9 | 13.9 |
| 14 | 93.9 | | | | | |
| 17 | | 92.0 | 88.1 | 94.9 | 41.2 | 48.8 |
| 19 | 98.3 | 99.4 | 98.0 | 98.6 | 70.8 | 87.1 |
| 23 | 100 | 100 | 100 | 100 | 92.4 | 97.3 |
| 26 | | | | | 100 | 100 |

## Results and Discussion

Tables 12 and 13 show the encapsulation rate of Diaparsis n.sp. eggs and larvae which occurred singly in host larvae. Encapsulation in this situation was lowest in 1974 and highest in 1973, and in all years larvae were encapsulated less than eggs. Overall encapsulation for the three years was 7.5% for eggs and 0.5% for larvae. The rate including both eggs and larvae occurring individually in the host was 1.8%.

Smooth eggs are more frequently encapsulated than knobbed eggs, Table 13. Perhaps the knob interferes in some way with encapsulation.

Encapsulation was higher when two parasitoids were found in the same host larva (Table 14), occurring more frequently in superparasitism than in multiparasitism with either Tetrastichus julis or Lemophagus curtus. In one case T. julis was found to be encapsulated, while in the other 54 cases of multiparasitism neither parasitoid was encapsulated.

It is possible that some of these encapsulations occurred prior to deposition of the second egg. This could not be determined since these are field data and the time of oviposition was not known. However, since encapsulation was more frequent in hosts which were superparasitized than in hosts with a single parasitoid, it appears that superparasitism triggers encapsulation of one parasitoid.

Table 12. Encapsulation of *Diaparsis* n.sp. eggs and larvae occuring singly in the host.

| Parasitoid stage | | 1973 | 1974 | 1975 | Total |
|---|---|---|---|---|---|
| Eggs | Encaps. | 2 | 1 | 17 | 20 |
| | Unencaps. | 31 | 49 | 168 | 248 |
| | % Encaps. | 6.0 | 2.0 | 9.2 | 7.5 |
| Larvae | Encaps. | 2 | 3 | 1 | 6 |
| | Unencaps. | 134 | 373 | 698 | 1205 |
| | % Encaps. | 1.5 | .8 | .1 | .5 |
| Totals | % Encaps. | 2.4 | .9 | 2.0 | 1.8 |

Table 13. Encapsulation of smooth vs. knobbed eggs occuring singly in the host.

| Egg Type | No. Encaps. | No. Unencaps. | $ Encaps. |
|---|---|---|---|
| Smooth | 8 | 67 | 11 |
| Knobbed | 12 | 181 | 6 |

Table 14. Encapsulation of *Diaparsis* n.sp. eggs and larvae with two parasitoids present.

| | | |
|---|---|---|
| **Superparasitism - 152 Cases** | | |
| Both Encaps. | 2 | 1.3% |
| One Encaps. | 85 | 55.9% |
| None Encaps. | 65 | 42.8% |
| **Multiparasitism - 62 Cases** | | |
| *Diaparsis* Encaps. | 7 | 11.3% |
| *T. julis* Encaps. | 1 | 1.6% |
| *L. curtus* Encaps. | 0 | 0% |
| None Encaps. | 54 | 87.1% |

From the data it appears that supernumaries in multiparasitism are more commonly eliminated by direct larval competition than by encapsulation. According to Askew (1971) this competition may be by fighting, with one larva physically destroying the other, or by destruction of excess larvae by toxins produced by one of the parasitoids, or by depletion of available $O_2$ by one of the parasitoids. In the latter case the excess larvae are destroyed by suffocation.

According to Dysart, et. al., (1973) _Diaparsis_ n.sp. most likely is the successful parasitoid in competition with _T_. _Julis_. In dissections of European material they observed the larvae of _Diaparsis_ biting _T_. _julis_ larvae, and in several instances healthy _Diaparsis_ were found with dead or wounded _T_. _julis_. However, in cases where the host larvae contain mature _T_. _julis_ larvae it may not be possible for _Diaparsis_ to destroy them. The outcome of _Diaparsis_ - _L_. _curtus_ competition is presently unknown.

The effects of super-and multiparasitism on encapsulation are shown in Table 15. In cases where _Diaparsis_ n.sp. was found in various combinations with other _Diaparsis_ n.sp., _T_. _julis_, or _L_. _curtus_ eggs or larvae, 43.9% had at least one _Diaparsis_ encapsulated. In contrast only 1.8% encapsulation occured in hosts containing one parasitoid.

Figure 29 shows the percentages of superparasitism and multiparasitism in each year, 1973-1975. In 1975

Table 15. Effect of superparasitism and multiparasitism on encapsulation rate of *Diaparsis* n.sp.

| | | | | |
|---|---|---|---|---|
| Cases of single parasitism | | | 1479 | 85.7% |
| Encaps. | 26 | 1.8% | | |
| Unencaps. | 1453 | 98.2% | | |
| Cases of super- or multiparasitism (1) | | | 246 | 14.3% |
| Encaps.(2) | 108 | 43.9% | | |
| Unencaps. | 138 | 56.1% | | |
| | | Total | 1725 | 100% |

(1) Superparasitism of *Diaparsis* n.sp. and multiparasitism with either *Tetrastichus julis* or *Lemophagus curtus*. In several cases all 3 were present.

(2) One *Diaparsis* encapsulated only.

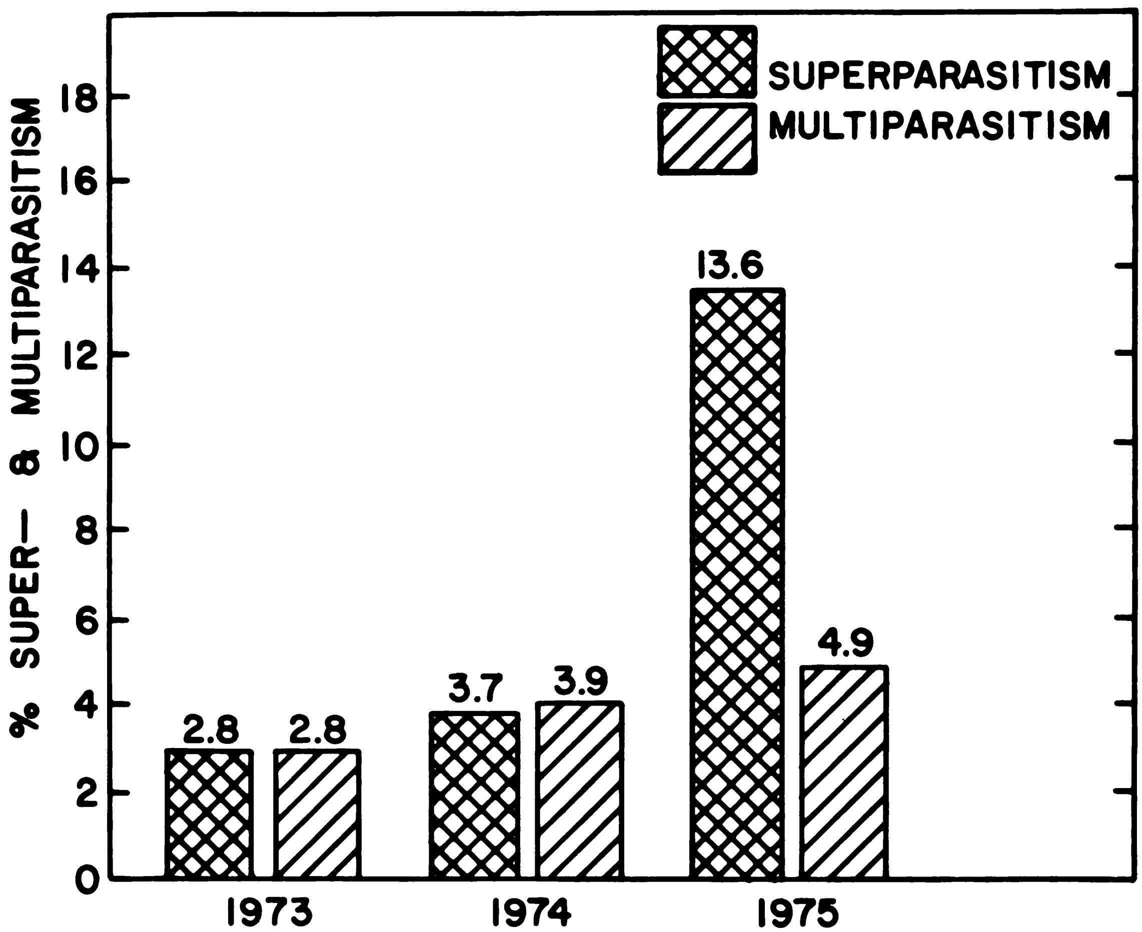



Figure 29. Percent superparasitism of CLB larvae by <u>Diaparsis</u> n.sp. and percent multiparasitism by <u>Diaparsis</u> n.sp. and <u>T. julis</u> or <u>L. curtus</u> at Niles, Michigan (1973-1975).

superparasitism increased more than 3X even though overall parasitism rates decreased slightly.

The overall rate of superparasitism for the three years of this study was 10.3% and multiparasitism was 4.9%. According to data from Dysart, et. al., (1973) superparasitism in Europe is 6.3%, but no data are given on multiparasitism.

The various combinations of superparasitism and encapsulation are shown in Table 16. The most common combination was two larvae in one host, 88% of which had one larva encapsulated. The next most common combination was an egg and a larva, in 30% of which the egg was encapsulated. In no case was the larva encapsulated and the egg viable.

Multiparasitism with Diaparsis and either T. julis, L. curtus or both occurred 76 times throughout this study. The number found each year for each species is shown in Table 17.

Since T. julis and L. curtus both occur in lower numbers in early and mid-season than at the end, an analysis was made of superparasitism and multiparasitism over time (Table 18, and Figure 30). Numbers were relatively small in 1973 and 1974, so the data shown is for 1975 only. The rates of superparasitism and multiparasitism were calculated for each sample day beginning on May 26.

The results show that superparasitism peaks in the first week of June and then declines to zero at the end of

Table 16. Combinations of superparasitism and encapsulation in Diaparsis n.sp. (2 parasitoids only).

| | | | | |
|---|---|---|---|---|
| Egg with Egg | | | 28 | 18.5% |
| One Encaps | 4 | 14.3% | | |
| Both Encaps. | 0 | 0% | | |
| None Encaps. | 24 | 85.7% | | |
| Egg with Larva | | | 49 | 32.2% |
| Egg Encaps. | 15 | 30.6% | | |
| Larva Encaps. | 0 | 0% | | |
| Both Encaps. | 1 | 2.0% | | |
| None Encaps. | 33 | 67.4% | | |
| Larva with Larva | | | 75 | 49.3% |
| One Encaps. | 66 | 88.0% | | |
| Both Encaps. | 1 | 1.3% | | |
| None Encaps. | 8 | 10.7% | | |

Table 17. Multiparasitism of CLB by *Diaparsis* n.sp. and *Tetrastichus julis* and *Lemophagus curtus*.

| | 1973 | 1974 | 1975 | Totals |
|---|---|---|---|---|
| With *T. julis* | 4 | 9 | 20 | 33 |
| With *L. curtus* | 1 | 7 | 28 | 36 |
| With *T. julis* & *L. curtus* | 0 | 2 | 5 | 7 |

Table 18. Seasonal trends of multi- and superparasitism of CLB larvae *Diaparsis* n.sp. at Niles, Michigan (1975).

| Sample date | May 26 | May 29 | June 2 | June 6 | June 12 | June 17 | June 19 | June 23 |
|---|---|---|---|---|---|---|---|---|
| Parasitized CLB | 13 | 126 | 176 | 256 | 384 | 149 | 113 | 22 |
| Super. | 0 | 13 | 37 | 46 | 43 | 10 | 3 | 0 |
| % Super. | 0 | 10 | 21 | 18 | 11 | 7 | 3 | 0 |
| Mult. | 0 | 17 | 13 | 12 | 5 | 2 | 3 | 10 |
| % Mult. | 0 | 14 | 7 | 5 | 1 | 1 | 3 | 46 |

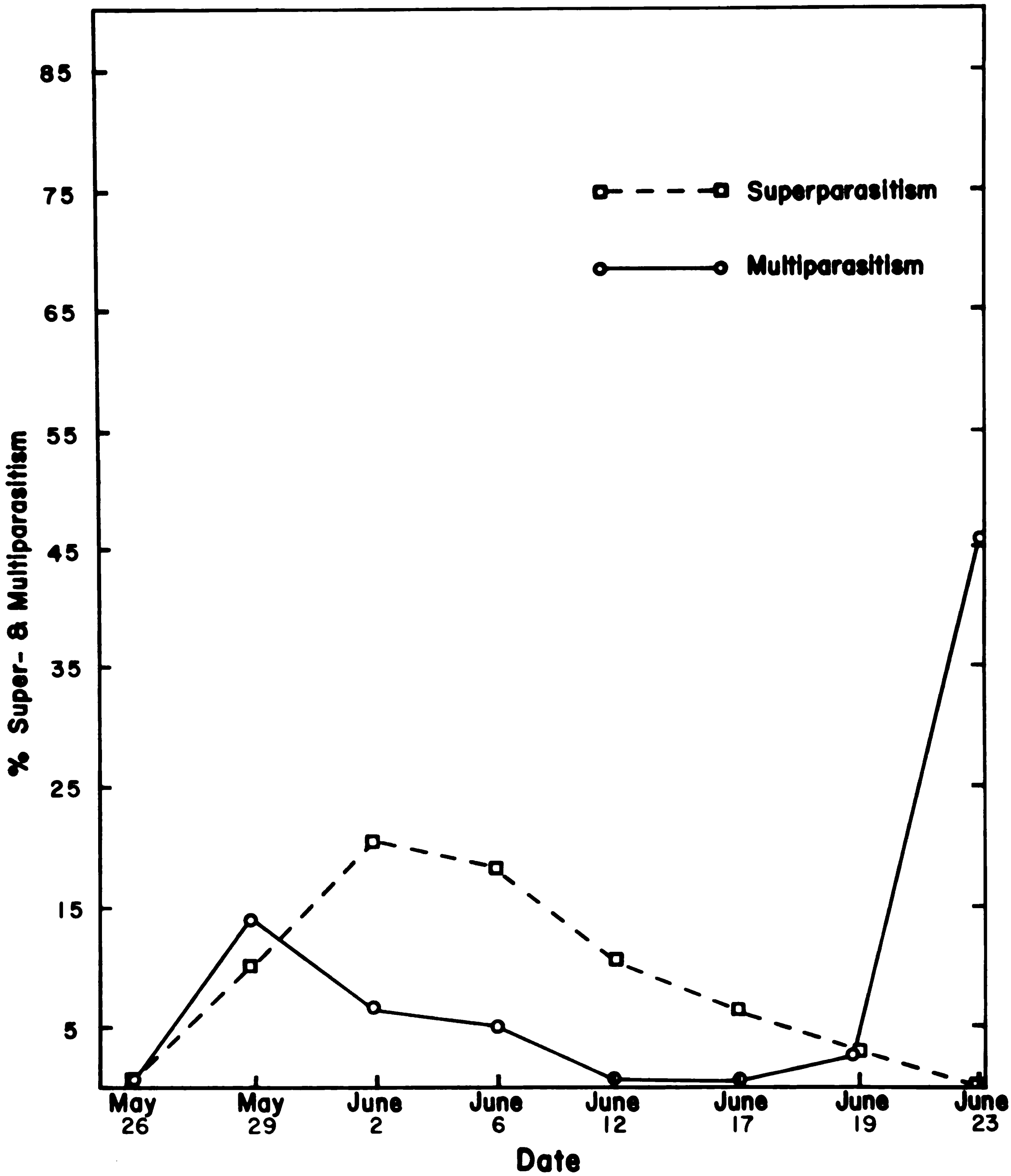



Figure 30. Seasonal trend of superparasitism of CLB larvae by Diaparsis n.sp. and multiparasitism by Diaparsis n.sp. and T. julis or L. curtus (1975).

the season. This peak corresponds well with the emergence of <u>Diaparsis</u> n.sp. which terminated on June 8 or 9 each year. This fact, considered with the fact that superparasitism was greatest in 1975 when parasitoid density was greatest, would indicate that superparasitism is a function of parasitoid density.

Multiparasitism shows a slightly higher rate early in the season than at mid-season when it drops to about one percent. However, late in the season it rises rapidly to a high level. This curve of multiparasitism follows the population emergence curve of <u>T. julis</u> reported by Gage and Haynes (1975). Malaise trap collections showed that <u>L. curtus</u> also became more abundant in mid-to late June. In addition the number of hosts available declined considerably at this time. Therefore the increase in multiparasitism seems to indicate that it is a function of the density both of the parasitoids and the CLB larvae.

## <u>Adult Parasitoid Density</u>

### Methods

Measurement of adult parasitoid density was attempted each year and several methods were tried. In 1973 and in 1975 a D-Vac, backpack model, was used as a sampling tool. Samples were taken twice weekly in each crop strip with each sample unit consisting of 20 $ft^2$. These were collected by carefully lowering the collecting hose with the one $ft^2$. collecting ring vertically into the crop and placing it on the ground. The material was then taken to the lab and either anesthetized with $CO_2$ or placed in a freezer overnight after which the Diaparsis n.sp. were sorted out.

The method yielded rather low numbers of parasitoids in 1973 with the result that a different sampling scheme was attempted in 1974. This was a large net on a lightweight frame covering an area 3 ft. by 6 ft. which was flopped down in the oats field with the aid of a long handle. The net was then propped up similar to an emergence trap so any parasitoids trapped inside would move upward to a trap located at the top of the net. This was then left for one to two hours before the parasitoids were removed.

Two problems were encountered with this method. The parasitoids did not seem as willing to move upward as had been expected, and late in the season when the grain was tall, it filled the net with a thick mass of vegetation which left very little room for the parasitoids to move freely. Consequently this method was not attempted again.

## Results and Discussion

The results from the 1973 and 1975 D-Vac samples are shown in Table 19.

The highest density obtained in 1973 was in second oats on June 13 when 0.25 parasitoids/$ft^2$ were caught. In

Table 19. Density of adult *Diaparsis* n.sp. at Niles, Michigan determined with a DVac sampling machine.

| | Oats-1 | Oats-2 | Oats-3 |
|---|---|---|---|
| | | 1973 | |
| June 6 | .1* | ** | |
| June 8 | .05 | | |
| June 11 | .05 | .05 | |
| June 13 | 0 | .25 | |
| June 16 | .05 | .05 | .1 |
| June 18 | .05 | | |
| | | 1975 | |
| June 5 | .78 | 1.0 | |
| June 10 | .52 | .28 | |
| June 12 | .20 | .28 | .32 |
| June 16 | .08 | .08 | .20 |
| June 19 | 0 | .04 | .16 |
| June 24 | 0 | 0 | 0 |

* Density expressed as individuals per $ft^2$.

**Blank space indicates no sample taken.

two of the remaining samples two parasitoids were caught and in the rest either one or none were taken.

In 1975 the highest density occurred on June 5 in second oats when $1/ft^2$ were caught. On the same date the first oats density was $0.78/ft^2$. The data from neither year can be interpreted as an absolute density since no data are available on the efficiency of the DVac in sampling for *Diaparsis* n.sp. However, they do indicate the relative densities in the two years.

Although the data collected in this study are minimal, they do reveal one aspect of the behavior of *Diaparsis* n.sp. which is that males do not move into the oat fields except in very low numbers. The total number of *Diaparsis* n.sp. caught by the DVac in the three strips of oats in two years was 121, and only four of these were males.

Through most of the season in the Niles insectary, males apparently remain on the flowers in the undisturbed stubble area where emergence takes place. Observations in Yugoslavia also showed that males spend nearly all their time on flowers growing at the borders of fields, with very few being in the grain field itself.

On two occasions in 1975 at Niles, males were found at the edge of Oats-3 in high numbers. This was about 75 yds. from the main emergence area where the males had been concentrated on flowers. The first time was June 5. It is difficult to estimate the numbers which were seen; however, the first time approximately 100-150 were flitting about low to the ground in the shade of a tree at the east end of the field. Although the shaded area was large, the area where the parasitoids were found was about one yd. across.

The second occasion when a similar concentration was seen was on June 11. This time it was on the north edge of the field and many more parasitoids were present. Again it is difficult to estimate the number, but, more than 500 were present. Again they were in a shaded area beneath a large tree growing on the edge where third oats and the previous year's wheat stubble met. At this point the stubble field was primarily covered with a volunteer timothy. The parasitoids were moving around in an area several yards in diameter both in the oats and the timothy. In the oats they were low to the ground, just above the crop canopy, but in the timothy they were higher, close to the top of the vegetation.

Activity in both cases seemed to be largely limited to flying around and resting on the foliage. Very few females were seen in the first group and none were seen in the second.

The reason for this behavior is not apparent, however, by this time the peppergrass blossoms on which they had been feeding were nearly all gone. This could have triggered a movement out of the area where they had been feeding, but it does not explain why they were aggregated as they were. The fact that both of these concentrations were in shaded areas may indicate that temperature or humidity or both may have been factors in this occurrence.

Overwinter Mortality

Methods

Overwinter mortality of *Diaparsis* n.sp. was investigated by taking 15 18"x36"x3" deep soil samples from the second oats stubble strip during the fall of 1974. The CLB pupal cells were removed from the sample by washing the soil through 1/8" hardware cloth baskets. The residue was then dumped into a tub of water and the floating material was skimmed off and refloated in clean water where the CLB pupal cases were sorted out. These were then dissected and the numbers of live and dead *Diaparsis* n.sp. prepupae were recorded for each sample.

The following spring an emergence trap was set up adjacent to each sample site, and emergence was monitored and recorded daily.

Results and Discussion

The total number of CLB cells containing *Diaparsis* n.sp. cocoons was 202, but 31 of these (15%) were dead (Table 20). This mortality occurred in the summer and its cause is unknown, however, a number of factors such as desiccation, pathogens, or heat may contribute to this mortality. Gage (1974) attributes a higher than normal mortality of *T. julis* summer generation to excessive heat and low soil moisture.

Table 20. Survival of Diaparsis n.sp. from cocoon formation to spring emergence at Miles, Michigan 1974.

| | #'s | % | $\bar{x}$ ± S.D. Per sample | Range |
|---|---|---|---|---|
| Alive | 171 | 85 | 11.7 ± 6.7 | 3-29 |
| Dead | 31 | 15 | 2.1 ± 1.9 | 0-6 |
| Total | 202 | | | |
| Adj. # Alive | 280 | | 18.7 ± 10.9 | 5.0-47.5 |
| Trap Catch x ½ (100% efficient) | 204 | | 13.6 ± 9.1 | 3.0-30.0 |
| Survival* | | 73*<br>62+ | | |
| Trap Catch x ½ (80% efficient) | 255 | | | |
| Survival* | | 91*<br>77+ | | |

*Survival overwinter.

+Survival from cocoon formation to adult emergence.

In order to calculate the mortality from this study, it was necessary to adjust the number alive upward by 39%. This was because Sawyer (1976) found that the washing technique used to recover CLB cells from the soil, recovers only 61% of those present. Thus, the total number of live parasitoids was adjusted to 280. Based on these figures, and assuming a 100% efficiency of the emergence traps, the survival rate over the winter was 73%, but if we include summer mortality, survival from cocoon formation until adult emergence was 62%.

In a similar study Casagrande (pers. comm.) also calculated a 73% survival of Diaparsis n.sp. overwinter. However, he determined the emergence traps to be only 80% efficient. If we consider the same trap efficiency in this study, then overwinter survival is 91%, and the survival from cocoon formation to adult emergence is 77%.

In the Casagrande study CLB cells containing parasitoids were placed in soil filled vials in the field in the fall and the following spring emergence traps were placed over them to trap the emerging parasitoids. After emergence the cells were recovered and dissected to determine the number of dead parasitoids. This number included those which were dead when the cells were placed in the field in the fall and the 73% survival rate compares well with the 77% survival rate found in this study.

## Flight Activity

### Methods

Flight activity of *Diaparsis* n.sp. was monitored for two years by the use of malaise traps (Figure 31) which were designed and constructed as outlined by Townes (1972). They were set up early in the year, at or just prior to parasitoid emergence, and were located as indicated in Figure 32. Locations were slightly different in 1975 from 1974 except for the two traps along the north edge of the insectary. The two traps in third oats in 1975 were modified to catch insects from only one direction, one from the south and the other from the north. The catching jars at the top were filled with ethylene glycol and were emptied daily for sorting and identification of *Diaparsis* n.sp.

The traps in 1974 were arranged at the locations shown in Figure 32 in order to determine, if possible, the direction of parasitoid migration out of the insectary. In 1975 it was hoped that the traps in third oats would give some idea of the direction of parasitoid movement between the emergence area and the oats where the host larvae were located.

### Results and Discussion

The total numbers of parasitoids trapped are given in Appendices IVa and IVb. Figures 33 and 34 show sight-fitted curves of the results of the trapping program as

Figure 31. Type of malaise trap used to monitor flight activity of Diaparsis n.sp.

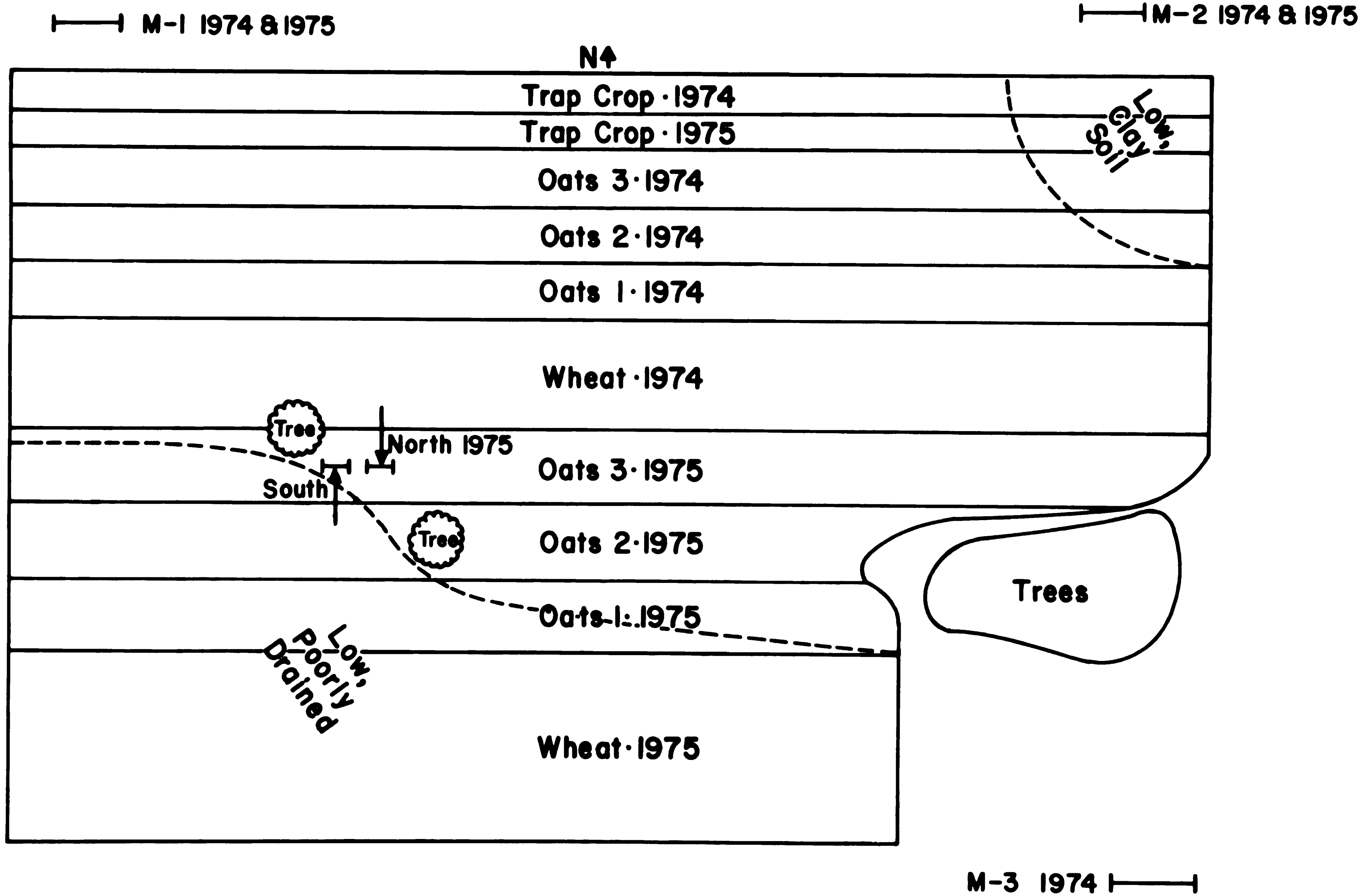



Figure 32. Diagram of the Niles insectary showing location of malaise traps and crops in 1974 and 1975.

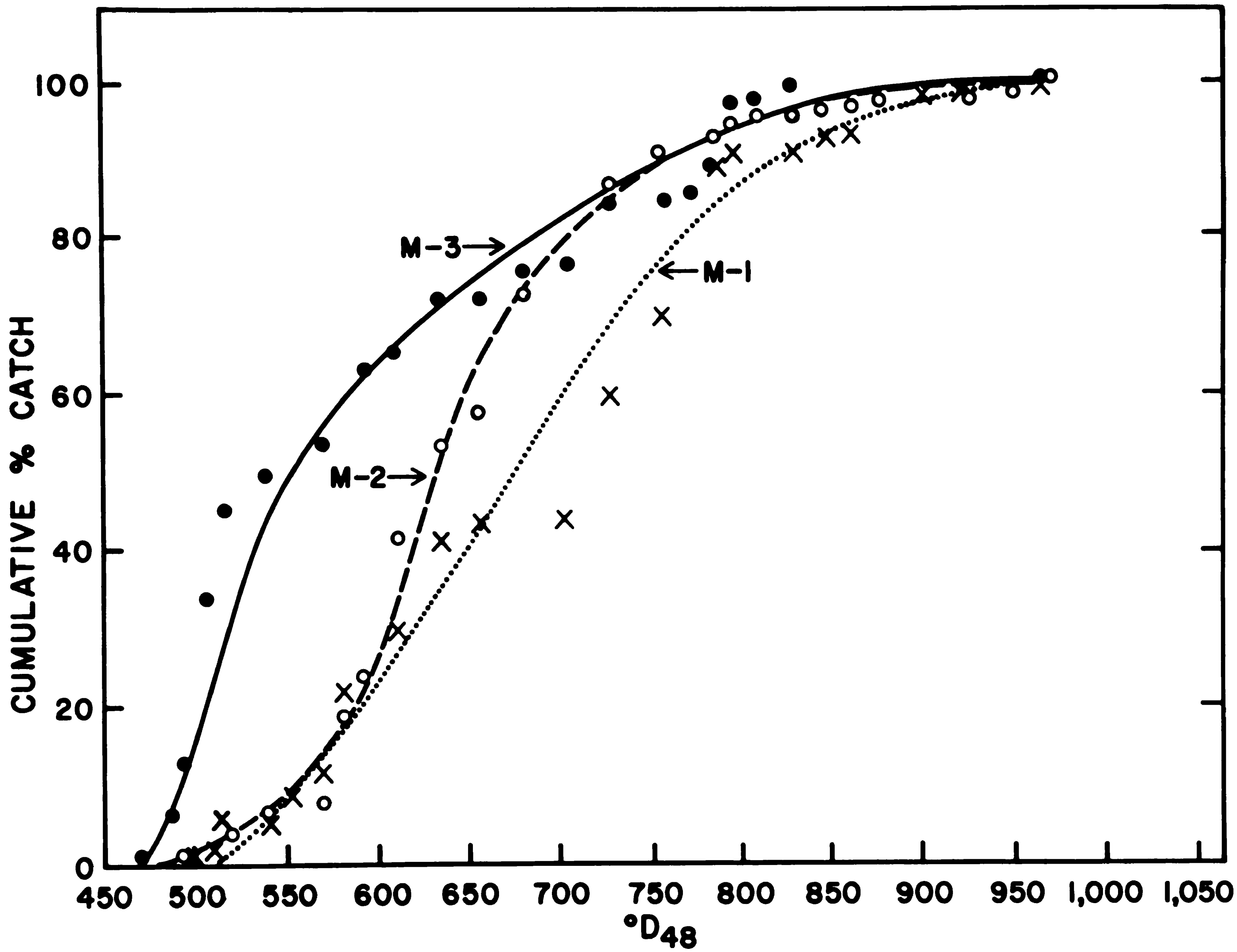



Figure 33. Cumulative percent malaise trap catch of <u>Diaparsis</u> n.sp. plotted over $^{o}D_{48}$ (1974).

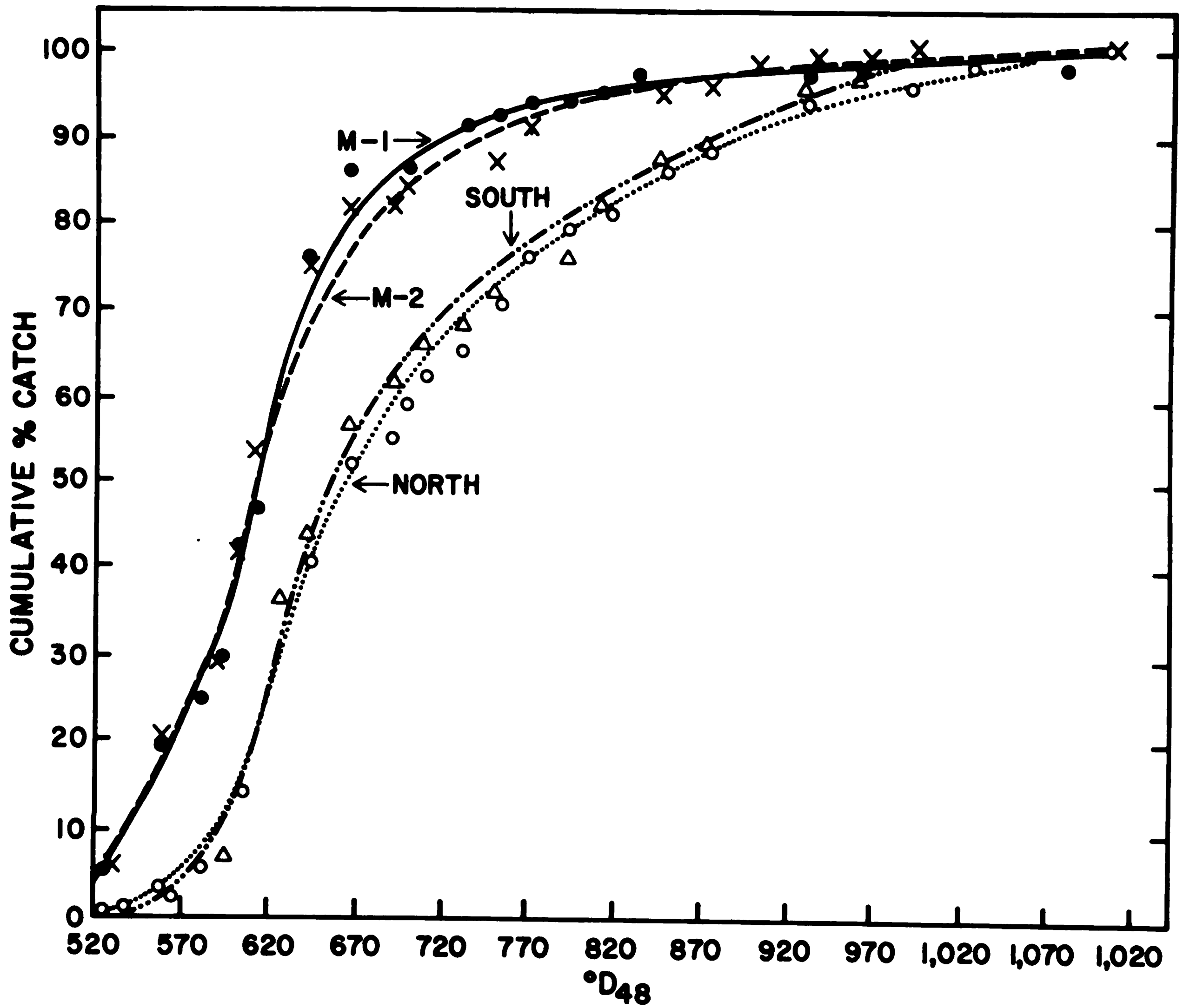



Figure 34. Cumulative percent malaise trap catch of Diaparsis n.sp. plotted over $^{o}D_{48}$ (1975).

daily cumulative percent graphed over $^{o}D_{48}$. The cumulative percentages were analyzed using the distribution free test by Friedman (Hollander and Wolfe, 1973) which shows differences or similarities in location of the curve without interference from differences in magnitude.

In 1974 all three traps showed a significant difference in location of the curve at the .05 level. Trap M-3 was the earliest and M-1 the latest with M-2 intermediate. The early catch of M-3 can be attributed to the location of the trap near the emergence area. The latter two traps were located beyond the oats where the host larvae occurred, and therefore most of the parasitoids had to move over or through this area before they came to the traps. The exception was those emerging from the trap crop stubble but this number was not large.

Besides a difference in location of the curve, M-2 also caught a much higher number of parasitoids (149) than M-1 (51) which can also be attributed to the location of the trap at the northeast corner of the insectary. Since prevailing winds are from the west and southwest in this area, it appears that the higher catch in M-2 was influenced by the wind.

Comparisons of the 1975 catches again showed significant differences at the .05 level between the various traps. Comparisons of M-1 and M-2 alone, however, showed that these were not significantly different in location of the curve (=time of capture), but M-2 caught many more than M-1 just as in 1974.

From Figure 34 it is apparent that curves M-1 and M-2 were very different in time of catch from curves South and North. The main reason for this earlier catch is because in 1975 M-1 and M-2 were near the emergence area, and as a result trapped the dispersing parasitoids much as M-3 did in 1974. In addition to catching parasitoids earlier than the north open and south open traps, M-1 and M-2 also caught far fewer parasitoids.

Comparison of the two traps in third oats (North and South open) shows them to be significantly different in total catch. The catch in the north open trap was much higher than in the south open, which may be attributed to its facing the emergence area. Since the trap catching from the direction of the CLB area caught only about half as many, it appears that movement between the areas was not uniform in both directions.

Graphs showing the fluctuation in numbers caught each day are shown in Figures 35 and 36. The early peak in M-3 is likely due to proximity to the emergence area, and the following fluctuations from day to day may be a response to weather factors such as wind, relative humidity, temperature, etc. However, no clear correlation can be found and it may be a combination of these factors.

The fluctuations in catch in 1975 were similar except for a very high peak early in the season. Aside from the

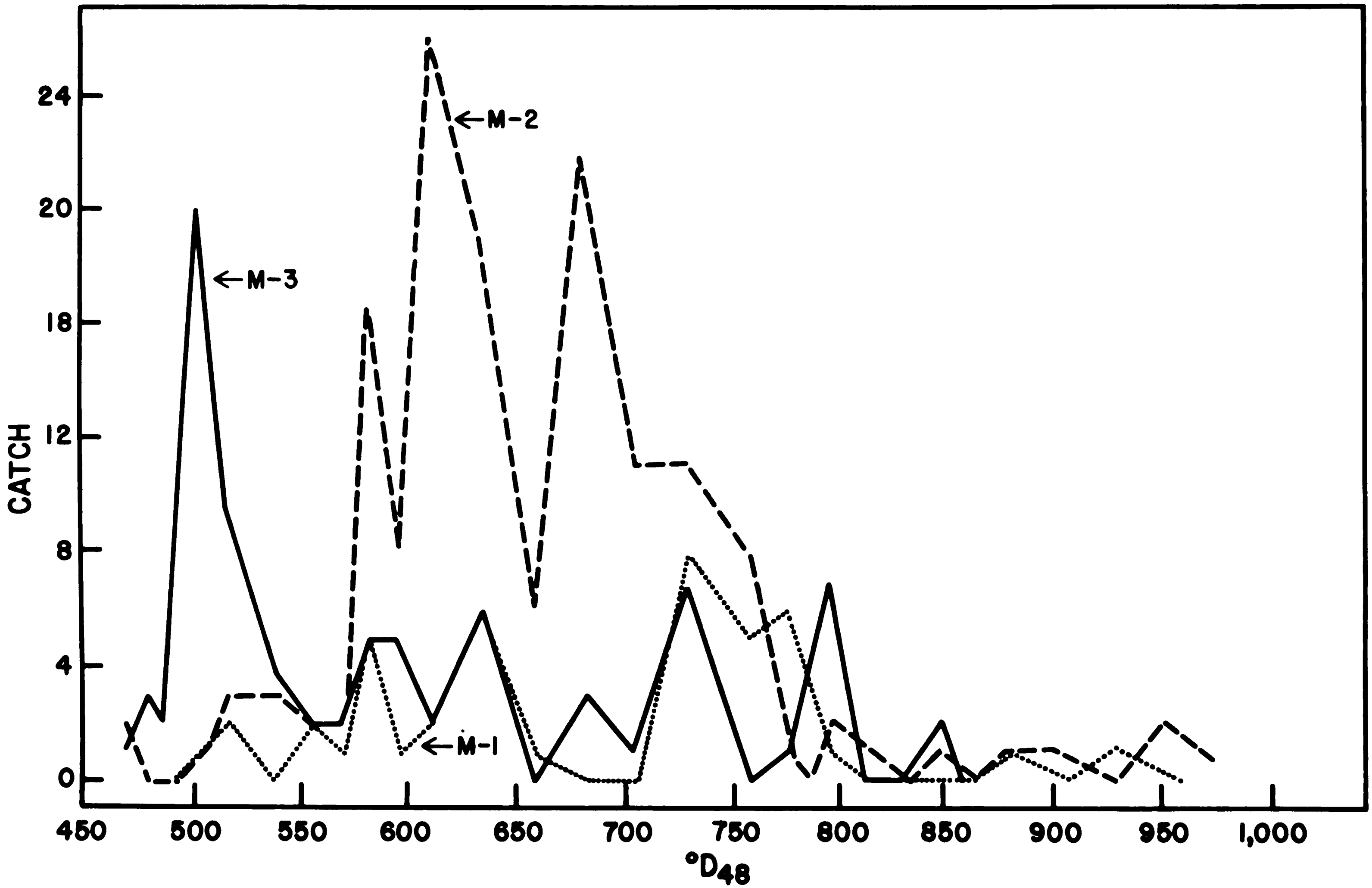



Figure 35. Total daily malaise trap catches of <u>Diaparsis</u> n.sp. over $^{o}D_{48}$ (1974).

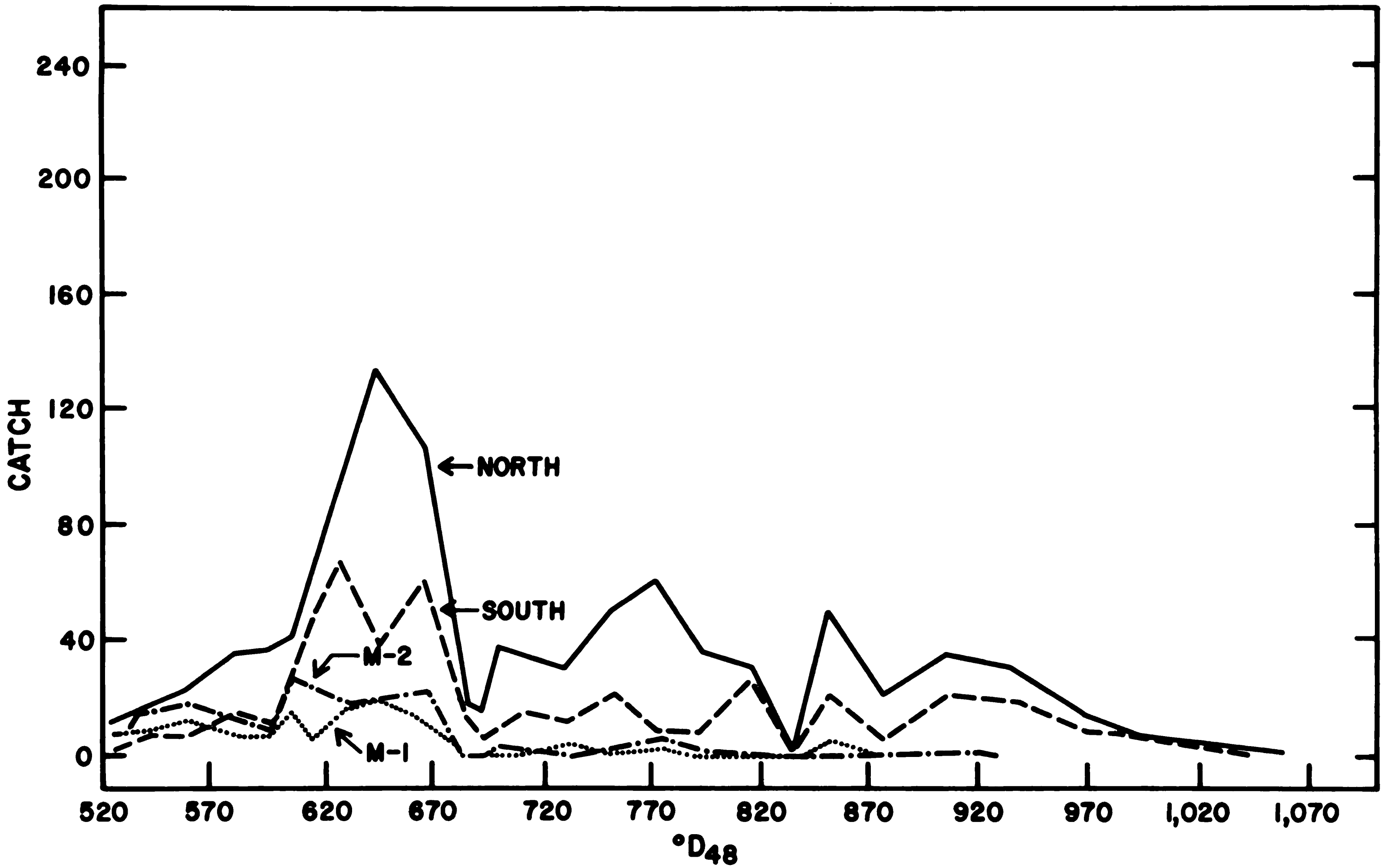



Figure 36. Total daily malaise trap catches of <u>Diaparsis</u> n.sp. over $^{o}D_{48}$ (1975).

weather factors discussed above, there are two possible hypotheses explaining the high peak with a subsequent drop.

One of these is that it is a reflection of behavior. If the parasitoids spend a period of time feeding just after emergence, followed by a period of more intense searching activity, a result such as this may be observed.

A second hypothesis is that this observation may be the result of increased host densities. Rogers and Hassell (1974) conclude that interference of parasitoids resulting from a high parasitoid density leads to emigration of the parasitoid from high density areas. This may have been the case with Diaparsis n.sp. in 1975. The 139% increase in emergence in 1975 over 1974 could have led to frequent interference between the adults with a resultant dispersal giving rise to the high malaise trap catches early in the season. This may also help explain the lower rate of parasitism in 1975 as compared to 1974 in spite of the much higher emergence density.

## Adult Parasitoid Food

Part of the overall biological control effort aimed at the CLB was to investigate the host-parasitoid relationships as they occur in areas where the CLB is native. In an unpublished summary of one of these studies (1972), Dr. Bjegović, of the Institute for Plant Protection in Belgrade, Yugoslavia, reported that Diaparsis spp. do considerable feeding on various wild flowers in the field.

As far as is known this is the first report of this being observed for _Diaparsis_ spp. although it is known for other species.

The species which Bjegović reported as food sources for adult _Diaparsis_ spp. in Yugoslavia are listed in Table 21. In addition to the plants listed, I also observed fair numbers of _Diaparsis_ spp. feeding on yarrow, _Achillea millefolium_, in Yugoslavia.

The order of importance of the plants listed in Table 21 was determined by Bjegović by observations in the laboratory of relative numbers of parasitoids visiting each plant.

Following the report of the findings in Yugoslavia, observations were made on flowers occuring in the Niles insectary. In 1974 _Diaparsis_ n.sp. was found feeding on yellow rocket (_Barbarea vulgaris_) and field cress (_Lepidium campestre_). In 1975 a more thorough examination was made in this area and seven species were found with _Diaparsis_ n.sp. feeding on them. These are listed in Table 22.

In addition to these, several pots of greenhouse-reared _Brassica kaber_ were set out in the field and several _Diaparsis_ n.sp. were observed to visit and feed on this.

Bjegović (1973) attempted to test the effect of the absence of flowers in the field by monitoring parasitism in two wheat fields, one of which was treated with herbicides to destroy the flowering plants. Parasitism was monitored

Table 21. Flowers reported by Bjegović as food plants for adult *Diaparsis* spp. (Listed in order of preference.)

| | |
|---|---|
| *Stellaria media* Vill. | Chickweed |
| *Sinapsis alba* L. | White mustard |
| *Lepidium draba* L. | Hoary cress |
| *Capsella bursa-pastoris* L. | Shepherds purse |
| **Lamimum amplexicaule* L. | Dead nettle |

*In the laboratory only.

Table 22. Flowers found at Niles, Michigan to serve as food plants for adult *Diaparsis* n.sp.

| | |
|---|---|
| *Barbarea vulgaris* (L.) | Yellow rocket |
| *Lepidium campestre* (L.) | Field cress |
| *Erigeron annuus* (L.) | Daisy Fleabane |
| *Stellaria media* L. | Chickweed |
| *Achillea millefolium* L. | Yarrow |
| *Viburnum* sp. | Viburnum |
| *Rubus* sp. | Dewberry |

by dissection of CLB larvae collected daily throughout the season and also by rearing CLB larvae.

The results of this investigation revealed a 5.9% parasitism rate in the field treated with herbicides and 9.7% in the untreated field. Although these data show a higher parasitism rate with flowers present, it was felt that since the fields were only about 500 feet apart there may have been some fly-over from one field to the other, which may have influenced parasitism. Also, since Diaparsis spp. appears to be more abundant in oats, more definitive results could perhaps have been obtained by using oats rather than wheat.

In the work at Niles, an attempt was also made to evaluate the effect of wild flowers on parasitism. The design of this test was outlined earlier in the discussion of parasitism rates. Initially it was not known that such a wide variety of flowers serve as a source of food for Diaparsis n.sp. Therefore, the proximity of various flowers to the test area could have affected the results and made them largely inconclusive.

In addition to the feeding which was observed on flowers, it was found in the laboratory that Diaparsis n.sp. also feeds on host-larval fluid at times. This has been reported for other parasitoids (Flanders, 1942, Leius, 1961) and likely occurs in the field as well as in the laboratory.

## Discussion

Observations in both Yugoslavia and Niles suggest that flowers are an important requisite for *Diaparsis* n.sp. Both males and females feed heavily on those mentioned above. At Niles, males were found more readily on the flowers growing in the emergence area and females were found more readily on flowers in or adjacent to the area where hosts were located. In Yugoslavia where food plants were nearly all in or bordering the crop, both males and females were found on them.

With regard to wild flowers and the effectiveness of *Diaparsis* n.sp., it appears reasonable to assume that there is a relationship. Such a relationship is indicated in the literature for other species. Wolcott (1942) attributes the successful introduction of a parasitoid of the mole cricket into Puerto Rico directly to the presence of two flowers on which the adult parasitoids feed. When these flowers were absent, establishment did not occur even though hosts were abundant.

A more recent study of parasitism in unsprayed non-commercial orchards (Leius, 1967) showed an 18X increase in parasitism of tent caterpillar pupae (*Malacosoma americanum* (F.)) in orchards with a rich growth of flowers over orchards with few or no flowers. Parasitism of the codling moth (*Carpocapsa pomonella* L.) was 5x greater with flowers present. van Emden and Williams (1974) feel that areas of floral diversity outside the crop should be maintained until we are certain that their beneficial capacities can be replaced by biological control or planned diversity. However, in the *Diaparsis* situation it appears that such diversity is a necessary component of biological control.

## Interaction with *Tetrastichus julis*

In an effort to establish effective biological control of the CLB in the U.S., three larval parasitoids have been established. The dynamics of one of these, *Tetrastichus julis*, has been studied by Gage (1974). The dominant parasitoids in areas where all three have been established are *T. julis* and *Diaparsis* n.sp. Since these are both larval parasitoids, it is important to look at their interaction. The following discussion is not meant to be definitive, but several observations may be made concerning interactions in the field.

The life cycles of these two parasitoids are essentially the same except that *T. julis* has a partial second generation. The emergence curves are compared in Figure 37 although the relationship between these curves may vary from year to year depending on weather factors. A general relationship is seen, however, in that first generation *T. julis* emerges earlier than *Diaparsis* n.sp.

There is considerable overlap between these parasitoids during the first generation of *T. julis* which might lead to a great deal of interference between them, however, multiparasitism is lowest early in the season.

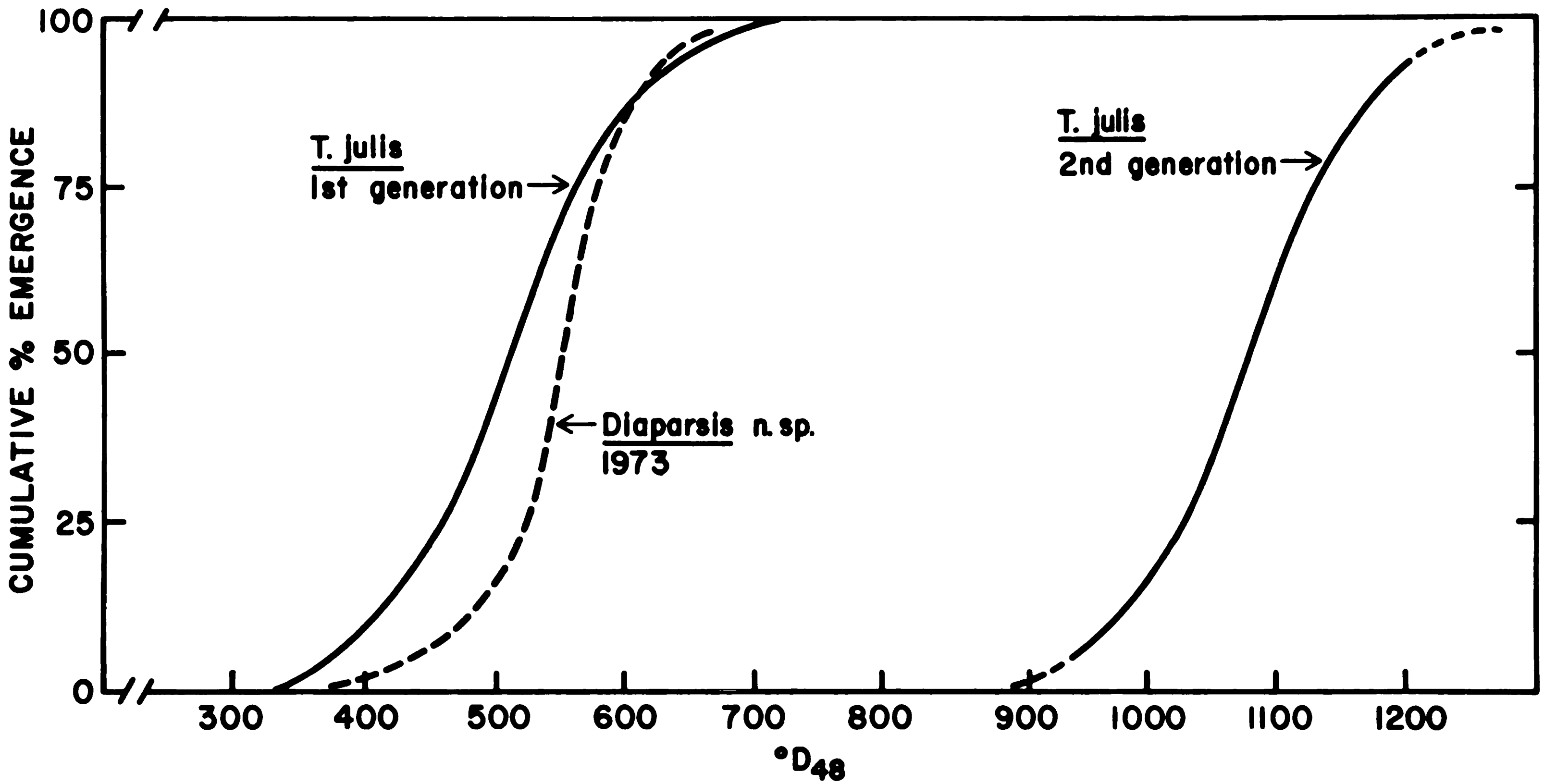



Figure 37. Comparison of emergence curves of Diaparsis n.sp. and T. julis. Adapted from Gage, 1974.

(See Figure 30). This is likely related to several factors. From emergence trap catches and from monitoring parasitism rates at Niles, it is evident that the first generation of T. julis is quite small (although it may be much larger at other localities). Therefore interference with Diaparsis n.sp. in the early part of the season is minimal. Host densities are also generally high early in the season, which helps to avoid multiparasitism.

According to Gage (1974) T. julis ends its first generation at about 850 $^{o}D_{48}$. So when Diaparsis n.sp. is most active, T. julis is not present.

Much more multiparasitism occurs late in the season as the second generation of T. julis appears and host densities decrease, but even here it is not serious. The total multiparasitism involving T. julis and Diaparsis n.sp. was only 36 (2.1%) in three years.

The following question needs to be answered, "Do these two parasitoids negate each other in any way?" This cannot be definitively answered at this point, but it does not appear that they do. Since T. julis is bivoltine and Diaparsis n.sp. fits essentially between the two generations, effects on each other are minimal.

The greatest effect of having Diaparsis n.sp. in the system from the viewpoint of management of the CLB if Diaparsis n.sp. is valued is that it narrows, if not closes, the biological window for insecticide application discussed and predicted by Gage (1974). Since Diaparsis n.sp. is present and active until *T. julis* begins its second generation, there is virtually no time when it would be feasible to apply insecticides to the larval population without affecting some of the parasitoid populations.

Although, from the observations above, it does not appear that *Diaparsis* n.sp. and *T. julis* have any great effect on each other, it seems that both have not attained high populations in the same area to date. In Michigan, both parasitoids were released at the MSU research farm at Gull Lake in Kalamazoo County and in the USDA insectary at Niles, in Berrien County. At both localities both parasitoids have been established, but at Gull Lake the dominant parasitoid has been *T. julis* and at Niles, only about 60 miles southwest, it has been *Diaparsis* n.sp. Also, in other insectaries established by the Niles laboratory in the Midwest and Northeast U.S. by sub-colonizations from the Niles insectary, a similar trend is developing (Burger, pers. comm.). In some of these *T. julis* is more abundant and in some *Diaparsis* n.sp. is more numerous.

In addition to the above considerations, it appears that a similar situation exists in Europe. In their European work Dysart, et. al., (1973), give the percent parasitism in a number of localities over a seven year period (Table 7). In nearly all cases parasitism by one parasitoid is high and the other low or both are low. In no cases were both high except in one locality in Sweden where parasitism by *Diaparsis* was 18.2% and by *T. julis* 27.2%.

It would appear that there is some ecological factor operating which favors one over the other in some areas and vice versa in other areas. Any attempt at this point to define what this factor might be would be mere speculation. More study is needed to evaluate the relationship of these two parasitoids and their effect on each other in various ecological conditions and population densities, but it does tend to support the position of many species introductions vs. single species introductions.

## EUROPEAN WORK

Part of the cooperative agreement with the United States Department of Agriculture under which this work was conducted, stipulated that an effort be made to find a means of separating the two species of parasitoids in the larval stage. This would make it possible to identify the species present in dissections of field-collected hosts and avoid the problem of rearing them to the adult stage.

To do this it was necessary to obtain parasitoids directly from Europe since only *Diaparsis* n.sp. is available in the U.S. Consequently, the 1974 field season, mid-April to mid-July, was spent in Europe, primarily for the purpose of collecting parasitoids, but also to make observations of the CLB and *Diaparsis* spp. in their native areas.

According to communications from the USDA Parasite Laboratory in Sevres, France, the area around Belgrade has a consistently high population of CLB. Therefore, approximately two months were spent in Zemun at the Biological Control Division of the Institute for Plant Protection of Belgrade.

Since a main objective in the European work was collection of material for laboratory use, considerable time was spent rearing CLB larvae to obtain parasitoid cocoons. Using a method developed by Gruber, et. al. (1972), a total of 19,500 larvae was reared. The cocoons obtained from these rearings were held for emergence of CLB's and non-diapausing parasitoids. The remaining cells were then sent via the USDA Laboratory in France to the quarantine lab at Newark, Delaware where they were held for emergence and subsequent shipment to MSU.

A few CLB cells were also collected in Central Denmark near Kolding which is where the CLB was reported as having the highest densities. In 1974, however, densities were very low and only 44 unemerged CLB cells were shipped to the U.S. Some of these contained *Tetrastichus julis* and some *Lemophagus curtus* so the actual number of *Diaparsis* spp. obtained was rather low.

The agricultural conditions and host behavior in Europe, were considerably different than expected, so not all the work could be carried out as planned. Some problems were also encountered due to an exceptionally rainy season, with the result that much of the information gathered is in the form of observational data rather than data subject to statistical analysis.

## Species Composition

The exact geographical distribution of the two species of *Diaparsis* in Europe is not currently known; however, both species occur in Yugoslavia with *Diaparsis* n.sp.

predominating. This was apparent in several of the sampling procedures, one of which was malaise traps. From a total of 4487 Diaparsis spp. caught in these, 85% were Diaparsis n.sp. and 15% were D. carinifer.

These figures are only relative since the sampling schemes may be selective in some way due to differences in species behavior. Also the mortality in lab rearing and emergence may differ between the two species. It seems clear, however, that Diaparsis n.sp. is the dominant species in Yugoslavia.

Although only a small number of larvae could be collected for rearing, the situation in Denmark appears to be different. All 15 adults received at MSU were found to be D. carinifer. This of course is not a large enough sample to rule out the possibility of Diaparsis n.sp. being present, but it does seem to indicate that D. carinifer is at least dominant in Denmark.

## Flight Activity

Part of the work in Yugoslavia was a Diaparsis spp. population monitoring program done with two malaise traps designed the same as those used at Niles (Figure 31).

The traps were set up on May 25 and were monitored daily for the remainder of the parasitoid season. Both traps were located between oat and wheat fields in narrow uncultivated areas. Trap 1, with the long axis north-south and thus open to the east and west, was located at the corner of a wheat field with a hedgerow containing trees and shrubbery behind and to one side of it. Trap 2 was in a more open area and was oriented in an east-west direction opening to the north and south (Figure 38).

The total catch of *Diaparsis* was 4487 with the highest catch occuring on May 28 when 730 were caught in one 24-hr. period. The total catch for the season was lower in Trap 1 (657) than in Trap 2 (3830).

In terms of sex ratio it was found that the catch of *Diaparsis* n.sp. is about equally divided with 47% of the total being males and 53% females, but in *D. carinifer* only 11% were males and 89% females.

Although the overall sex ratio was nearly 1:1 in *Diaparsis* n.sp. it appeared that males predominated early in the season. Consequently the catches of both species for the first 15 days of trap operation were analyzed to see if a relation existed between percent of males captured and the total catch. The initial test used was Kendall's tau which is a coefficient of rank correlation. The result of this showed no significance in correlation of percent males to total catch for *D. carinifer*, but for *Diaparsis* n.sp. the correlation was significant at the .002 level.

This was further analyzed using a least squares linear regression statistic by which the equation of the regression line (Figure 39) was determined to be $Y=13.18+0.091x$ with an $R^2$ value of .5665. The T value (HO:B=O) for this regression was 4.122 which is significant at the .001.

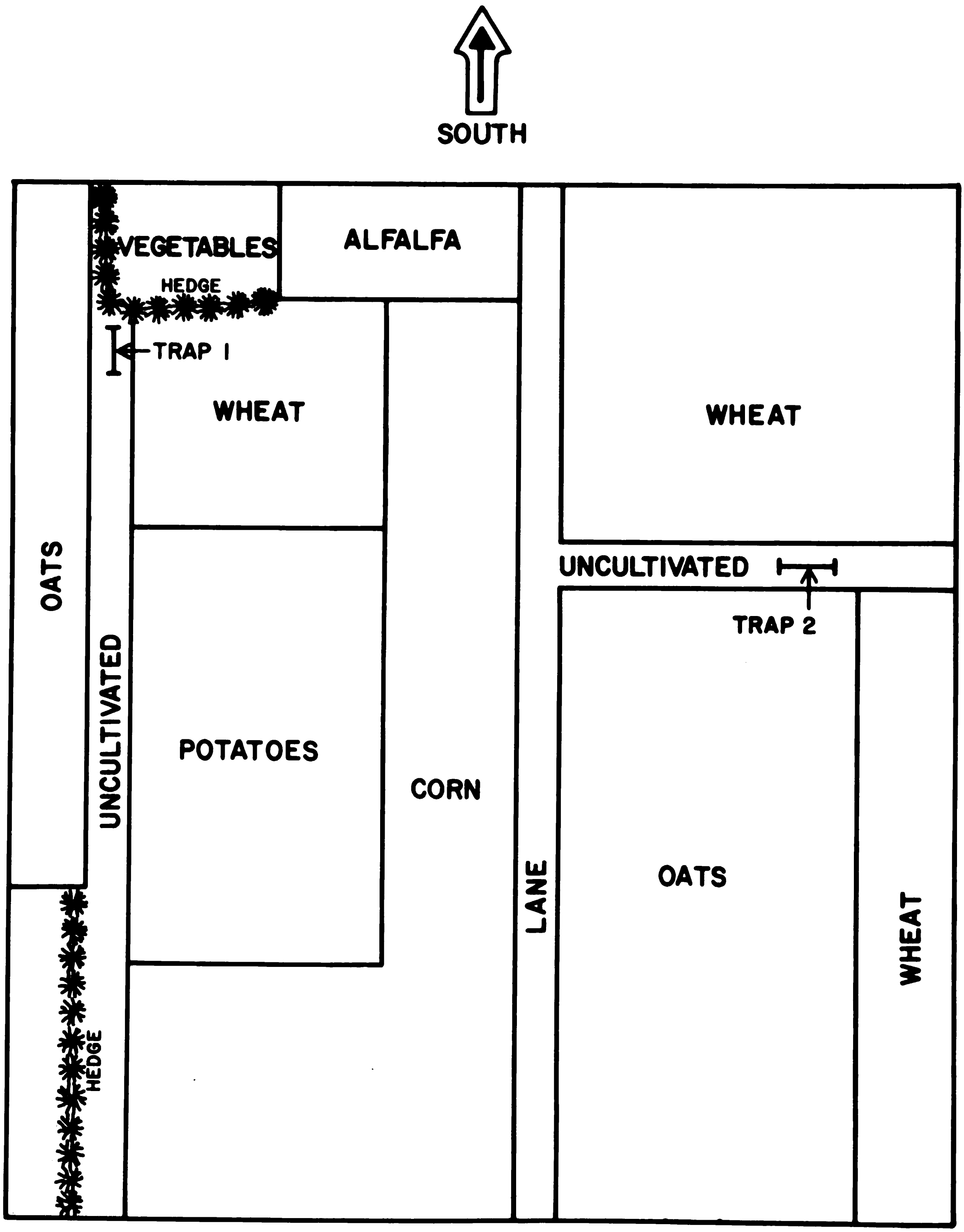



Figure 38. Diagram of the study area in Yugoslavia and the location of the malaise trap.

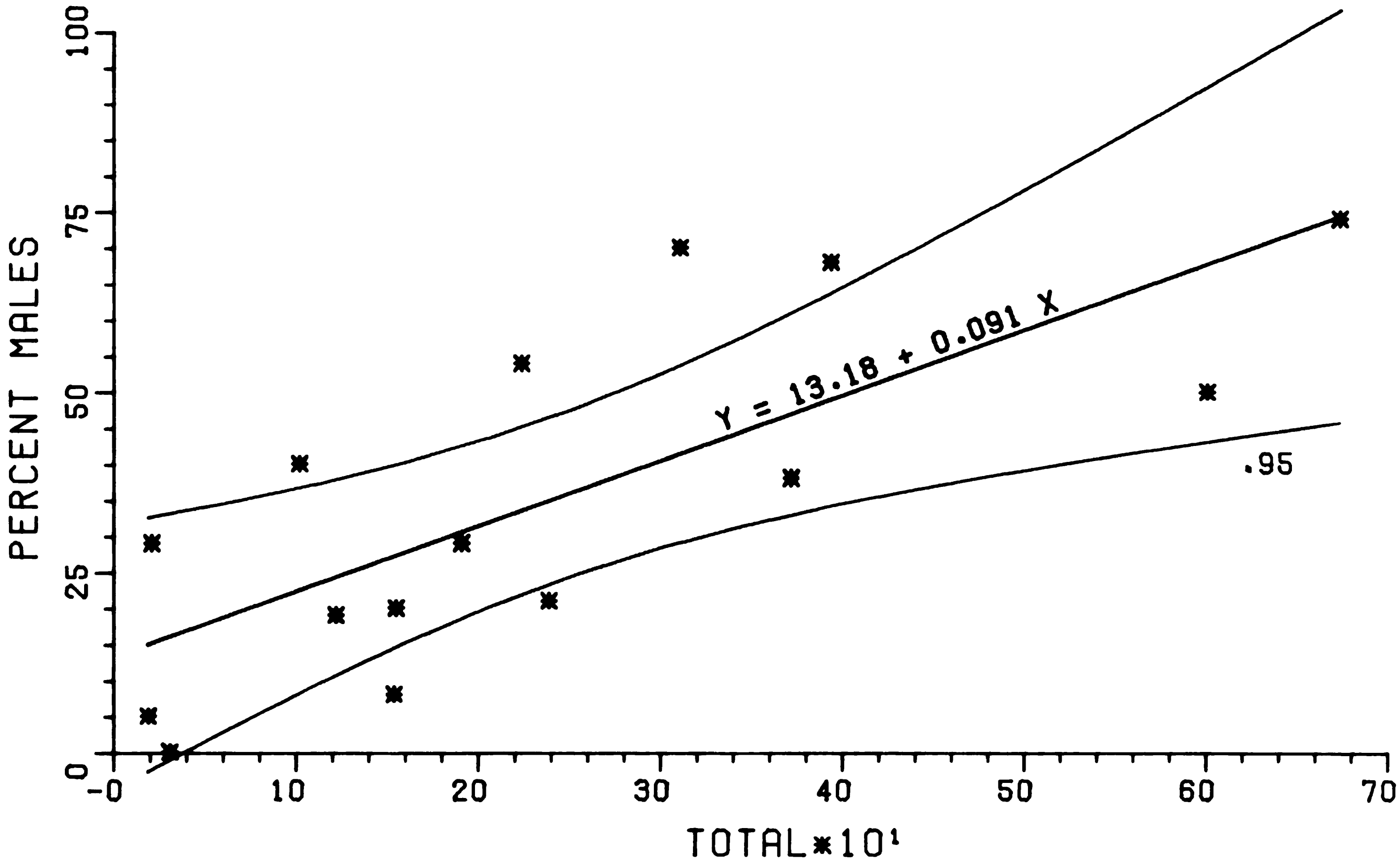



FIG. 39. REGRESSION LINE AND EQUATION WITH .95 CONFIDENCE INTERVAL SHOWING RELATION OF PERCENT MALES TO TOTAL CATCH OF DIAPARSIS N.SP. IN MALAISE TRAPS IN YUGOSLAVIA.

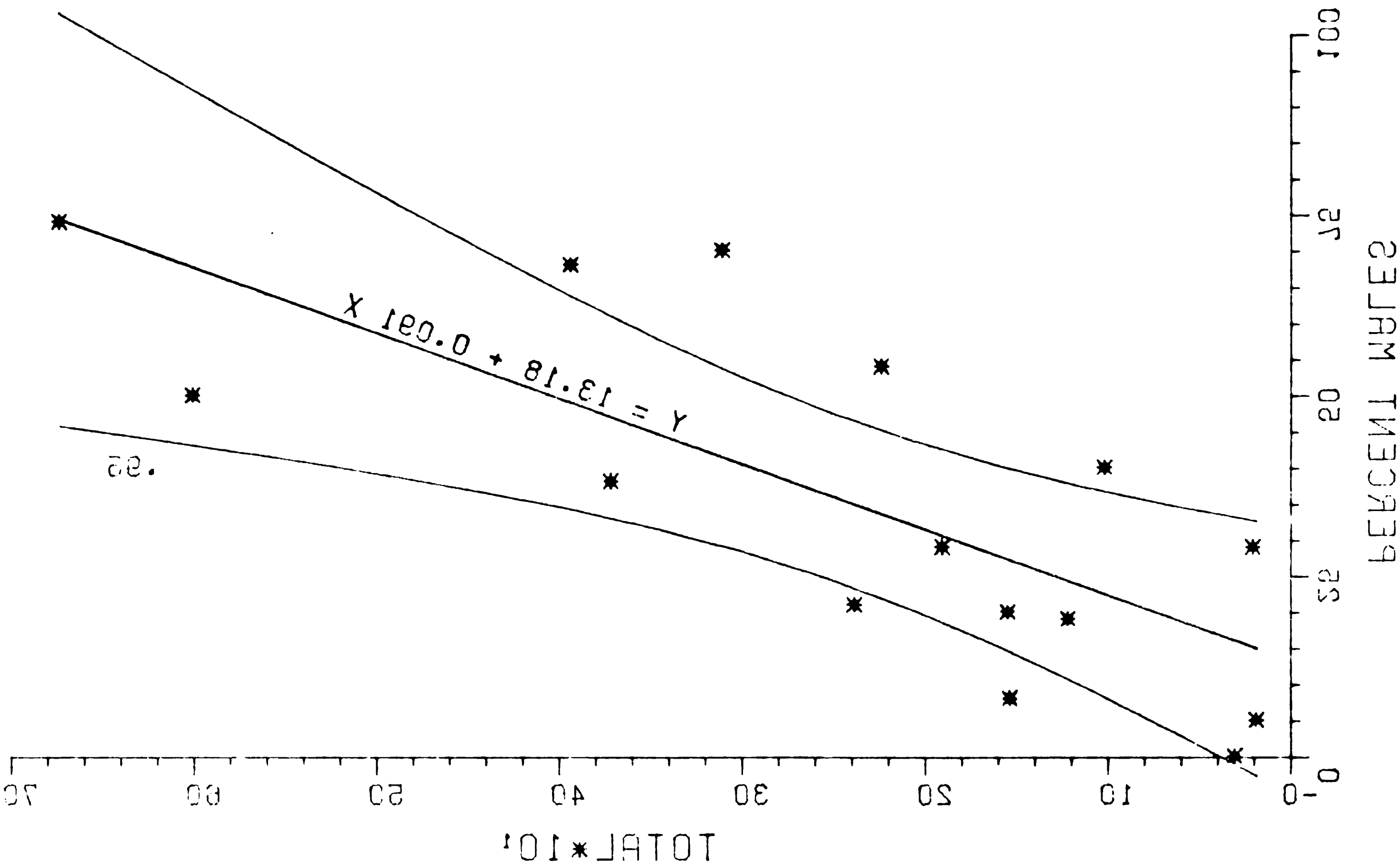



FIG. 39. REGRESSION LINE AND EQUATION WITH .95 CONFIDENCE INTERVAL SHOWING RELATION OF PERCENT MALES TO TOTAL CATCH OF DIAPARSIS N.SP. IN MALAISE TRAPS IN YUGOSLAVIA.

This shows quite clearly that early in the season malaise traps did not sample the population accurately with respect to sex ratio. The explanation for this apparently lies in the differing behavior of the two sexes, the females tending to remain within the fields where hosts are located and the males tending to be more active in searching for flowers outside the grain fields. Sampling within the grain fields substantiated this since of 158 parasitoids caught only one was a male.

Emergence

Twenty-three emergence traps were set up on May 8 which was somewhat later than originally planned, but circumstances did not permit an earlier starting date.

The traps were made according to a design received from Klaus Carl of the Commonwealth Institute of Biological Control in Delemont, Switzerland. The trap consisted of a wood base frame 3 in. high and 1 $yd^2$, with a fine mesh netting sewn in the shape of a pyramid stapled to it. The top of the netting was taped to the mouth of a plastic funnel, the base of which was inserted into a hole in the bottom of a one pint freezer box and sealed with silicone rubber.

The trap was erected by placing it over a heavy gauge wire stuck into the ground with a horizontal loop supporting the funnel and thus expanding the trap. The boxes at the top were partially filled with ethylene glycol to preserve the trapped parasitoids.

Since no uncultivated stubble fields were available, it was necessary to place most of the traps in grassy areas along field borders. Sixteen traps were placed in such areas and the remaining seven were placed in an oats field which was already growing to see if Diaparsis spp. could emerge from a cultivated field. All the traps were checked daily for approximately 2½ weeks.

Only two or three Diaparsis spp. were caught during the entire period of monitoring them, which is not surprising as far as those traps located in field borders is concerned. Numbers of CLB larvae are not high enough in wild grasses to make trapping of parasitoids possible.

As noted earlier, the malaise traps revealed that a high population of parasitoids were present. Since they could not have come from field borders and other areas of wild grasses, they must have emerged from fields which were cultivated.

The failure of the emergence traps in the oat field to catch emerging parasitoids may be due to mislocation of the traps. At the time they were set up it was assumed that oats had been planted in the field the previous year. If, however, it was planted to some other crop, the parasitoid population would have been low or non-existent. This would explain why these traps caught no parasitoids. Since only one field season was available for work in this area, it was not possible to modify plans for a second year.

Adult Density

The relative density of adult parasitoids in oat fields was sampled several times through the season using a standard sweep net with 100 sweeps constituting one sample. Two areas, one brown and the other green, were sampled each time. The brown areas of the field were areas where most of the crop foliage had been destroyed by CLB larvae, while green areas still appeared a normal color but had a high larval density.

The results of these sweep samples are given in Table 23 and show that consistently more parasitoids occur in areas of the field where host larvae occur. *Diaparsis* n.sp. showed a greater difference between the two areas than *D. carinifer* did and was much more numerous in all the samples except one. The slight difference on the last date may have been partially due to a declining population, but it was likely also due to the fact that nearly the entire field was turning brown from larval feeding. With little green area left it would seem that the parasitoids had dispersed in search of hosts.

This spatial movement of the parasitoids in response to a corresponding shift of the host population is indicative of a well synchronized host parasitoid relationship and also indicates an effective searching ability on the part of the parasitoid.

The fact that of the 209 parasitoids caught, only one was a male shows that few males occur in the fields where

Table 23. Numbers of adult[5] *Diaparsis* spp. caught in sweepnet samples in oats at Zemun, Yugoslavia.

| Date | Brown Area[1] *Diaparsis carinifer* | Brown Area[1] *Diaparsis* n.sp. | Green Area[2] *Diaparsis carinifer* | Green Area[2] *Diaparsis* n.sp. |
|---|---|---|---|---|
| June 5 | 11[3] | 16 | 7 | 29 |
| June 8 | 2 | 6 | 8 | 49 |
| June 10 | 1 | 2 | 2 | 22 |
| June 14 | 3 | 2 | 10 | 20 |
| June 19[4] | 3 | 5 | 4 | 7 |

1. Areas where most crop foliage was destroyed by CLB larval feeding.

2. Areas where crop foliage was still green but with a high population of host larvae.

3. Numbers of parasitoids are numbers/100 sweeps.

4. Most of the sample field was brown by this date.

5. All were females except one.

the host is found as was pointed out in the discussion on malaise trap catch.

Field Observation

In an area with a high parasitoid density such as Zemun, it is possible to spend time in the field observing them in their natural environment. This was done a number of times in the hope that these observations would reveal some useful information concerning attack rate in the field.

Observations were most often made in the evening since similar attempts in the morning showed that little activity occurred before dew had dissipated. Later in the day when there was generally a breeze, it was difficult to keep track of the parasitoids since they searched lower in the oats and also were more subject to being blown about by the wind.

Observations were made by simply standing in the field in one spot until a parasitoid was seen. It was then followed and timed with a stop watch from first sighting until it was lost from sight. During this time notation was made of hosts located and ovipositions attempted.

The results of these observations are given in Table 24. Most of these observations were made in mid-to late season when many host larvae were large. Few, if any, of the oviposition attempts noted above were successful since the defense of the larger CLB larvae is to release

Table 24. Observations of searching and attack by *Diaparsis* spp. in the field at Zemun, Yugoslavia.

| o # | Time Observed | Hosts Located | Ovipositions Attempted |
|---|---|---|---|
| 1 | 5 min. 15 sec. | 1 | 1 |
| 2 | 9 min. 0 sec. | 6 | 5 |
| 3 | 9 min. 10 sec. | 5 | 4 |
| 4 | 20 min. 0 sec. | 0 | 0 |
| 5 | 4 min. 30 sec. | 2 | 2 |
| 6 | 11 min. 0 sec. | 2 | 2 |
| 7 | 6 min. 50 sec. | 2 | 1 |
| 8 | 2 min. 30 sec. | 1 | 1 |
| 9 | 3 min. 0 sec. | 0 | 0 |
| 10 | 3 min. 0 sec. | 1 | 1 |

from the leaf when contacted by the parasitoid, and fall to the ground.

Searching by the parasitoids was mostly by flight within the crop canopy at the level of the foliage. Flight was slow and somewhat erratic as leaves and stems were approached. Periodically the parasitoid landed upon a leaf and searched it more thoroughly by walking.

Much time was also spent cleaning and grooming. Parasitoids which sat for as much as ten minutes with no activity other than grooming were observed. One was also observed to search repeatedly for 20 minutes a small area of a single leaf which did not contain any larvae.

For the most part, however, *Diaparsis* spp. appeared to search efficiently. Judging from the times recorded in Table 24, hosts were located about once every 3.5 to 4 minutes during the times of day when they were searching for hosts.

Another characteristic of *Diaparsis* spp. attack observed at Zemun and later at Niles, Mich. was that small larvae were often turned on their backs in the process of oviposition (Figure 40) and were left in this position sticking to the leaf by the fecal coat.

This is typical of *Diaparsis* n.sp. but it is not thought that it occurs very often with *D. carinifer* due to the difference in oviposition. Since these larvae, especially the very small, are not able to right themselves, this is a mortality factor to both the host and

(a)

(b)

Figure 40. Photographs showing Diaparsis n.sp. attacking an early instar larva (a) and the same larva left sticking on its back on the leaf (b).

the parasitoid, but it needs further study to determine its significance.

Host Behavior

One of the greatest differences found in the situation in Europe as compared to the U.S. concerns the behavior of the CLB which was found to be much more gregarious than in the U.S. Early in the season a number of larval density measurements were made by randomly selecting ten one-ft.$^2$ plots in a field and counting all the larvae in each plot. The results of these (Table 25) show that the densities varied widely within a field.

The gregariousness of the adult CLB becomes increasingly evident as the season progresses and areas of original infestations turn brown from larval feeding with other areas remaining green. During the course of a season it is possible to walk through a field and find areas of complete foliage destruction due to larval feeding with a few large larvae only. Around this the field is greener and larvae are smaller and farther out they become smaller still, the foliage appears normal, and eggs and adults may be present. Still farther only eggs and adults are found and finally no sign of CLB's is present. No behavior such as this has been recorded in the U.S. but rather entire fields are more uniformly infested. If an area of low or high infestation does occur within a field it tends to remain at about the same level throughout the season.

Table 25. CLB larval densities in oats and wheat at Zemun, Yugoslavia (1974).

| | Oats | | | Wheat | | |
|---|---|---|---|---|---|---|
| Date | Low | High | $\bar{X}$ | Low | High | $\bar{X}$ |
| May 9 | 4[1] | 61 | 18.8 | | | |
| May 10 | | | | 1 | 191 | 54.3 |
| May 13 | 3 | 168 | 33.8 | | | |
| May 21 | 3 | 522 | 92.3 | 5 | 226 | 51.8 |
| May 30 | 140 | 620 | 290.9 | | | |

[1]Density is expressed as number of larvae/ft$^2$.

The CLB is apparently characterized by this gregarious behavior throughout Europe. According to Dysart, et. al., (1973), outbreaks seen in Europe were characterized by circular areas in the fields where plant leaves were whitened by larval feedings. Klaus Carl of the Commonwealth Institute of Biological Control also indicates this to be the case in Switzerland and other areas he has observed (pers. comm.) and he refers to these as "hot spots". Sajo (1893) describes these areas as well defined spots which gradually dilate like oil drops until they merge.

In the very low densities in Denmark this aggregation was also noted. Although no adult beetles were seen, most of the larvae found in a field occurred in a relatively small area. Densities were too low to be termed an outbreak or a hot spot but there was a definite tendency to aggregate.

## DISCUSSION

This investigation revealed a number of characteristics of the biology and behavior of *Diaparsis* n.sp. which help to evaluate the potential this parasitoid has as a biocontrol agent. The situation in which field research was conducted was artificial, in that the insectary was geared to maximum parasitoid production with crop production being secondary. Therefore the question of the impact of *Diaparsis* n.sp. on CLB populations cannot be evaluated.

Several characteristics of *Diaparsis* n.sp. seem to indicate that this parasitoid has the potential of being a good biological control agent. It appears that with a minimal effort toward management practices favoring *Diaparsis* n.sp., it could become a valuable component in the CLB management program.

One of the most obvious favorable factors is the good synchrony with the host which was exhibited each year. Evidence for this synchrony was particularly prevalent in 1975 when emergence of the parasitoid was early in terms of degree days based on air temperature. However, it still closely followed host larval buildup which was also early.

In addition to good synchronization, it was also found that _Diaparsis_ n.sp. persists for almost the entire CLB larval season. This persistence is due partially to longevity of the parasitoid, but also due to the length of the population emergence time. Emergence time was found to be about two and one half weeks which effectively stretches parasitoid activity to cover almost the entire time that host larvae are present. Besides making more larvae available for parasitization, this also minimizes intra-specific interference by lowering the frequency of superparasitism.

A third favorable characteristic is the high fecundity rate of _Diaparsis_ n.sp. Gage (1974) found a fecundity of about 60 eggs per female for _Tetrastichus julis_. In contrast, _Diaparsis_ n.sp. fecundity is approximately 3x greater, averaging about 185 eggs per female. In terms of hosts utilized per female, _Diaparsis_ n.sp. would be more than 3x as effective as _T. julis_ since the latter deposits more than one egg per host, while _Diaparsis_ n.sp. only deposits one per host. However, it is not known if _Diaparsis_ n.sp. is able to take full advantage of its fecundity in the field.

_Diaparsis_ n.sp. apparently has a good overwinter survival rate which is important since the host is successful in northern latitudes. In addition, it is apparent that this parasitoid can increase its population quite rapidly as evidenced by increases in emergence density of 30% and 139% in successive years.

A highly desirable characteristic of parasitoids of agricultural pests is the ability to survive normal agricultural practices. It was shown that *Diaparsis* n.sp. is able to survive these practices but at a considerably reduced level. This may be of considerable value in developing a management scheme for the CLB.

*Diaparsis* n.sp. also appears to be compatible with *T. julis*. Although there is some overlap between these two parasitoids, the life cycles are such that competition is minimal. The spring generation of *T. julis* emerges prior to the emergence of *Diaparsis* n.sp. and attacks the early CLB larvae. The second generation of *T. julis* appears as *Diaparsis* n.sp. is declining and thus helps to attack the later host larvae. The main activity of *Diaparsis* n.sp. occurs between the two generations of *T. julis*.

In analysis of multiparasitism, it was found that about 5% of the host larvae are multiparasitized by *Diaparsis* in combination with either *T. julis* or *Lemophagus curtus* or both. In addition to this, a multiparasitism involving *L. curtus* with *T. julis* occurred in nearly 6% of the parasitizations over the three years covered by this study. The majority of these (92%), occurred after June 22 which indicates that the main interference of *L. curtus* with *T. julis* occurs with the second generation of *T. julis*. It is not definitely known if *L. curtus* is intrinsically superior to T. julis; however, it seems to be a reasonable assumption since it is a solitary parasitoid similar to Diaparsis n.sp. which is known to be superior. Thus, it appears that L. curtus may reduce the numbers of T. julis. Since the density of the spring generation depends partly on the parasitism which occurs late in the previous season, any interference at this time is realized as fewer T. julis the following year. Despite the fact that T. julis is doing extremely well, this interference, along with the fact that in Europe (Dysart, et. al., 1973) L. curtus only occasionally exceeds 15% parasitization, gives rise to the question whether or not it should have been released in the U.S. Although it is too late to do anything about it, it appears that little, if anything, was accomplished by releasing it.

A final valuable characteristic of Diaparsis n.sp. is its ability to survive over a wide area. According to reports from the USDA mentioned earlier, it has been established in six states and eleven counties. Since these areas are widely scattered in the midwest and northeastern U.S. it is hopeful that Diaparsis n.sp. will be able to eventually inhabit the entire range of the CLB in the U.S.

## Some Management Considerations

As was mentioned above, it is hoped that the findings reported here will help to develop an effective management program for the cereal leaf beetle. With respect to this there are several considerations which would be of value in development of such a program.

One of these is to provide favorable overwinter and emergence conditions for the parasitoids in order to maximize spring emergence. This would take the form of leaving oats stubble undisturbed from harvest until after parasitoids have emerged in the spring. Since this is not compatible with existing cropping practices, a modification of this may be necessary. One alternative is to leave strips of stubble to help augment the population of parasitoids which survive tillage.

Another alternative discussed by Gage (1974) suggests the seeding of oat fields to a crop such as alfalfa. Since the objective is to allow the soil to remain undisturbed, any rotation system which follows oat crops with a forage crop would be useful. Harcourt and Guppy (1977) suggested that a crop rotation scheme such as this was a factor in the rapid buildup and dispersal of _T. julis_ populations in Canada.

A second practice which should receive consideration is a reduction in the use of herbicides in and near oats fields in order to conserve wild flowers. The value of these various flowers in enhancing the effectiveness of _Diaparsis_ n.sp. as a control agent needs to be evaluated further. It appears that it may be valuable to allow some plants on which _Diaparsis_ is known to feed to remain in areas where this parasitoid is established and where efforts are being made to establish it.

The logical place to begin to leave these food plants is in field borders, although in very large acreages this may not be sufficient to allow the parasitoids to fly from the flowers to the central areas of the field. In such fields it may be necessary to leave a narrow strip(s) of the field untreated by herbicides to attract the parasitoids.

In fields, either large or small, where oats is planted in successive years, the ideal practice would be to leave a strip of stubble untilled to maximize emergence and then leave the natural flora of this strip undisturbed to provide a favorable environment for parasitoid activity.

Such practices may be difficult to implement since growers are conditioned to favor clean, weed-free fields uninterrupted by fence rows, or other untilled areas. The problem needs to be examined by an economist to evaluate the economic tradeoffs between reduced yield and reduced cost of pesticide application. Various other factors would also need to be considered in making such an evaluation. These include the desirability of reduced pesticides from an environmental viewpoint and the value in maintaining floral diversity wherever possible to foster the natural enemies of other agricultural pests.

A third management consideration of importance is to avoid destroying the effectiveness of *Diaparsis* n.sp. by the injudicious use of insecticides. Without doubt there will be times when it will be necessary to spray for control of the cereal leaf beetle. The time of application of this spray is critical to the survival and effectiveness of both *Diaparsis* n.sp. and *T. julis*. Gage (1974) discusses the "biological window" concept with regard to pesticide application. This is an effective management scheme in spraying for CLB control when *T. julis* only is present. However, with the addition of *Diaparsis* n.sp. this biological window is nearly closed, since parasitoids are present nearly the entire season. In this situation, a better management practice would be to spray early in the season prior to parasitoid emergence using a short lived chemical which will kill the adult CLBs before they oviposit. An alternative, if the above time is not feasible, would be to spray late in the CLB larval season, just prior to second generation *T. julis* emergence. *Diaparsis* n.sp. populations are declining at this point and damage would be not as great as in mid-season. It could, in fact, be beneficial if it minimizes multiparasitism with *T. julis* since this would foster a higher population of *T. julis* in the following spring. However, timing of this would be very critical since it would need to be done just prior to *T. julis* emergence.

The other alternative to those mentioned above would be to spray during the time *Diaparsis* n.sp. is most active. This of course would be very damaging to the parasitoid population, however, if it is necessary to apply an insecticide at this time, damage could be minimized by spraying only the most heavily CLB infested areas of the field. No doubt the majority of the parasitoids would be concentrated in these areas; however, some would be elsewhere and spraying in selected areas would preserve these as a reservoir to rebuild the population in following seasons.

The CLB - Europe vs. U.S.

A significant finding in this research was the difference in behavior which was observed between European and the U.S. populations of the CLB. As indicated earlier this habit of aggregating in well defined areas of a field was evident everywhere in Europe but has not been observed in the U.S. There was no apparent difference in the crop, topography, or other external factors to which this difference could be attributed. The most plausible explanation at this point is that there is a genetic difference in the two populations.

There are two other evidences for this at this point. One of these is that in Europe parasitization does not seem to be a limiting factor. In a letter (1974) to Dr. Stehr of Michigan State University, Dr. Carl said that in a survey of Switzerland, Germany and Austria, parasitism of 30,000 larvae dissected was only about 1%. Yet the CLB was not a problem anywhere. From this Dr. Carl concludes that there is some self-regulating mechanism operable which broke down when the species was introduced into North America. If this pest was introduced as only a few individuals bringing in a very small gene pool it is possible that a genetically different strain has developed which did not have this self-regulating characteristic.

A second evidence for the U.S. having a different genetic strain is the fact that _D. carinifer_ is apparently subject to an extremely high encapsulation rate in the U.S. which is the most obvious explanation for why this parasitoid has not been recovered in the U.S.

If it is true that the U.S. CLB is a different genetic strain the question arises concerning the meaning of this to the CLB management program in North America. At this point it is not possible to make valid suggestions except to say that this hypothesis should be investigated further by studying the CLB more thoroughly in its native areas. In addition tests should be made to determine if a genetic difference really does exist. If this proves to be the case, it may be useful to enlarge the genetic pool of the U.S. population by the introduction of additional CLB's collected from a wide area of Europe. This would perhaps establish the self-regulating mechanism which appears to be present in Europe. The result would be to prevent the CLB from becoming a more serious pest in the U.S. and perhaps reduce it to non-pest status altogether.

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# APPENDICES

Appendix I(a): Emergence of *Diaparsis* n.sp. by date and crop strip at Niles, Mich. 1973.

| | Wheat | | Oats 1 | | Oats 2 | | Oats 3 | | Trap Crop | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F |
| May 21 | | | 2 | | | | | | | |
| 22 | | | | 1 | | | | | | |
| 23 | | | | 1 | | | | | | |
| May 24 | | | 1 | | | | | | | |
| 25 | | | | 1 | | | | | | |
| 26 | | | | | | | | | | |
| May 27 | | | 2 | | | | | | | |
| 28 | | | | | 2 | | 1 | | | |
| 29 | | | | | 5 | | | | | |
| May 30 | | | | | 1 | | 1 | 1 | | |
| 31 | | | | | 4 | 2 | | | | |
| June 1 | | | | | 4 | 1 | 4 | 1 | | |
| 2 | | | | | | | 5 | 4 | | |
| 3 | | | | | 1 | 3 | 1 | 1 | | |
| June 4 | | | | | | 3 | 1 | 3 | | |
| 5 | | 1 | | | | | | | | |
| 6 | | | | | | | | 1 | | |
| June 7 | | | | | | | | 1 | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | 1 | | | | |

Appendix I(b): Emergence of *Diaparsis* n.sp. by date and crop strip at Niles, Mich. 1974.

| | Wheat | | Oats 1 | | Oats 2 | | Oats 3 | | Trap Crop | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F |
| May 21 | | | | | | | 1 | | | |
| 22 | 1 | | | | | 1 | 1 | | | |
| 23 | | | | | | | | | | |
| May 24 | 2 | | 1 | | | | | | | |
| 25 | 1 | | | | | | | | 2 | |
| 26 | 1 | | 1 | 1 | 1 | | | | 1 | |
| May 27 | | | 1 | | | | | | 2 | |
| 28 | 1 | | | | 1 | | | | 4 | 3 |
| 29 | 1 | 1 | | 2 | 1 | | | | 7 | 2 |
| May 30 | 1 | 1 | 1 | | 1 | | 2 | | 3 | 2 |
| 31 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 3 | 5 |
| June 1 | 2 | | | | | | 1 | | | 1 |
| 2 | 1 | 2 | | 1 | | | | 1 | | 2 |
| 3 | | 2 | | 1 | 2 | | | | | 1 |
| June 4 | 1 | 2 | | 2 | | | | 1 | | 4 |
| 5 | | 2 | | | | | | 1 | | |
| 6 | | 2 | | 1 | | | | 1 | | |
| June 7 | | | | | | | | | | 2 |
| 8 | | | | | | | | 1 | | |
| 9 | | | | | | | | | | |

Appendix I(c): Emergence of Diaparsis n.sp. by date and crop strip at Niles, Mich. 1975.

| | Wheat | | Oats 1 | | Oats 2 | | Oats 3 | | Trap Crop | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F |
| May 21 | | | | | | | | | | |
| 22 | | | 1 | | 2 | | | | | |
| 23 | | | | | | | 1 | | | |
| May 24 | | | 4 | 1 | 18 | 1 | 12 | 4 | | |
| 25 | Traps not checked | | | | | | | | | |
| 26 | | | 21 | 9 | 57 | 32 | 9 | 12 | | |
| May 27 | 1 | | 14 | 23 | 20 | 42 | 5 | 11 | | |
| 28 | | | 3 | 14 | 23 | 33 | 0 | 4 | | |
| 29 | | | 1 | 17 | 35 | 33 | 0 | 7 | | |
| May 30 | | | 0 | 4 | 10 | 29 | 2 | 1 | | |
| 31 | | | | 1 | 5 | 11 | | | | |
| June 1 | | | | 3 | 6 | 21 | | | | |
| 2 | | | 1 | | 0 | 6 | | | | |
| 3 | | | | | 1 | 13 | | | | |
| June 4 | | | | | 0 | 5 | | | | |
| 5 | | 1 | | | 0 | 2 | | | | |
| 6 | | | | | 0 | 2 | | | | |
| June 7 | | | | | 0 | 0 | | | | |
| 8 | | | | | 1 | 0 | | | | |
| 9 | | | | | | | | | | |

Appendix II: Comparative emergence of <u>Diaparsis</u> n.sp. from tilled and untilled plots of oats stubble - Niles, Mich. 1974 and 1975.

<u>1974</u>

| | | Tilled Males | Tilled Females | Tilled Total | Untilled Males | Untilled Females | Untilled Total |
|---|---|---|---|---|---|---|---|
| May | 24 | | | | 2 | | 2 |
| | 25 | 1 | 1 | 2 | 4 | | 4 |
| | 26 | 1 | | 1 | 6 | 1 | 7 |
| | 27 | | | | 8 | 2 | 10 |
| | 28 | 2 | 3 | 5 | 15 | 10 | 25 |
| | 29 | 5 | 3 | 8 | 8 | 9 | 17 |
| | 30 | 3 | 2 | 5 | 2 | 3 | 5 |
| | 31 | 1 | 3 | 4 | 8 | 28 | 36 |
| June | 1 | 1 | 3 | 4 | 2 | 4 | 6 |
| | 2 | 3 | 7 | 10 | | 12 | 12 |
| | 3 | | | | 3 | 4 | 7 |
| | 4 | 1 | 2 | 3 | | 3 | 3 |
| | 5 | 2 | 1 | 3 | | 3 | 3 |
| | 6 | | 2 | 2 | | 2 | 2 |
| | 7 | 1 | 1 | 2 | 2 | 1 | 3 |
| | 8 | 1 | | 1 | | 1 | 1 |
| | 9 | | 2 | 2 | | | |
| | 10 | | 1 | 1 | | | |
| | 10<br>14 | | 1 | 1 | | | |
| | 14<br>18 | | 1 | 1 | | | |

<u>1975</u>

| | | Tilled Males | Tilled Females | Tilled Total | Untilled Males | Untilled Females | Untilled Total |
|---|---|---|---|---|---|---|---|
| May | 24 | | | | 1 | | 1 |
| | 25 | | | | | | |
| | 26 | 1 | | 1 | 7 | | 7 |
| | 27 | | 1 | 1 | 11 | 3 | 14 |
| | 28 | | | | 8 | 3 | 11 |
| | 29 | | | | 3 | 2 | 5 |
| | 30 | | 1 | 1 | 4 | 12 | 16 |
| | 31 | | 2 | 2 | | 1 | 1 |
| June | 2 | 1 | | 1 | | 2 | 2 |
| | 3 | | 1 | 1 | | | |
| | 4 | | 2 | 2 | 1 | 2 | 3 |

Appendix III: $^{o}D_{48}$ accumulations at Niles, Mich. 1973-1975.

| | | 1973 | 1974 | 1975 |
|---|---|---|---|---|
| May | 21 | 425 | 438 | 386 |
| | 22 | 441 | 458 | 406 |
| | 23 | 453 | 474 | 428 |
| | 24 | 467 | 484 | 457 |
| | 25 | 483 | 492 | 484 |
| | 26 | 495 | 501 | 508 |
| | 27 | 509 | 510 | 524 |
| | 28 | 520 | 524 | 538 |
| | 29 | 528 | 546 | 558 |
| | 30 | 533 | 563 | 580 |
| | 31 | 546 | 578 | 594 |
| June | 1 | 565 | 590 | 606 |
| | 2 | 587 | 603 | 615 |
| | 3 | 613 | 620 | 629 |
| | 4 | 637 | 644 | 647 |
| | 5 | 659 | 668 | 669 |
| | 6 | 681 | 692 | 687 |
| | 7 | 701 | 716 | 695 |
| | 8 | 727 | 742 | 703 |
| | 9 | 756 | 770 | 714 |
| | 10 | 784 | 788 | 734 |
| | 11 | 817 | 798 | 756 |
| | 12 | 844 | 809 | 774 |
| | 13 | 866 | 824 | 796 |
| | 14 | 885 | 844 | 818 |
| | 15 | 907 | 862 | 838 |
| | 16 | 938 | 870 | 854 |
| | 17 | 962 | 876 | 880 |
| | 18 | 987 | 894 | 908 |
| | 19 | 1014 | 918 | 940 |
| | 20 | 1038 | 942 | 971 |
| | 21 | 1061 | 966 | 1001 |
| | 22 | 1081 | 983 | 1033 |
| | 23 | 1099 | 992 | 1063 |
| | 24 | 1117 | 1003 | 1091 |
| | 25 | 1137 | 1014 | 1115 |
| | 26 | 1167 | 1030 | 1140 |
| | 27 | 1192 | 1047 | 1166 |
| | 28 | 1213 | 1065 | 1195 |
| | 29 | 1231 | 1085 | 1223 |
| | 30 | 1249 | 1109 | 1249 |

Appendix IV(a): Malaise trap catches of <u>Diaparsis</u> n.sp. at Niles, Mich. 1974.

| | 1974 | | | | | |
|---|---|---|---|---|---|---|
| | M-1 | | M-2 | | M-3 | |
| | M | F | M | F | M | F |
| May 24 | 0 | 0 | 0 | 2 | 0 | 1 |
| 25 | 0 | 0 | 0 | 0 | 3 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 2 |
| 27 | 0 | 0 | 0 | 0 | 7 | 0 |
| 28 | 0 | 1 | 1 | 0 | 12 | 8 |
| 29 | 1 | 1 | 1 | 2 | 7 | 3 |
| 30 | 0 | 0 | 0 | 3 | 4 | 0 |
| 31 | 1 | 1 | 1 | 1 | 2 | 0 |
| June 1 | 1 | 0 | 1 | 1 | 2 | 0 |
| 2 | 3 | 2 | 11 | 6 | 4 | 1 |
| 3 | 1 | 1 | 4 | 4 | 5 | 0 |
| 4 | 2 | 0 | 23 | 3 | 1 | 1 |
| 5 | 5 | 1 | 15 | 3 | 1 | 5 |
| 6 | 1 | 0 | 2 | 4 | 0 | 0 |
| 7 | 0 | 0 | 8 | 14 | 2 | 1 |
| 8 | 0 | 0 | 5 | 6 | 1 | 0 |
| 9 | 4 | 4 | 5 | 6 | 5 | 2 |
| 10 | 2 | 3 | 3 | 5 | 0 | 0 |
| 11 | 2 | 4 | 0 | 1 | 1 | 0 |
| 12 | 1 | 3 | 0 | 0 | 3 | 1 |
| 13 | 0 | 1 | 0 | 2 | 4 | 3 |
| 14 | 0 | 0 | 1 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 1 | 0 | 0 | 0 | 1 | 1 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 1 | 0 | 1 | 0 | 0 |
| 20 | 0 | 2 | 0 | 1 | 0 | 0 |
| 21 | 0 | 1 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 3 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 0 | 1 |

Appendix IV(b): Malaise trap catches of Diaparsis n.sp. at Niles, Mich. 1975.

| | | 1975 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | M-1 | | M-2 | | South | | North | |
| | | M | F | M | F | M | F | M | F |
| May | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 28 | 2 | 4 | 10 | 1 | 0 | 0 | 0 | 0 |
| | 29 | 2 | 5 | 11 | 4 | 2 | 4 | 3 | 12 |
| | 30 | 4 | 7 | 11 | 7 | 1 | 5 | 16 | 17 |
| | 31 | 1 | 5 | 9 | 3 | 0 | 14 | 3 | 33 |
| June | 1 | 2 | 4 | 6 | 2 | 3 | 8 | 25 | 12 |
| | 2 | 6 | 9 | 13 | 13 | 4 | 23 | 10 | 31 |
| | 3 | 4 | 1 | 14 | 9 | 5 | 42 | 6 | 56 |
| | 4 | 11 | 3 | 11 | 7 | 24 | 45 | 38 | 57 |
| | 5 | 14 | 6 | 8 | 11 | 14 | 24 | 15 | 118 |
| | 6 | 7 | 5 | 14 | 9 | 8 | 53 | 22 | 85 |
| | 7 | 0 | 0 | 0 | 0 | 5 | 8 | 8 | 10 |
| | 8 | 0 | 0 | 0 | 0 | 1 | 4 | 12 | 3 |
| | 9 | 0 | 0 | 3 | 1 | 3 | 7 | 25 | 13 |
| | 10 | 2 | 0 | 1 | 2 | 3 | 12 | 6 | 29 |
| | 11 | 3 | 1 | 1 | 1 | 1 | 11 | 5 | 26 |
| | 12 | 0 | 1 | 0 | 3 | 5 | 17 | 12 | 39 |
| | 13 | 1 | 1 | 2 | 5 | 3 | 6 | 21 | 41 |
| | 14 | 0 | 0 | 1 | 2 | 1 | 9 | 5 | 31 |
| | 15 | 0 | 1 | 0 | 2 | 2 | 25 | 3 | 28 |
| | 16 | 0 | 3 | 0 | 1 | 0 | 13 | 2 | 25 |
| | 17 | 0 | 3 | 0 | 1 | 0 | 9 | 1 | 23 |
| | 18 | 0 | 0 | 0 | 2 | 0 | 6 | 0 | 21 |
| | 19 | 0 | 0 | 0 | 3 | 0 | 21 | 0 | 36 |
| | 20 | 0 | 0 | 1 | 0 | 0 | 19 | 0 | 31 |
| | 21 | 0 | 1 | 0 | 0 | 0 | 9 | 0 | 15 |
| | 22 | 0 | 0 | 0 | 2 | 0 | 8 | 0 | 7 |
| | 23 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 4 |
| | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 25 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 26 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix V(a): CLB larval densities and parasitism rates at Niles, Mich. - 1973

| Oats 1 | | | Oats 2 | | | Oats 3 | | |
|---|---|---|---|---|---|---|---|---|
| | Larvae /ft$^2$ | % Para. | | Larvae /ft$^2$ | % Para. | | Larvae /ft$^2$ | % Para. |
| June 4 | 11.3 | 0 | June 11 | 41.0 | 20 | June 18 | 22.3 | 4 |
| 11 | 34.6 | 24 | 18 | 29.7 | 18 | 25 | 26.6 | 4 |
| 18 | 41.4 | 10 | 25 | 16.9 | 12 | July 2 | 2.2 | 2 |
| 24 | 14.8 | 4 | July 2 | 0.5 | 3 | | | |
| July 2 | 1.4 | 0 | | | | | | |

Appendix V(b): CLB larval densities and parasitism rates at Niles, Mich. - 1974

| Oats 1 | | | Oats 2 | | | Oats 3 | | |
|---|---|---|---|---|---|---|---|---|
| | Larvae $/ft^2$ | % Para. | | Larvae $/ft^2$ | % Para. | | Larvae $/ft^2$ | % Para. |
| June 3 | 0.8 | 37 | June 10 | 17.0 | 34 | June 10 | 9.3 | 28 |
| 6 | 5.5 | 58 | 13 | 25.3 | 36 | 13 | 17.5 | 28 |
| 10 | 20.6 | 32 | 17 | 39.7 | 30 | 18 | 20.8 | 40 |
| 13 | 28.2 | 48 | 20 | 39.0 | 34 | 20 | 28.3 | 26 |
| 17 | 46.6 | 52 | 24 | 47.8 | 46 | 24 | 26.9 | 30 |
| 20 | 27.9 | 30 | 27 | 35.8 | 46 | 27 | 27.4 | 32 |
| 24 | 20.7 | 40 | July 1 | 14.1 | 30 | July 1 | 16.2 | 40 |
| 27 | 21.6 | 50 | 5 | 4.0 | 16 | 5 | 10.0 | 26 |
| July 1 | 4.7 | 44 | 8 | 0.3 | 10 | 8 | 2.5 | |
| 5 | 0.5 | 16 | | | | | | |

Appendix V(c): CLB larval densities and parasitism rates at Niles, Mich. - 1975

| Oats 1 | | | Oats 2 | | | Oats 3 | | |
|---|---|---|---|---|---|---|---|---|
| | Larvae $/ft^2$ | % Para. | | Larvae $/ft^2$ | % Para. | | Larvae $/ft^2$ | % Para. |
| May 26 | 32.0 | 8 | May 26 | 26.8 | 18 | June 12 | 33.4 | 16 |
| 29 | 60.9 | 18 | 29 | 30.7 | 22 | 14 | 41.6 | |
| June 2 | 64.0 | 34 | June 2 | 54.5 | 30 | 17 | 59.0 | 26 |
| 6 | 58.3 | 34 | 6 | 39.2 | 43 | 19 | 66.5 | 22 |
| 9 | 41.6 | 44 | 9 | 42.5 | | 23 | 48.6 | 8 |
| 12 | 46.5 | 62 | 12 | 27.8 | 59 | 26 | 17.1 | 6 |
| 14 | 40.7 | | 14 | 23.2 | | 28 | 7.4 | |
| 19 | 15.4 | 60 | 19 | 22.7 | 12* | | | |
| 23 | 6.0 | 14 | 23 | 4.3 | | | | |

*Late dissection of frozen sample may have resulted in lower than actual figures.

Appendix VI: Data from soil samples and spring emergence for analysis of overwinter mortality of *Diaparsis* n.sp. at Niles, Mich. 1974.

| | Fall Samples | | | Spring Emergence |
|---|---|---|---|---|
| Sample # | # Alive | # Dead | Total | $\frac{\text{Total Catch}}{2}$ |
| 1 | 29 | 1 | 30 | 23 |
| 2 | 6 | 2 | 8 | 6 |
| 3 | 11 | 3 | 14 | 2.5 |
| 4 | 19 | 6 | 25 | 11.5 |
| 5 | 7 | 4 | 11 | 25 |
| 6 | 15 | 0 | 15 | 30 |
| 7 | 10 | 0 | 10 | 8 |
| 8 | 6 | 0 | 6 | 10.5 |
| 9 | 13 | 1 | 14 | 6.5 |
| 10 | 4 | 0 | 4 | 5 |
| 11 | 10 | 3 | 13 | 2.5 |
| 12 | 3 | 5 | 8 | 13 |
| 13 | 12 | 4 | 16 | 12.5 |
| 14 | 9 | 1 | 10 | 3 |
| 15 | 17 | 1 | 18 | 22.5 |