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INTEGRATED CONTROL OF THE ALFALFA WEEVIL, HYPERA POSTICA  
(GYLLENHAL) (COLEOPTERA: CURCULIONIDAE), IN ONTARIO

A Thesis

Presented to  
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## ABSTRACT

INTEGRATED CONTROL OF THE ALFALFA WEEVIL, HYPERA POSTICA  
(GYLLENHAL) (COLEOPTERA: CURCULIONIDAE), IN ONTARIO

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University of Guelph, 1976

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The distribution of the established parasitoids of the alfalfa weevil, Hypera postica (Gyllenhal) in western Ontario was determined during 1973 to 1975. Potasson luna (Girault), a parasitoid of eggs, was found in 13 out of 17 counties surveyed, but parasitism was only 0.7-17.0%. Tetrastichus incertus (Ratz.), a parasitoid of the larva, was recovered from only two sites and parasitism was less than 4.0% at both places. Bathyplectes curculionis (Thomson), another parasitoid of the larva, was widespread and parasitism ranged from 4.4 to 75.0%. Microctonus aethiopoides Loan, a parasitoid of the adult weevil, was recovered at only one site where parasitism was 33.3% in 1973, 56.6% in 1974, and 76.6% in 1975.

The relationships between the alfalfa weevil and each of its two major parasitoids in Ontario, B. curculionis and M. aethiopoides, were investigated. Parasitism by B. curculionis was only 6.3-33.3% at the peak of the host population, but increased to 56.0-68.0% later in the season, when host population was low. The incidence of diapause as well as winter survival of B. curculionis were highest among parasitoid cocoons obtained towards the end of the season. The populations of the first generation adults of M. aethiopoides was synchronized well with those of the overwintered adult weevils, resulting in parasitism of 76.6-82.0%. The second generation of the adult parasitoids and the

summer adult weevils overlapped for about two weeks and parasitism was 41.6-60.0%. Some adults of M. aethiopoides were present in the field when hosts were unavailable and this may have significance in the establishment of the parasitoid at new release sites. Parasitism by M. aethiopoides did not significantly affect the overwintering mortality of the alfalfa weevil.

The toxicity of five insecticides, carbofuran, carbaryl, malathion, phosmet, and methoxychlor was determined by topical application in the laboratory on adult B. curculionis, adult M. aethiopoides, third-instar larvae of H. postica, third-instar larvae of H. postica parasitized by B. curculionis, adult H. postica and adult H. postica parasitized by M. aethiopoides. The effect of these five insecticides in an integrated control program was assessed, using selectivity ratios calculated as the  $LD_{50}$  of the parasitoid divided by the  $LD_{50}$  of weevil larva or adult. Each of the insecticides was more toxic to the two parasitoids than to the host larvae but, comparatively, carbofuran and carbaryl were 2 to 3 times safer to the parasitoids than malathion, phosmet, or methoxychlor. Carbofuran, the only insecticide which was selective in favour of both parasitoids over the adult weevils, had selectivity ratios 2 to 4 times those of malathion, phosmet, carbaryl, and methoxychlor.

Parasitized larvae and adults of H. postica were more susceptible to the insecticides than were their non-parasitized counterparts and most of the parasitoid larvae died within the susceptible hosts. The weevil larvae were much more tolerant to all the insecticides than were the adults.

Based on the selectivity ratings, carbofuran and carbaryl are

preferable to malathion, phosmet, or methoxychlor in the integrated control of the alfalfa weevil. Because adults are more susceptible than larvae and since there is a period in the spring when adult weevils are active in the field while adult parasitoids are not, the feasibility of chemical control against the adult weevil should be investigated further. Such a recommendation could have application in alfalfa seed production where early cutting is not an alternative and control is usually required each year.



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## INTRODUCTION

The alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae), is a major pest of alfalfa, a valuable forage crop in North America. In Ontario, this pest was first reported by MacLachlan (1967) and damage to alfalfa had reached economic levels by 1970 in many counties (Goble, 1970).

Control measures adopted in Ontario to reduce damage by this insect were early harvesting of alfalfa, the release of parasitoids and the use of insecticides when necessary. Despite its advantages, early cutting of the crop was often impractical because of wet weather and the inability of growers to meet harvest deadlines. Some parasitoids of the weevil have established in Ontario but their effect is still unknown. At present, there is no conclusive evidence that the parasitoids can suppress populations of the alfalfa weevil below the economic threshold. Hence, the most reliable alternative, when early cutting is not possible, is chemical control. To avoid some of the usual hazards associated with chemical control, and to obtain the maximum benefit from the parasitoids, an integrated control program is advisable.

Van den Bosch and Stern (1962) discussed the objectives of integrated control of arthropod pests and suggested that insecticides should be used only when biological control is inadequate. They stressed that there should be minimum disruptive effects of pesticides on the parasitic insects. Thus, for an integrated control program, basic information on the relationships between the pest and its parasitoids is essential. One must also know how the parasitoids are

affected by insecticides applied against the pest. These aspects of the control of the alfalfa weevil have received little attention in Ontario.

The objectives of this study were:

- (1) to determine the distribution of the established parasitoids of the alfalfa weevil in western Ontario;
- (2) to investigate the relationships between the alfalfa weevil and each of its major parasitoids;
- (3) to compare the susceptibilities of the alfalfa weevil and its major parasitoids to insecticides recommended in Ontario;
- (4) to evaluate the data and make recommendations for an integrated control program for the alfalfa weevil in Ontario.

## REVIEW OF LITERATURE

## HISTORY OF THE ALFALFA WEEVIL IN NORTH AMERICA

The alfalfa weevil was detected in western United States of America in 1904 (Titus, 1910) and about 50 years later in the eastern states (Poss and Bissell, 1953). Since then it has spread to cover all the 48 conterminous states (Cothran, 1972). The western and eastern populations of H. postica differ in food consumption (Koehler and Gyrisco, 1963) and mating habits (Blickenstaff, 1965). The weevil has been recorded in Canada in Alberta (Hobbs, 1954), Saskatchewan (McMahon et al., 1954), Ontario (MacLachlan, 1967), and Quebec (Perron, 1969).

## BIOLOGY OF THE ALFALFA WEEVIL IN ONTARIO

The biology of the alfalfa weevil in Ontario was outlined by Miller and Guppy (1971). They observed one germination during the growing season of the crop, from early spring to summer. The eggs, laid in clusters inside the stems of alfalfa, hatched within 8 to 12 days. The larvae passed through 4 instars in 10 to 14 days while they fed on the leaves of alfalfa and then pupated. The pupal stage lasted 6 to 10 days and the new adults fed on alfalfa leaves for about 3 weeks and then aestivated in the summer. They resumed feeding for a short period in the fall, mated, laid a few eggs and went into diapause until the following spring. Overwintered adults became active and resumed laying eggs in late April to early May (Miller and Guppy, 1971; Bereza, 1972).

## CONTROL OF THE ALFALFA WEEVIL IN ONTARIO

## Cultural Control

Early cutting of alfalfa just before the first bloom opened was recommended by the Ontario Department of Agriculture and Food (1971). This usually saved the crop from further damage and killed most of the larvae of the weevil through dessication and starvation. However, Miller and Guppy (1971) pointed out that wet weather and numerous farm operations often prevented growers from harvesting the crop at the right time.

## Biological Control

In attempts to suppress the populations of the weevil by biological means, several species of parasitoids were imported from Europe and released in the eastern United States and subsequently in Ontario. The release of parasitoids in Ontario began in 1970 with the following 6 species (Williamson, 1971) (Fig. 1):

Bathyplectes anurus (Thomson) (Hymenoptera: Ichneumonidae)

B. curculionis (Thomson) (Hymenoptera: Ichneumonidae)

B. stenostigma (Thomson) (Hymenoptera: Ichneumonidae)

Tetrastichus incertus (Ratz.) (Hymenoptera: Eulophidae)

Microctonus aethiopoidea Loan (Hymenoptera: Braconidae)

(= M. aethiops of authors) (Loan, 1975)

M. colesi Drea (Hymenoptera: Braconidae)

It may be deduced from Miller and Guppy (1971) that B. curculionis and T. incertus were already present in Ontario before the releases in 1970, probably as a result of their spread from release sites in the United States. B. anurus and B. stenostigma were recovered only at one

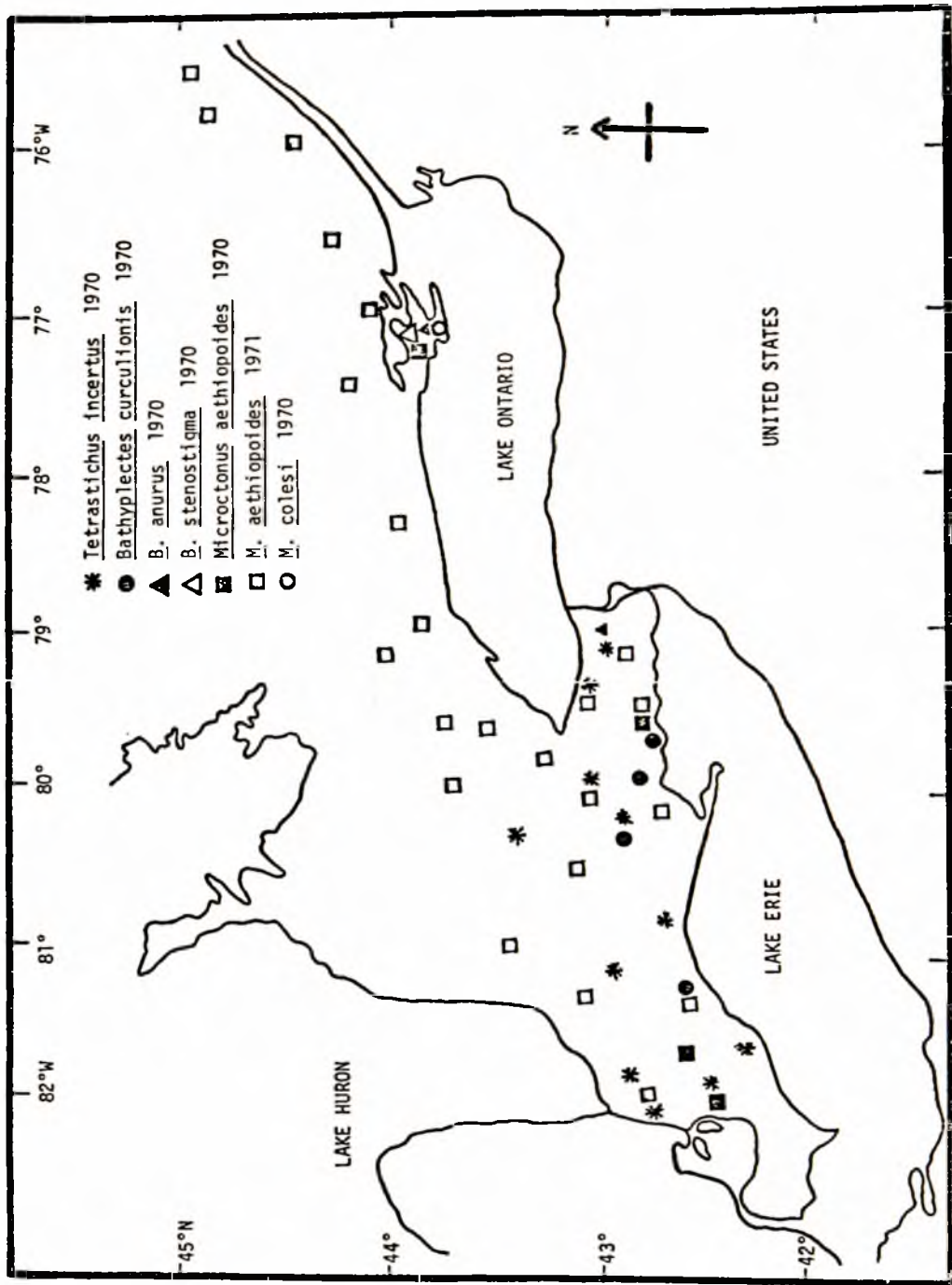
site whereas M. aethiopoides was recovered at 4 of the 5 release sites (Kelleher and Loan, 1971). There was no recovery of M. colesi. Subsequent releases of M. aethiopoides were made in 1971 over a wider area (Fig. 1) but no recoveries were reported.

Two other parasitoids reared from eggs of the alfalfa weevil in Ontario were Potasson luna (Girault) (Hymenoptera: Mymaridae) (Miller and Guppy, 1971; Ellis, 1973) and Fidiobia rugosifrons (Crawford) (Hymenoptera: Platygasteridae) (Ellis, 1973). The latter parasitoid was reared mainly from overwintered eggs and was apparently of little significance on weevil eggs laid in the spring.

#### Chemical Control

Five insecticides were recommended for emergency control of the alfalfa weevil (Ontario Department of Agriculture and Food, 1972). If early cutting was not possible and if the infestation was high, a pre-harvest treatment of the first crop was recommended. After the first-cut a damaging infestation sometimes occurred on the regrowth and a post-harvest spray was needed to protect the crop. The recommendation specified that methoxychlor, phosmet, and carbofuran could be used only as post-harvest sprays whereas malathion and carbaryl could be applied either before or after the first harvest. The specification on which insecticides were to be used as pre-harvest or post-harvest spray was based on the recommended post-treatment interval before harvest. The minimum post-treatment days before cutting were, 1 day for malathion and carbaryl, 7 days for methoxychlor and phosmet, and 14 days for carbofuran. Thus, applying any of the last three pesticides on the first growth of alfalfa, would require a grower to wait

Figure 1. Sites where parasitoids of Hypera postica were released in Ontario in 1970 and 1971.



for a longer period before harvesting the crop, a situation that would reduce the protein content of the crop.

Chemical control was economical for fields in which the stand was at least 50% alfalfa (Bereza, 1972). Such fields were to be treated only when 25-50% of the upper third of the leaves showed injury and early cutting was not possible.

#### BIOLOGY OF THE PARASITOIDS

##### Potasson luna

Potasson luna was introduced into Utah State during the years 1911-1913 and 1925-1928 (Brunson and Coles, 1968). Streams and Fuester (1966) and Brunson and Coles (1968) reported the recoveries of this parasitoid in various areas of the eastern United States. There were no releases of P. luna in Canada and it was believed that the parasitoid spread into Ontario from the United States (Ellis, 1973).

Various aspects of the biology of P. luna were given by Chamberlin (1924), Streams and Fuester (1966), Blickenstaff et al. (1972), and Ellis (1973). In the field, the parasitoid oviposited in eggs of the alfalfa weevil within dead or fresh alfalfa stems (Chamberlin, 1924; Ellis, 1973). Percentage parasitism was usually higher in dead stems than in fresh stems because the parasitoid could enter dead stems to reach many host eggs (Chamberlin, 1924) but in fresh stems, parasitism was limited to a few eggs around the oviposition puncture of the weevil (Ellis, 1973). Blickenstaff et al. (1972) reported that, in the laboratory, the life cycle of P. luna required about 16 days from egg to adult and the species reproduced generation after generation without diapause. The parasitoid overwintered in weevil eggs laid

in the fall (Streams and Fuester, 1966; Brunson and Coles, 1968; Ellis, 1973). Streams and Fuester (1966) found that mortality was high among these host eggs and, consequently, only a few parasitoids survived the winter. The low population of adult P. luna in the spring, and the parasitoid's inability to reach many of the host eggs within fresh alfalfa stems, were probably responsible for the low percentage parasitism reported by Streams and Fuester (1966), Brunson and Coles (1968), and Ellis (1973).

#### Tetrastichus incertus

The introductions of T. incertus into the United States were started in the late 1930's but there was no evidence that the parasitoid established prior to subsequent importations in 1960 (Coles and Puttler, 1963). Coles and Puttler (1963), Streams and Fuester (1967), Brunson and Coles (1968), and Schroder et al. (1969) reported the recoveries of this parasitoid in several areas of the eastern United States. T. incertus had already established in Quebec (Mailloux and Pilon, 1970a), and probably Ontario before the releases in 1970.

The biology of T. incertus has been investigated well in the eastern United States. Streams and Fuester (1967) reported that the female parasitoid preferred to oviposit in the third- and fourth-instar larvae of the weevil. About 10 days after parasitization, the host larva died and the adult parasitoid emerged 4 to 7 days later from the dried mummy of the host. Blickenstaff et al. (1972) observed that, in Pennsylvania, T. incertus had 4 generations per season with some larvae of the parasitoid entering diapause at each generation. These diapausing parasitoids overwintered in the mummified host.

Streams and Fuester (1967) noted that when the larval population of weevil was high in May, parasitism by T. incertus was 0-5% but increased to 100% in late summer when host population was low. This high percentage parasitism in late summer had advantage in some areas of the eastern States because it reduced the population of overwintering weevils (Brunson and Coles, 1968).

Despite its rapid spread and higher populations in the early 1960's, T. incertus is presently of little significance in the control of the alfalfa weevil (Streams and Fuester, 1967). Factors that could have contributed to the decline of this parasitoid included 1) an interspecific competition for host between T. incertus and B. curculionis in which the latter was the superior because of its preference for first- and second-instar larvae (Miller, 1970), and 2) high overwintering mortality (51-64%) (Horn, 1971).

#### Bathyplectes curculionis

The history of B. curculionis and its importation into the United States was documented by Chamberlin (1926). The parasitoid was first introduced into Utah State in 1911. Since then, there have been subsequent importations, releases, and recoveries in many parts of the eastern United States (Brunson and Coles, 1968). Michelbacher (1940) reported on its establishment in the western States. B. curculionis had already been recorded in Canada prior to 1970 releases; in Alberta by Hobbs et al. (1959) and in Quebec by Mailloux and Pilon (1970b).

Several aspects of the biology of B. curculionis has been investigated in the eastern United States. The female parasitoid oviposited in the alfalfa weevil, preferring first-, second-, and

third-instar larvae to fourth-instar larvae (Brunson and Coles, 1968; Miller, 1970; Duodu and Davis, 1974b). Van den Bosch (1964) and Puttler (1967) reported on the ability of larvae of H. postica to encapsulate the eggs of B. curculionis; a phenomenon which led to the death of the parasitoid egg within the host and enabled the latter to complete its development successfully. However, if the host larva was superparasitized, encapsulation could not cope with all the eggs of the parasitoid and, hence, parasitism was effective. Encapsulation was more prevalent in the eastern strain than in the western strain of the alfalfa weevil (Puttler, 1967). In the absence of encapsulation, the incubation period of parasitoid eggs was 72 hours at 23°C (Van den Bosch, 1964) and development from the first-instar larva to pupation required 13.6 days at 26°C (Duodu and Davis, 1974a).

Parasitized weevil larvae consumed less alfalfa than did non-parasitized ones, but whereas Armbrust et al. (1970) observed that the differences in food consumption was not significant, Duodu and Davis (1974c) reported that it was significant. Apparently, apart from the possible differences in laboratory conditions, the two investigations involved different strains of the alfalfa weevil; the eastern strain in the former and the western strain in the latter. This may explain the discrepancy in the results.

Bathyplectes curculionis had one complete generation and a partial second generation in the eastern United States. Some of the first-generation parasitoid larvae developed, without diapause, to adulthood and gave rise to second-generation larvae most of which went into diapause (Brunson and Coles, 1968; Blickenstaff et al., 1972). B. curculionis overwintered as diapausing larvae within cocoons on the

ground. Cocoons containing diapausing larvae were darker and of heavier construction than those containing non-diapausing larvae (Chamberlin, 1926). Armbrust et al. (1972) observed that winter survival was low among those parasitoids which entered diapause in the first generation. Hyperparasitism of the cocoons of B. curculionis was reported in the United States by Puttler (1966), Day (1969), and Pike and Burkhardt (1974).

Armbrust (1970) reviewed the performance of B. curculionis and concluded that this parasitoid was responsible for an apparent decline in the populations of the alfalfa weevil. However, in a 6-year study of B. curculionis, Blickenstaff et al. (1972) observed that parasitism began near the peak of the population of the weevil larvae and thereafter percentage parasitism increased as the number of hosts declined. Consequently, even though high parasitism of more than 70% (Brunson and Coles, 1968) was reported in several areas, damage by the weevil persisted.

#### Microctonus aethiopoides

Microctonus aethiopoides was introduced into the United States as a parasitoid of the sweet-clover weevil, Sitona cylindricollis Fahr. during the years 1948-1958 (Coles and Puttler, 1963). Similarly, the parasitoid was imported into Manitoba, Canada, against the sweet-clover weevil during 1952-1954, but there was no establishment in that province (Loan, 1961). Coles and Puttler (1963) reported, after a series of tests in field cages, that H. postica would be a good host for M. aethiopoides. Several releases were made and the parasitoid is now well established in the eastern United States (Brunson

and Coles, 1968; Day et al., 1971).

There was a similarity in the biology of M. aethiopoides on Sitona cylindricollis (Loan and Holdaway, 1961) and on Hypera postica (Coles and Puttler, 1963; Neal and Bickley, 1971). The parasitoid oviposited in the adult alfalfa weevil, through the membranous area around the anus. Incubation of the parasitoid egg required 4 to 5 days at about 25°C and larval instars 1 to 5 were completed in 8 to 11 days. The fifth-instar larva emerged through the anus of the host to pupate in the soil. The pupal stage lasted about 12 days.

Loan and Holdaway (1961) reported that after the parasitoid egg hatched the trophamnions surrounding the embryo dissociated to form small, white pear-like teratocytes. They also noted that there was a degeneration of ovaries and a shrinking of mature eggs of parasitized, female weevils. In sexually mature females, parasitism by M. aethiopoides reduced oviposition by 80% within 48 hours (Van Driesche, 1975). Parasitism had no immediate effect on the potency of males of S. cylindricollis (Loan and Holdaway, 1961), but in males of H. postica in diapause, Microctonus spp. caused castration (Drea, 1968).

M. aethiopoides had two generations a year (Brunson and Coles, 1968). Parasitoid larvae emerged from overwintered H. postica in the spring and pupated. The adults of M. aethiopoides that emerged, oviposited in the non-parasitized, overwintered weevils still in the field and completed development in these hosts. The resulting second brood of M. aethiopoides parasitized the new generation of adult weevils. The parasitoid went into diapause as first-instar larvae in these new adult weevils. Several observations indicated that the diapause of M. aethiopoides depended on the physiological age of the host

(Loan and Holdaway, 1961; Coles and Puttler, 1963; Drea, 1968; Day, 1971). Fusco and Hower (1973) found that the incidence of diapause in M. aethiopoides decreased linearly with increased host age. However, they observed exceptions in the 7-day-old and 49-day-old weevils. Previously, Neal et al. (1970) had suggested that diapause in this parasitoid was related to the amount of hormone in the host weevil. Bowers and Blickenstaff (1966) terminated diapause of the alfalfa weevil with the synthetic hormone 10,11-epoxy-farnesenic acid methyl ester. Since then, several investigators (Day, 1971; Neal and Bickley, 1971; Fusco and Hower, 1973; Hollaway et al., 1973) have found it useful, in the laboratory, to apply hormones to parasitized, diapausing weevils to cause earlier emergence of M. aethiopoides.

Although the biology of B. curculionis and M. aethiopoides is fairly well known in the United States, there is little information on how these parasitoids affect the populations of the alfalfa weevil in Ontario. Host-parasitoid synchrony and overwintering mortalities of the two parasitoids have not been investigated in this province.

#### TOXICITIES OF INSECTICIDES TO PESTS AND THEIR PARASITOIDS

The literature on the toxicities of insecticides to pests and parasitoids was reviewed by Ripper (1956), Van den Bosch and Stern (1962), and Croft and Brown (1975). They concluded that most insecticides were detrimental to the survival of parasitoids. The lack of selective insecticides, favouring parasitoids, has been the major factor limiting the integration of chemical and biological control. However, some parasitoids have shown exceptional tolerances to certain insecticides (Bartlett, 1964).

It was apparent from Croft and Brown (1975) that most of the responses of parasitoids to insecticides have been observed qualitatively in the field rather than measured quantitatively. They pointed out that although qualitative observations were useful in evaluating the general effects of an insecticide, it was impossible to distinguish between the direct and indirect effects.

A number of investigators have reported the toxicities of several insecticides to the larvae and adults of the alfalfa weevil in the laboratory (Bennett and Thomas, 1963; Bass and Blake, 1964; Harrendorf et al., 1967; Van Meter and Pass, 1970). There are no quantitative data on toxicities of insecticides to B. curculionis. However, Van den Bosch et al. (1956), Stern (1961), Davis (1970), Wilson and Armbrust (1970), Dondale (1972), and Surgeoner (1973) reported that at the rates which various insecticides gave adequate control of the alfalfa weevil in the field, B. curculionis was not affected significantly. There are virtually no quantitative or qualitative data on the effects of insecticides on M. aethiopoides.

## MATERIALS AND METHODS

### HANDLING OF THE ALFALFA WEEVIL AND PARASITOIDS IN THE LABORATORY

All the developmental stages of the alfalfa weevil and the parasitoids were maintained in the laboratory at  $25 \pm 2^{\circ}\text{C}$ , 12-hour photoperiod and  $70 \pm 5\%$  RH, unless stated otherwise.

#### Rearing of the Alfalfa Weevil

Eggs of the alfalfa weevil were obtained by caging overwintered adults on weevil-free alfalfa stems, raised in the greenhouse. Eggs were dissected from the stems each day and incubated in 6 x 2 cm petri dishes. Larvae emerging on the same day were reared together and later used for insecticide tests.

Weevil larvae, swept from the field or raised from eggs in the laboratory, were reared in 20 x 12 x 12 cm plastic cages with screen lids. There were up to 50 larvae per cage. Fresh bouquets of alfalfa were provided every other day until the larvae pupated or when cocoons of B. curculionis were formed. Pupae of H. postica and B. curculionis were later removed from the debris of alfalfa leaves.

Pupae of the weevil were transferred to new cages and kept moist. Emerging adults were placed in plastic gallon jars and fed on fresh bouquets of alfalfa. Later, such adults were caged with female M. aethiopoides prior to being stored, or used for other purposes.

#### Method for Parasitizing Weevil Larvae by B. curculionis

The procedure for parasitizing larvae of H. postica by B. curculionis was modified from Duodu and Davis (1974a). Larvae to be

parasitized were raised from eggs in the laboratory, and the adult parasitoids were reared from field-parasitized weevil larvae. Two days after emergence, larvae were caged with day-old, mated females of B. curculionis for 24 hours. The host:parasitoid ratio was 50:2.

#### Method for Parasitizing Adult Weevils by M. aethiopoides

Two physiological types of adult, alfalfa weevils were obtained and parasitized in the laboratory:

- (1) new adults, reared from field-collected larvae of the alfalfa weevil;
- (2) overwintered adults collected from the field in the spring.

Adult weevils obtained by either method usually contained some small individuals. Fusco and Hower (1973) observed that, after parasitization by M. aethiopoides, smaller host-weevils had higher mortality than did larger ones. The smaller weevils were therefore discarded by screening them through a standard No. 12 sieve (Barnes and Ratcliffe, 1967; Fusco and Hower, 1973).

Fifty weevils were placed in a 12.7 cm high x 13 cm diameter plastic cage and covered with a 20-mesh screen lid. A day-old, mated female of M. aethiopoides, obtained from field-parasitized weevils from Aylmer or South Bay, was placed in the cage for 24 hours. Fusco and Hower (1974) found that a host-parasitoid ratio of 50:1 and an exposure period of 24 hours resulted in the lowest mortality after weevils had been parasitized by M. aethiopoides.

#### Emergence Cage for Larvae of M. aethiopoides

Parasitized, adult weevils were transferred to cages designed

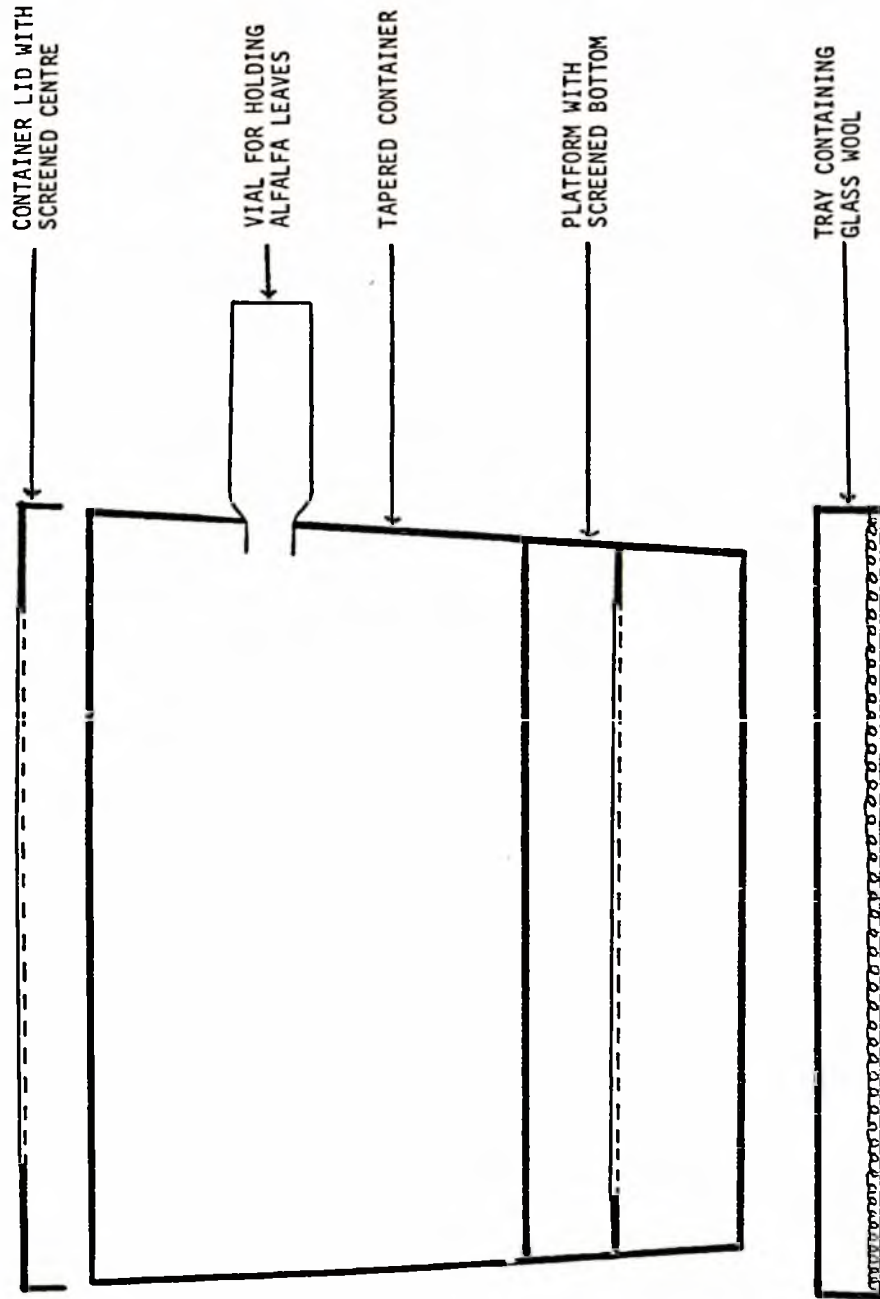
for the emergence of larvae of M. aethiopoides (Loan and Holdaway, 1961) (Fig. 2). The cage was constructed from a round, tapered plastic container 12.7 cm high and 13 cm in diameter at the top. A section, 1.5 cm high, was cut from the bottom of the container, inserted through the top opening and wedged tightly at a point about 2 cm from the open bottom, thus forming a platform within the cage. A hole, 10 cm in diameter, was made in the centre of the platform and covered with a 16-mesh plastic screen. Another hole, 10 cm in diameter, cut in the centre of the container lid, was covered with a 20-mesh screen. A plastic tray, 13 cm in diameter, containing a layer of glass wool, was placed at the open bottom of the cage. Fifty weevils were held in each cage and provided with bouquets of alfalfa every other day. Emerging parasitoid larvae dropped through the mesh platform and pupated on the layer of glass wool. Weevils were held in these cages for 30 days by the end of which time all non-diapausing parasitoid larvae emerged from the hosts (Neal and Bickley, 1971).

#### Handling Pupae of B. curculionis and M. aethiopoides

Cocoons of B. curculionis were placed on moist filter papers within 20 x 12 x 12 cm cages. The filter papers were sprayed with water once a week. Non-diapausing B. curculionis usually emerged 8-12 days after pupation.

Layers of glass wool, on which M. aethiopoides pupated, were removed daily from the bottom of the larval emergence cages. Pupae were carefully removed from the wool and incubated in the manner described for B. curculionis. M. aethiopoides emerged within 7-10

Figure 2. Emergence cage for the larvae of Microctonus aethiopoulos.



days after pupation.

#### Handling Adults of B. curculionis and M. aethiopoides

Cages containing cocoons of B. curculionis and M. aethiopoides were examined daily and adult parasitoids were aspirated into 2 dram vials. The mouths of the vials were plugged with cotton soaked with a solution of 50% honey (1 part of commercial liquid honey diluted with 1 part of water). Males and females were aspirated into separate vials. If mated females were required, several males and females were kept together in the same vial for 24 hours.

#### Storage of Cocoons of B. curculionis

Most of the B. curculionis, reared from weevil larvae in the summer of 1974, remained in diapause. The cocoons of the parasitoid were placed in petri dishes with screen lids, and stored in a growth cabinet at  $1.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , 8-hour photoperiod and 80-95% RH for at least 3 months to break diapause (Duodu and Davis, 1974c). When adult B. curculionis were required for insecticide tests the cocoons were transferred to  $25^{\circ}\text{C}$ , 12-hour photoperiod and 70% RH. The adult parasitoid emerged 10 to 17 days later.

#### Storage of New Adult Weevils Parasitized by M. aethiopoides

Newly-emerged adults of the alfalfa weevil were parasitized by M. aethiopoides during July to August, 1974 and stored for 1-3 months, to provide a steady source of adult M. aethiopoides for the insecticide tests. Batches of 200-300 parasitized weevils were placed in gallon jars, with 20-mesh screen lids, and supplied with pieces of

soda straw, 10 cm in length and 5 mm in diameter, to serve as artificial hibernacula (Neal and Bickley, 1971). After they had fed for about 4 weeks, most of the weevils entered the straw. The jars were kept in the same growth cabinet used for storing cocoons of B. curculionis. A piece of cotton was placed at the bottom centre of each jar. Every 2 weeks, the cotton was sprayed with a 2% sucrose solution to provide food for those weevils that were not yet in diapause. Byrne (1965) and Day (1971) observed that the survival of adult alfalfa weevils in storage was much greater when bouquets of alfalfa or 2% sucrose solution was provided at least every 4 weeks. The latter author concluded that sucrose was more convenient and more economical. When adult parasitoids were required, the weevils were removed from cold storage and treated with hormone to break diapause of both the host and the parasitoid. The fifth-instar larvae of M. aethiopoides emerged from the treated host within 9 to 14 days and pupated. The pupal stage lasted from 10 to 13 days.

#### Applying Synthetic Hormone to Diapausing Adult Weevils

Each adult weevil was treated with 100 µg of the synthetic, juvenile hormone, viz: trans,trans-10,11-epoxy-farnesenic acid methyl ester (obtained from Calbiochem, P.O. Box 54282, Los Angeles, California 90054). The hormone was applied in 0.5 µl of acetone to the venter of the abdomen using an ISCO micro-applicator.

#### SURVEY FOR THE DISTRIBUTION OF PARASITIDS

A survey was carried out in western Ontario during May and June of 1973. The objective was to obtain current information on the

establishment and distribution of the parasitoids of the alfalfa weevil. Samples of eggs, larvae, and adults of H. postica were collected from 17 counties located approximately west of longitude 79°W and south of latitude 44°N. The samples were obtained mostly from places where parasitoids of the alfalfa weevil had been released in 1970 and 1971 (Fig. 1). At each of the places where M. aethiopoidea was previously recovered (Kelleher and Loan, 1971), samples of adult weevils were collected from an area of about a 10-mile radius around the original release site. These additional samples provided information on the spread of this parasitoid to neighbouring alfalfa fields.

Fresh alfalfa stems from square-foot samples, taken randomly from fields in each of the 17 counties, were dissected to obtain egg clusters of H. postica. These egg clusters were placed in gelatin capsules and kept in the laboratory for 15-20 days and then examined for emergence of P. luna.

Larvae and adults of the weevil were collected from the fields by sweep nets. In the laboratory, third- and fourth-instar larvae were reared until they pupated or when cocoons of B. curculionis formed. Weevil larvae that died during rearing were transferred, individually, into gelatin capsules and examined later for emergence of T. incertus. Percentage parasitism by M. aethiopoidea was determined by dissecting the adult alfalfa weevils.

#### BIOLOGY OF THE PARASITIDS

##### Synchronization of B. curculionis with H. postica

Two fields of alfalfa, one each at the University of Guelph

Research Stations at Elora and Arkell, were sampled weekly during May to August of 1974 and 1975. The sampling device was a standard, 15-inch diameter, insect net. Five hundred sweeps, each consisting of a swing of the net through approximately 180° angle (Davis, 1970) were taken on each sampling day. The end of the net was placed in a killing bottle for about 10 minutes to kill all insects in the sample. Larvae of the alfalfa weevil and adults of B. curculionis were counted later in the laboratory.

Another 500-sweep sample was taken to obtain living larvae of the alfalfa weevil to estimate parasitism by B. curculionis. The percentage parasitism was determined from the number of third- and fourth-instar larvae reared and the number of B. curculionis cocoons obtained. Davis (1970) observed that dissecting weevil larvae to determine percentage parasitism by B. curculionis had no advantage over rearing. The latter method was used in the present investigation because it saved time and provided cocoons for investigating winter mortality of the parasitoid.

#### Synchronization of M. aethiopoides with H. postica

Preliminary surveys for the distribution of M. aethiopoides in southern Ontario, undertaken in 1973 and 1974, showed that this parasitoid established at two of the release sites of 1970; Aylmer in Elgin County and South Bay in Prince Edward County.

Adults of H. postica and M. aethiopoides were collected weekly from these two sites, with a sweep net as described previously. The sampling was from mid-April to mid-September, 1975. On some occasions, particularly in early spring and mid-summer, no adults of either the

weevil or parasitoid could be obtained, even from as many as 2000 sweeps. Percentage parasitism was determined by dissecting all the adult alfalfa weevils in the sample. The developmental stages of the parasitoids were recorded, based on Loan and Holdaway's (1961) description of the immature stages of M. aethiopoides.

#### Overwintering Mortality of B. curculionis

Third- and fourth-instar larvae of the alfalfa weevil were collected weekly from the Elora Research Station in the summer of 1973 and 1974. Cocoons of B. curculionis obtained from rearing these weevils were held in the laboratory for at least one month to allow all non-diapausing parasitoids to emerge.

Cocoons of parasitoids that had not emerged by October 1 were examined and those that were wrinkled or showed other visible signs of deformity were discarded. The remaining cocoons were counted and put on soil within plastic petri dishes, 6 x 2 cm, which had 34-mesh window-screen bottoms. The dishes were left in an alfalfa field at Elora from October 1 to May 1 of the succeeding year. Birds and ants were prevented from picking the cocoons by covering the dishes with 20-mesh window-screen tops. However, the dishes were left open during January to March when there was continuous cover of snow on the field.

Each petri dish was brought into the laboratory on May 1 and the soil was washed off through a 34-mesh sieve to recover the cocoons. Emergence of adult B. curculionis was recorded daily. All parasitoids that had not emerged by June 20 were assumed to be dead.

Effect of Parasitism by M. aethiopoides on Overwintering Mortality of H. postica

This experiment was designed to test whether or not parasitism by M. aethiopoides affects the overwintering mortality of the diapausing host.

Summer adults of the alfalfa weevil were obtained by rearing third- and fourth-instar larvae collected from the Elora Research Station during the period, July 21-28, 1974. Emerging adults were divided randomly into two groups. Those in one group were caged with female M. aethiopoides, as previously described, and thereafter designated as parasitized. The other group of weevils were handled in a similar way except that they were not caged with parasitoids and hence they were referred to as non-parasitized weevils.

Weevils of both groups were stored in the laboratory in plastic cages containing moist paper towels until October 1, 1974. Before they were transferred to cages in the field, samples of the parasitized weevils were dissected. The average parasitism was 61.1%.

The procedure for releasing weevils to overwinter in field cages was modified from Peterson (1960). On October 1, 1974, 40 open-bottom plastic cages, 20 x 12 cm and 12 cm high, were placed in pairs over crowns of alfalfa in a plot at Elora. Each cage was forced about 3 cm into the soil and the area inside was vacuumed to remove any weevils that might be present. Twenty parasitized adults of H. postica were dropped into one cage of each pair and 20 non-parasitized adults were dropped into the other. Thus, there were 400 parasitized and 400 non-parasitized weevils. The cages were covered with 20-mesh plastic screens to prevent weevils from crawling out, but these lids

were removed during January to March, 1975 when there was continuous cover of snow on the field.

The soil, plants, and litter enclosed in each cage, to a depth of 2.5 cm, were removed to the laboratory on May 1, 1975. The contents of each cage were washed through a 20-mesh sieve. Materials caught on the sieve were decanted into a bowl of water. Dead and living weevils floated on the water and were collected and counted. The percentage parasitism of the living weevils of the parasitized group was determined by dissection. Mortalities recorded for both parasitized and non-parasitized groups were analysed by the student t-test.

#### Effect of Parasitism by M. aethiopoides on the Mortality of H. postica During Low Temperature Storage

To maintain a colony of M. aethiopoides for subsequent field releases, several investigators have stored parasitized, as well as non-parasitized, adult weevils at temperatures ranging from 1.7°C (Day, 1971) to 10°C (Neal and Bickley, 1971; Hollaway et al., 1973). Day (1971) stored parasitized and non-parasitized adult weevils at various temperatures for 16 weeks and reported that survival of M. aethiopoides in diapause was not affected by any of the temperatures. However, the best weevils survival occurred at 1.7°C. Owing to the long cold months, lasting from October to April in most parts of Ontario, it may be necessary to store parasitized and non-parasitized weevils for as long as 7 or 8 months. A laboratory experiment was designed to test whether or not the survival of non-parasitized and parasitized H. postica would be affected by such a long storage. The result also served as a supplement to the field test on the survival

of parasitized and non-parasitized overwintering weevils.

Batches of 250 parasitized and non-parasitized summer adults, from the same groups used for the field experiment, were placed in plastic, gallon jars and provided with 50 pieces of soda-straw sections. Four pairs of these batches were stored from October 1, 1974 to May 1, 1975 in the growth cabinet programmed at  $1.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , 8-hour photoperiod and 80-95% RH. There were 1000 parasitized and 1000 non-parasitized weevils. Two per cent sucrose was provided throughout the storage period.

Most of the weevils crawled out of the straw sections when the cages were transferred to  $25^{\circ}\text{C}$  on May 1. The rest were removed by gently tapping the straws. Living and dead weevils in each cage were counted and mortalities among parasitized and non-parasitized groups were analysed by the t-test. All living weevils in the parasitized batches were dissected to determine parasitism.

#### TOXICITY OF INSECTICIDES TO THE ALFALFA WEEVIL AND ITS PARASITOIDS IN THE LABORATORY

##### The Insecticides

Five insecticides, chosen for these tests, were those recommended for the control of the alfalfa weevil in Ontario (Ontario Department of Agriculture and Food, 1972). Technical grade materials supplied by the manufacturers were used (Table I). The purity of methoxychlor was not supplied by the manufacturer but was assumed to be 95.0%.

##### The Insects

Toxicities of the five insecticides were determined by topical

Table I. Chemical name, purity and source of five technical grade insecticides applied topically to Hypera postica, Bathyplectes curculionis, and Microctonus aethiopooides.

Insecticide *	Chemical name *	Purity (%)	Source
carbaryl	1-naphthyl methylcarbamate	99.9	Union Carbide Corporation, New York, N.Y.
carbofuran	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate	99.0	Niagara Chemical Division, FMC Corporation, Middleport, N.Y.
malathion	diethyl mercaptosuccinate s-ester with O,O-dimethyl phosphorodithioate	95.0	Cyanamid of Canada
methoxychlor	1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane	---	DuPont of Canada Limited
phosmet	O,O-dimethyl phosphorodithioate s-ester with N-(mercaptomethyl) phthalimide	95.8	Stauffer Chemical Company, Richmond, California

\* After, List of Pesticides and Chemical Names. In Pesticide Research Report 1974, pages 393-413. The Canada Committee on Pesticide Use in Agriculture, Ottawa, Canada. 443 pp.

application on adults of B. curculionis, adults of M. aethiopoides, third-instar larvae of H. postica, third-instar larvae of H. postica parasitized by B. curculionis, overwintered adults of H. postica, and overwintered adults of H. postica parasitized by M. aethiopoides. Samples of these insects were anesthetized with CO<sub>2</sub> and weighed on a Cahn electrobalance which had a sensitivity of 0.1 µg. The mean weights are presented in Table II.

The adult parasitoids were used for the tests within 24 to 48 hours after emergence. Sufficient numbers of male B. curculionis could not be obtained and only females were tested. Male and female M. aethiopoides were treated separately because a t-test indicated that their mean weights were significantly different at the 5% level (Table II).

Parasitized and non-parasitized larvae were treated with insecticides when they were 6 days old, by which time they were in the third instar. The former had been parasitized 4 days prior to treatment. Dissection of samples indicated parasitism of 30-60%.

The adult weevils were used 7 days after exposure to parasitoids. Parasitism was 50-70%. Parasitized and non-parasitized groups were collected and handled in a similar manner except that the latter were not caged with parasitoids.

#### Application of Insecticides

Acetone was used as the solvent for the insecticides and all insects to be treated were anesthetized with CO<sub>2</sub>. Preliminary investigations showed that the combination of CO<sub>2</sub> and acetone was not toxic to any of the insects at the dosages used.

Table II. Mean weights of insects used for the determination of LD<sub>50</sub> values for five insecticides by topical application.

Insect	Weight (mg) ± standard deviation
<u>Bathyplectes curculionis</u>	
Female	1.44 ± 0.24
<u>Microctonus aethiopoides</u>	
Female	0.61 ± 0.09
Male	0.65 ± 0.07
	(significantly different at 5%)
Third-instar larvae of <u>Hypera postica</u>	
Parasitized by <u>M. aethiopoides</u>	7.90 ± 1.58
Non-parasitized	8.08 ± 1.01
	(not significantly different)
Adults of <u>H. postica</u>	
Parasitized by <u>B. curculionis</u>	8.45 ± 1.69
Non-parasitized	8.84 ± 1.27
	(not significantly different)

Approximate LD<sub>50</sub> values of each of the insecticides were determined on each of the insects in preliminary tests. Thereafter, 5 concentrations; 1 equal to the approximate LD<sub>50</sub> value and 2 above and 2 below it, were applied to the insects. Each replicate also included a control, using acetone only. The stock solution, from which the desired concentrations were obtained by dilution, was prepared on the day the test was performed and any remaining solution was discarded. The solutions were applied by means of an ISCO micro-applicator and a 0.25 ml tuberculin syringe (B-D Yale syringe No. 20001) fitted with a No. 27 hypodermic needle. The volumes of solutions, dropped on the dorsum of the thorax of each insect, were 0.5 µl for the parasitoids and 1.0 µl for the larvae and adults of the alfalfa weevil. There were 10 insects per each concentration of each insecticide and all treatments were replicated 10 times except for the larval and adult weevils which had been caged with parasitoids prior to the test. In the latter group of weevils, the topical applications were replicated up to 20 times. Parasitism of these larval and adult weevils was about 50% and the 20 replicates ensured treatment of an adequate number of parasitized individuals.

Recovery cages for treated parasitoids were round, tapered ice-cream cartons, 12 cm high and 7.5 cm in diameter at the top, covered with 34-mesh nylon cloth. A piece of cotton soaked with a 50% honey solution was placed on the nylon cloth to serve as food for the parasitoids. Treated larvae and adults of H. postica were transferred into 20 x 12 x 12 cm plastic cages, containing bouquets of alfalfa, and were covered with 20-mesh nylon screens. All the recovery cages were held in the laboratory at 25°C and 70% RH for

48 hours. The numbers of moribund and dead insects were combined in estimating mortality. Larval and adult weevils that were exposed to parasitoids before treatment were dissected and only individuals containing parasitoid larvae were used to estimate mortality. Mortality of larvae and adults of the weevil was estimated as:

$$\frac{\text{No. of dead weevils containing parasitoid larvae}}{\text{Total no. of weevils containing parasitoid larvae}}$$

All the replicates of the dosage-mortality data for each insect and each insecticide were pooled and subjected to probit analysis after Finney (1971) using an APL programming on an IBM 370 computer.

## RESULTS AND DISCUSSION

## DISTRIBUTION OF PARASITOIDS

Potasson luna was recovered from 13 of the 17 sites surveyed (Fig. 3), but parasitism was only 0.7 to 17.0% (Table III). In most cases only 1 to 3 weevil eggs in a whole cluster were parasitized. T. incertus was obtained from only two places, Bramalea in Peel County and Arkell in Wellington County with parasitism of 1.4% and 3.2%, respectively. B. curculionis was present at all the sites surveyed (Fig. 3) and parasitism ranged from 4.4 to 75.0% (Table IV). Microctonus aethiopoides was recovered only from Aylmer in Elgin County where parasitism was 33.3% and 56.6% in 1973 and 1974, respectively. Kelleher and Loan (1971) had previously recovered M. aethiopoides from two other places in western Ontario (Lythmore in Haldimand County and Chatham in Kent County). In 1973, 10.0% of adult weevils collected from an alfalfa field 4 miles west of the original release site at Aylmer, were parasitized by M. aethiopoides. Parasitism at this farm increased to 30.0% in 1974. No other alfalfa field was found in the vicinity of the release site at Aylmer.

There was no evidence of establishment of M. aethiopoides at any of the 1971 release sites in western Ontario. Similarly, in eastern Ontario, Kelleher and Loan (1973) reported that M. aethiopoides was not recovered from any of the 1971 release sites whereas there was 88% parasitism at South Bay in Prince Edward County (Fig. 3), where the parasitoid was released in 1970. In 1970, adult M. aethiopoides were released, whereas in 1971, adults of H. postica parasitized by M. aethiopoides were released (Kelleher and Loan, 1971). Hollaway

Figure 3. Sites where parasitoids of Hypera postica were recovered in western Ontario in 1973.

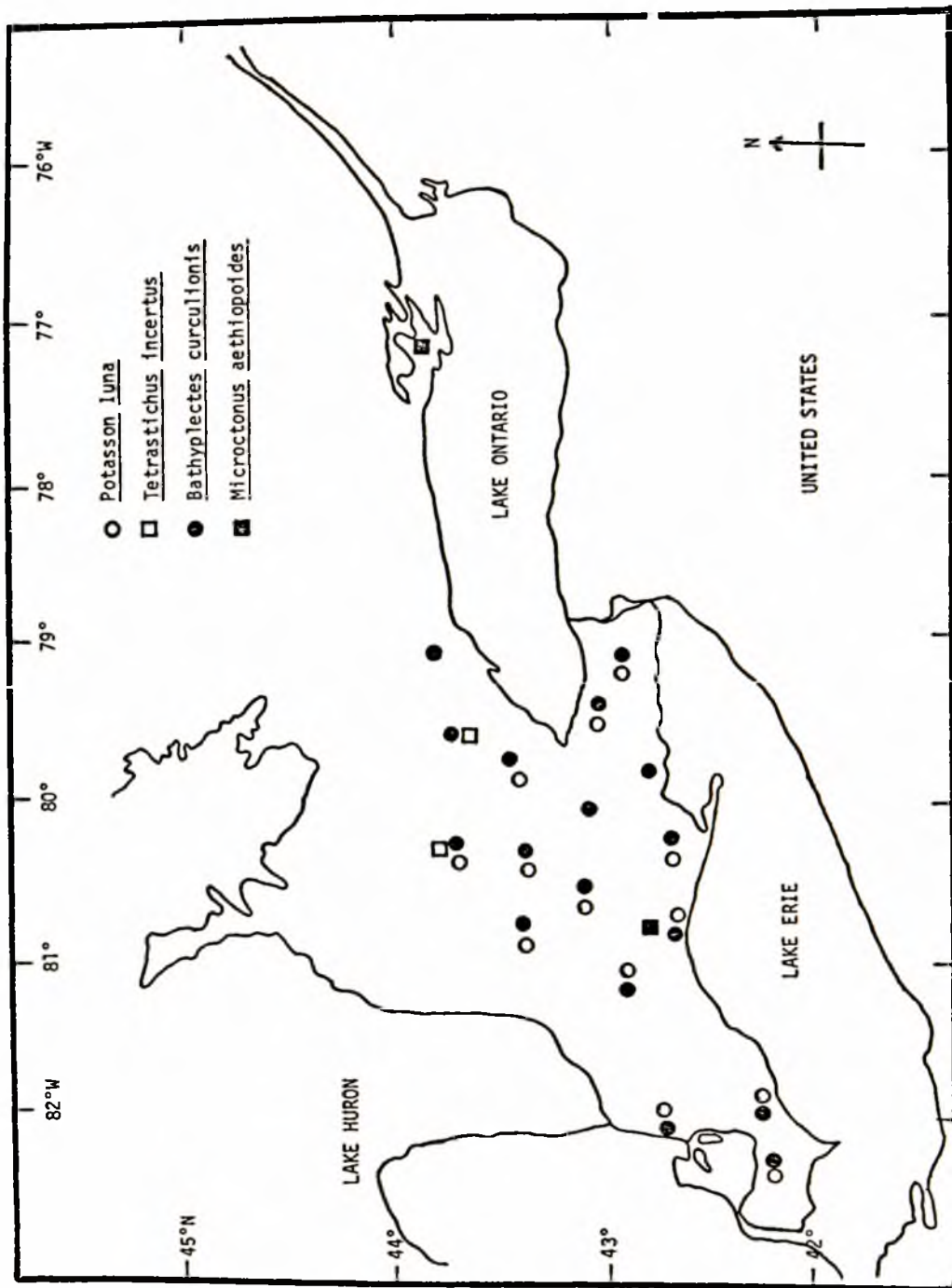


Table III. Number of eggs of Hypera postica collected, number parasitized by Potasson luna, and percentage parasitism in counties and localities surveyed in western Ontario in 1973.

County	Locality	Date of collection	No. of eggs collected	No. of eggs parasitized	Parasitism (%)
Essex	Comber	May 31	217	8	3.7
Kent	Merlin	" 30	120	1	0.8
Lambton	Port Lambton	" 31	85	2	2.4
Middlesex	Middlemiss	June 7	228	11	4.8
Elgin	Aylmer	May 31	144	2	1.4
Perth	Kirkton	June 7	183	5	2.7
Oxford	Woodstock	" 8	8	1	12.5
Norfolk	Simcoe	" 5	307	16	5.2
Welland	Fenwick	" 5	221	17	8.0
Lincoln	Fulton	" 5	280	2	0.7
Waterloo	Galt	" 8	217	7	3.3
Halton	Burlington	" 11	47	8	17.0
Wellington	Arkell	" 6	179	12	6.7

Table IV. Number of larvae of *Hypera postica* collected, number parasitized by *Bathyplectes cureulionis*, and percentage parasitism in counties and localities surveyed in western Ontario in 1973.

County	Locality	Date of collection	No. of larvae collected	No. of larvae parasitized	Parasitism (%)
Essex	Comber	May 31	280	34	12.1
Kent	Chatham	" 30	23	4	17.4
Lambton	Port Lambton	" 31	148	52	35.1
Middlesex	Middlemiss	June 7	95	16	16.8
Elgin	Aylmer	May 31	26	10	38.5
Perth	Kirkton	June 7	20	2	10.0
Oxford	Woodstock	" 8	60	10	16.8
Norfolk	Simcoe	" 5	85	4	4.7
Haldimand	Kohler	" 14	22	3	13.6
Welland	Fenwick	" 5	84	10	11.9
Brant	Brantford	" 8	40	10	25.0
Lincoln	Fulton	" 5	15	6	40.0
Waterloo	Galt	" 8	45	2	4.4
Halton	Burlington	" 11	60	15	25.0
Wellington	Rockwood	" 11	20	15	75.0
Peel	Bramalea	" 11	70	22	31.4
York	Schomberg	" 12	60	19	31.7

et al. (1973) tested the two methods under field conditions and concluded that the establishment of M. aethiopoidea was more successful if the adult parasitoids were released. This could explain the failure of M. aethiopoidea to establish from the 1971 releases in Ontario.

The results of this survey indicate that P. luna and T. incertus are presently of little significance in the control of the alfalfa weevil. The period of collecting the weevil larvae could be partly responsible for the failure to obtain T. incertus from most of the sites surveyed. Streams and Fuester (1967), Brunson and Coles (1968), Horn (1971), and Blickenstaff et al. (1972) reported that parasitism by T. incertus was highest in late summer. B. curculionis was well distributed in western Ontario, but there was much variation in the percentage parasitism at the various sites. Apart from the high percentage parasitism, M. aethiopoidea also has the potential for rapid spread once established (Day et al., 1971).

#### BIOLOGY OF THE PARASITIDS

##### Synchronization of B. curculionis with H. postica

The results of field samplings for B. curculionis and H. postica at Arkell for the 1974 and 1975 growing seasons are presented in Figs. 4 and 5 respectively. The results obtained for similar investigations at Elora during 1974 and 1975 are given in Figs. 6 and 7 respectively. Generally, the populations of host and parasitoid, as well as percentage parasitism by B. curculionis at Arkell and Elora in the two years, were similar. Larvae of H. postica were first observed in the samples during the third week of May. The numbers of weevil larvae were less than 10

per 100 sweeps until the end of May. Thereafter, the populations increased sharply and reached maxima in the last week of June. An exception was noted in 1974 at Arkell where the peak occurred at mid-June (Fig. 4), about two weeks ahead of Elora during the same season (Fig. 6). The peaks of larval populations recorded in 1974 (207.8 and 197.6 larvae per 100 sweeps at Arkell and Elora, respectively) were higher than those of 1975 (186.6 and 175.5 at Arkell and Elora, respectively). Larval numbers per 100 sweeps declined to less than 20 within four weeks and then declined gradually to less than 10 for the remainder of the seasons. These decreases in populations resulted from pupation of the larvae and minimal egg laying in July and August.

Adults of B. curculionis were found in the samples from the end of May to mid-August, coinciding with populations of the larvae of H. postica. However, numbers of the adult parasitoid were low (0-13 per 100 sweeps) throughout the season.

Parasitism of weevil larvae by B. curculionis was noted 1 to 2 weeks after the adult parasitoids had been observed in the field samples, but there was no marked increase in percentage parasitism until July when larval populations started to decline. Consequently, parasitism was very low at the time of larval peaks but increased to maxima of 56.6% (Fig. 4) to 68.0% (Fig. 6) in August when host populations were less than 10 per 100 sweeps.

During the growing seasons of 1973-1975, 40.5-95.0% of B. curculionis cocoons, obtained by rearing parasitized larvae of weevils from each week's sample at Elora, contained parasitoids that were in diapause (Table V). Diapause of parasitoids obtained from Elora



Figure 4. Numbers of larvae of Hypera postica, numbers of adults of Bathyplectes curculionis and percentage parasitism at Arkell, Ontario, in 1974.

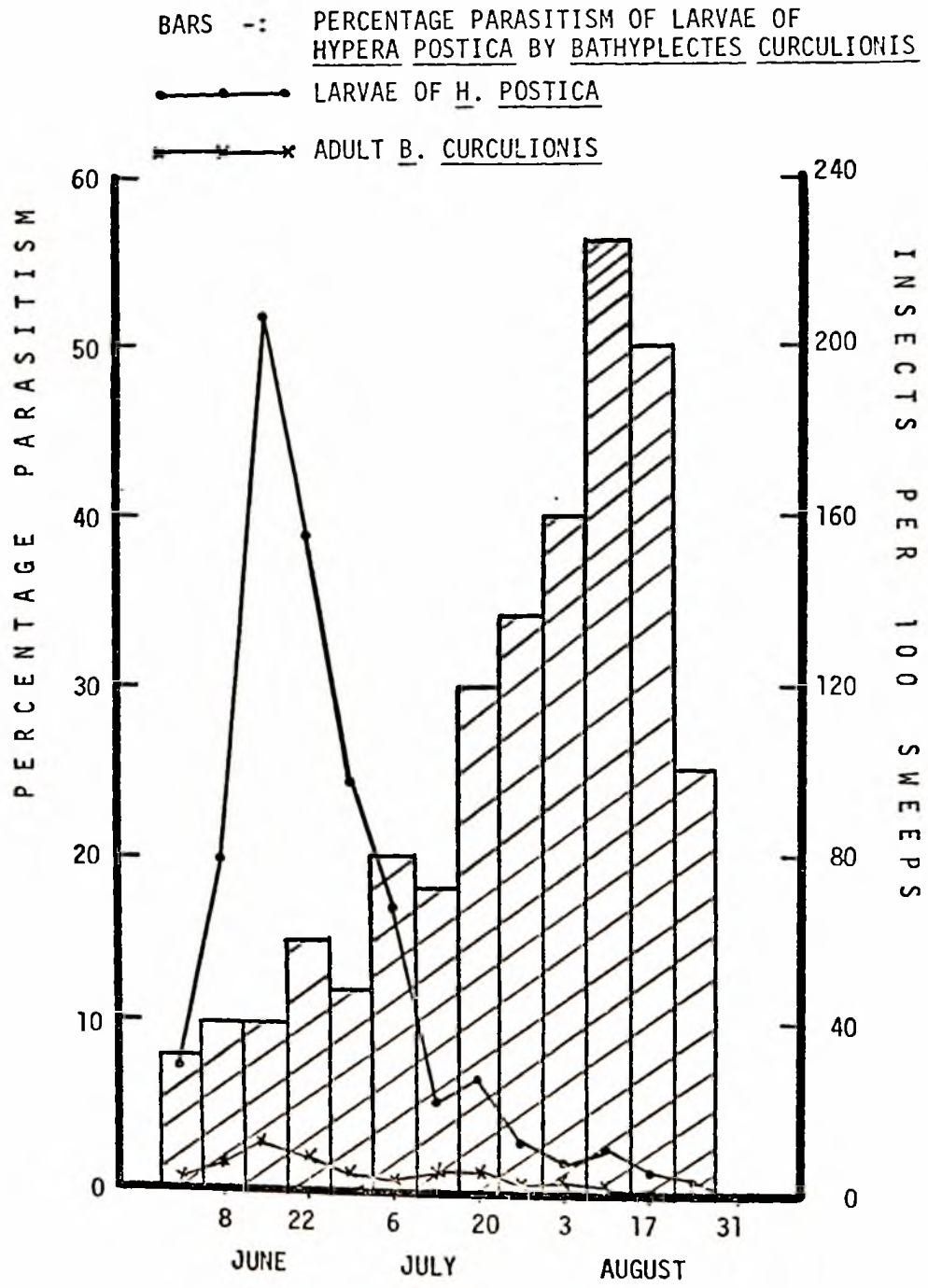




Figure 5. Numbers of larvae of Hypera postica, numbers of adults of Bathyplectes curculionis and percentage parasitism at Arkell, Ontario, in 1975.

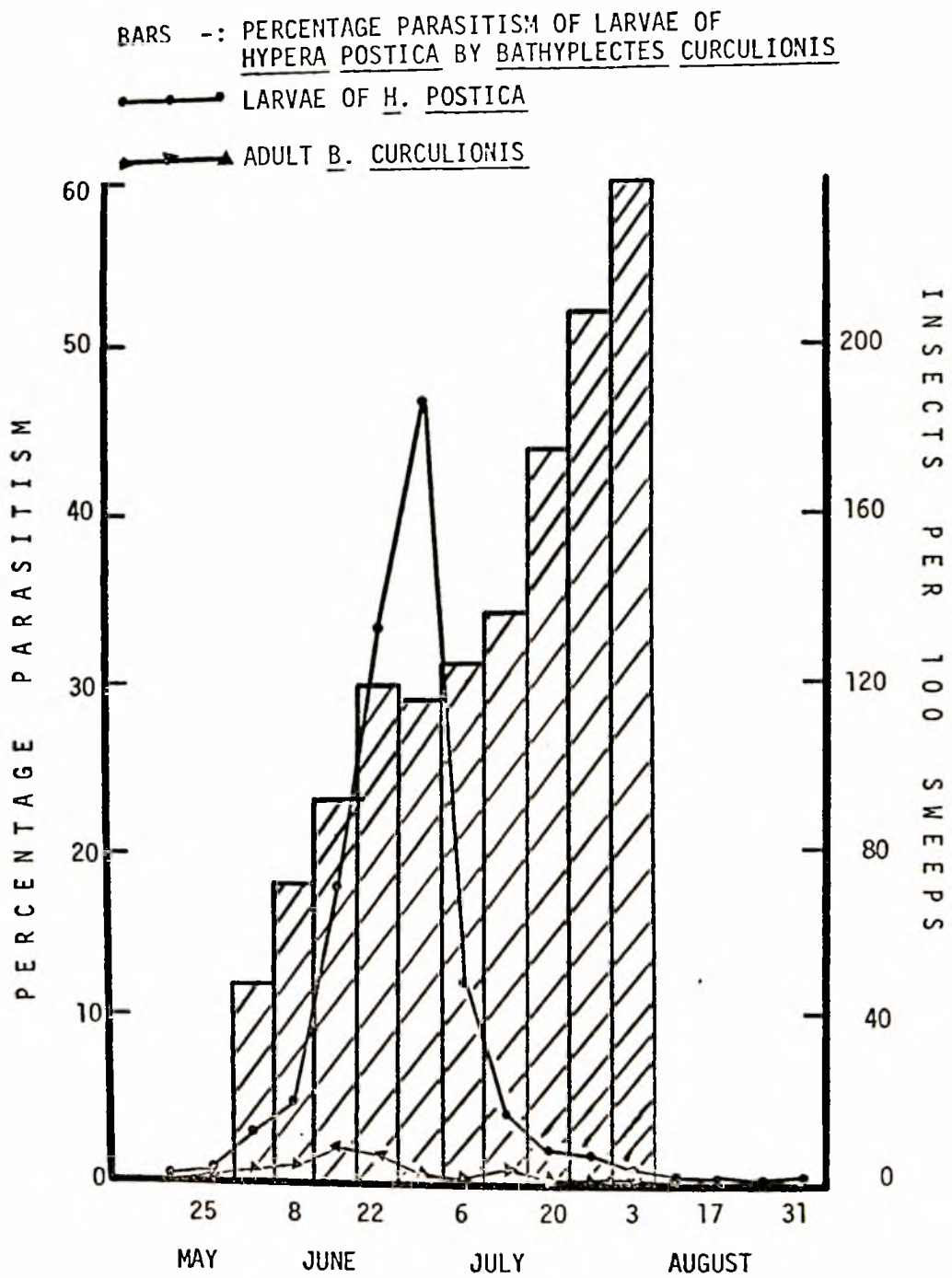


Figure 6. Numbers of larvae of Hypera postica, numbers of adults of Bathyplectes curculionis and percentage parasitism at Elora, Ontario, in 1974.

BARS --: PERCENTAGE PARASITISM OF LARVAE OF  
HYPERA POSTICA BY BATHYPLECTES CURCULIONIS  
 ●●●● LARVAE OF H. POSTICA  
 ▲▲▲▲ ADULT B. CURCULIONIS

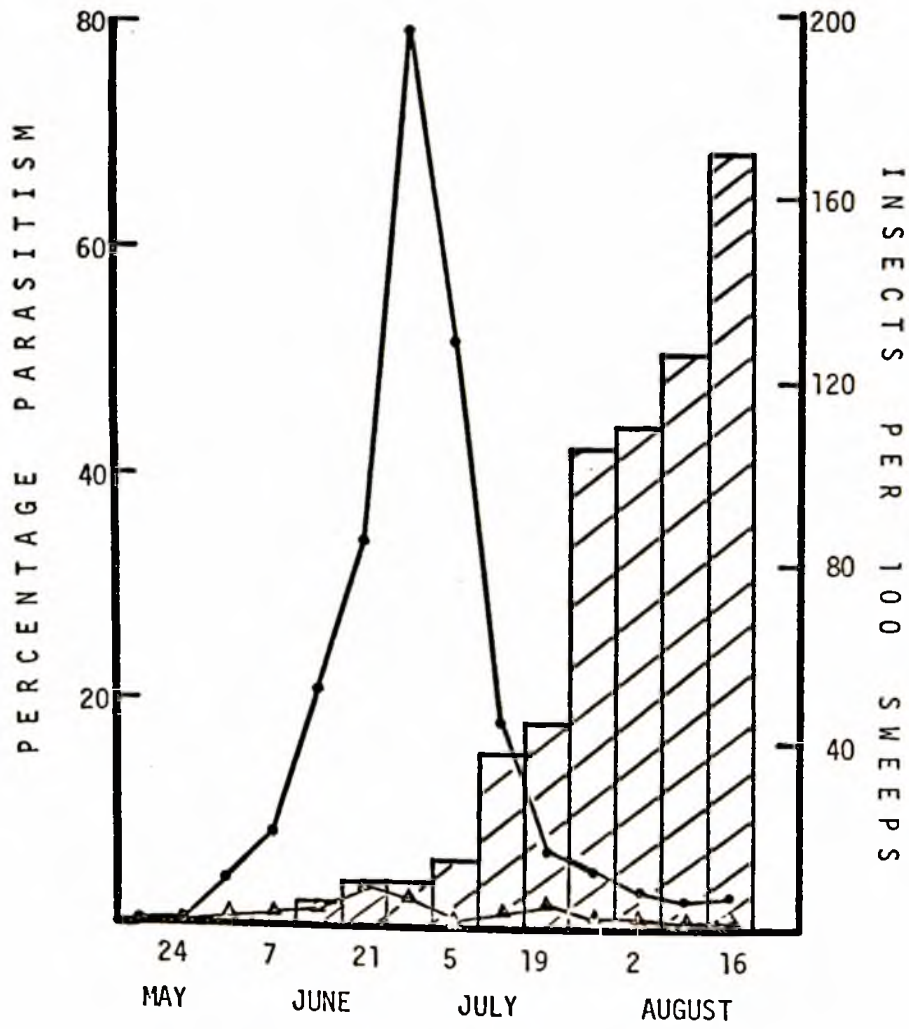
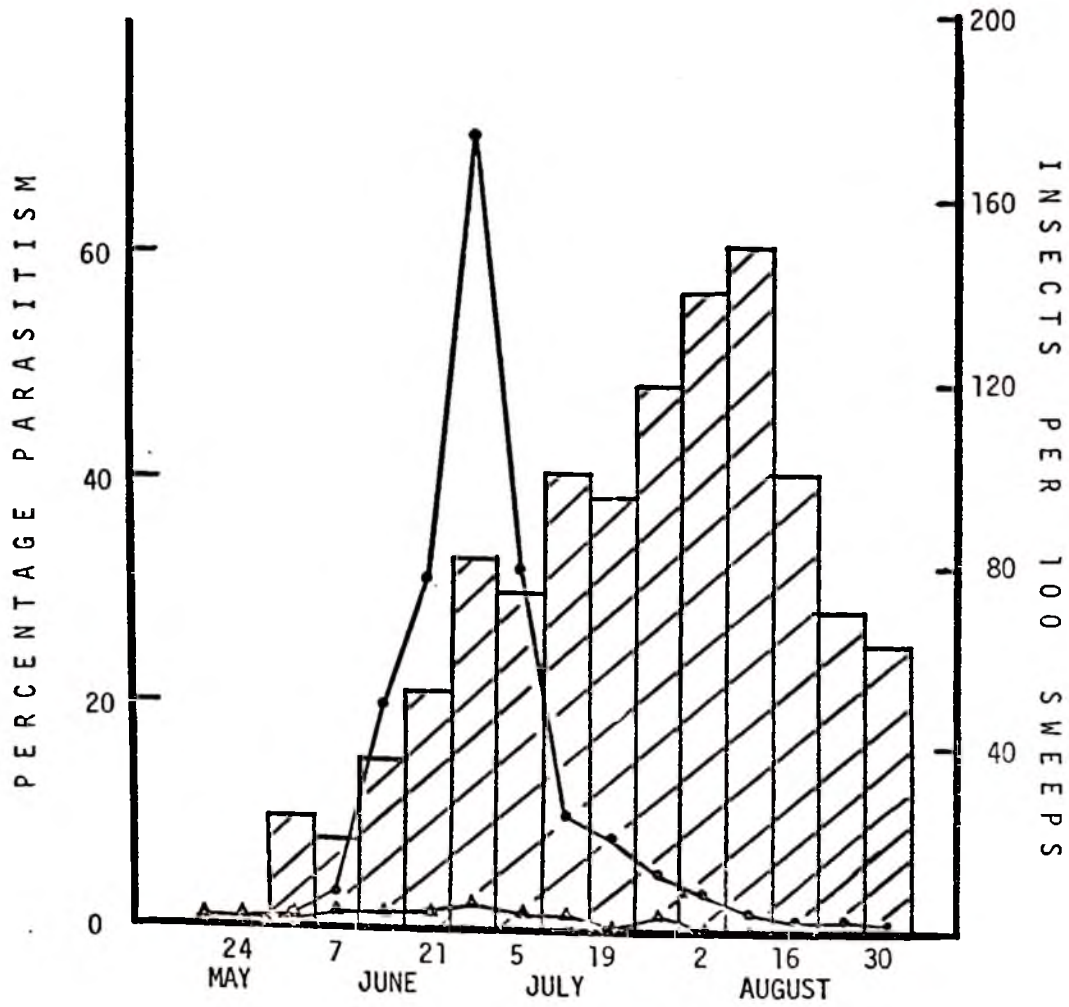


Figure 7. Numbers of larvae of Hypera postica, numbers of adult Bathyplectes curculionis and percentage parasitism at Elora, Ontario, in 1975.

BARS --: PERCENTAGE PARASITISM OF LARVAE OF  
HYPERA POSTICA BY BATHYPLECTES CURCULIONIS  
 —●— LARVAE OF H. POSTICA  
 —▲— ADULT B. CURCULIONIS



(Table V) and Arkell (Table VI) in 1974-1975 followed similar trends. At both places diapause was more than 70% among parasitoids collected after June 15. The high incidence of diapause early in the season was responsible for the minimal increase in the number of adult B. curculionis to affect the rising host population. This relationship between B. curculionis and H. postica is similar to what was observed in the eastern United States by Blickenstaff et al. (1972) and Smilowitz et al. (1972). The increase in percentage parasitism after June was due to lower host:parasitoid ratios resulting from the natural decline in larval populations. Davis (1974) reported that a lower host:parasitoid ratio was conducive to higher percentage parasitism.

#### Synchronization of M. aethioides with H. postica

The fluctuations in the populations of H. postica and M. aethioides as well as changes in percentage parasitism at Aylmer and South Bay in 1975 are shown in Figs. 8 and 9. Adult weevils were not found in the samples until the first week of May. The number of weevils in the fields at that time was low (1.1 per 100 sweeps at Aylmer and 0.7 per 100 sweeps at South Bay). Within 3 to 4 weeks the populations increased to peaks of 43.7 and 38.1 weevils per 100 sweeps at Aylmer and South Bay, respectively. These rapid increases in the populations resulted from the resumed activity and return of overwintered adult weevils to the alfalfa fields. These weevils had hibernated in the soil and surrounding vegetation (Tysowsky and Dorsey, 1970). A sharp decline in the population of adult weevils occurred from late May to mid-June. This decline was the result of mortality from natural causes and from parasitism by M. aethioides. Van Driesche (1975)

Table V. Dates when larvae of Hypera postica were collected from Elora, Ontario, and reared and percentage of diapausing Bathyplectes curculionis obtained.

Date of collecting larvae of <u>H. postica</u>		<u>B. curculionis</u> remaining in diapause (%)			
		1973	1974	1975	Mean
June	7	47.7	--	33.3	40.5 ± 10.2
	14	50.0	70.0	60.0	60.0 ± 10.0
	21	76.6	80.0	74.0	76.9 ± 3.0
	28	84.0	95.5	87.7	89.1 ± 5.9
July	5	75.0	84.7	80.0	79.9 ± 4.9
	12	80.0	100.0	78.0	82.9 ± 8.9
	19	85.0	93.8	75.2	84.7 ± 9.3
	26	90.0	75.0	90.0	85.0 ± 8.7
August	2	90.0	90.0	82.4	87.5 ± 4.4
	9	86.0	90.6	78.0	84.9 ± 6.4
	16	90.0	100.0	95.0	95.0 ± 5.0

Table VI. Dates when larvae of Hypera postica were collected from Arkell, Ontario, and reared and percentage of diapausing Bathyplectes curculionis obtained.

Date of collecting larvae of <u>H. postica</u>		<u>B. curculionis</u> remaining in diapause (%)		
		1974	1975	Mean
June	1	33.3	53.4	43.3 ± 14.2
	8	50.0	50.0	50.0 ± 0.0
	15	66.3	66.7	66.5 ± 0.3
	22	80.0	90.0	85.0 ± 7.1
	29	76.3	100.0	88.1 ± 16.8
July	6	75.0	75.5	75.2 ± 0.3
	13	78.5	92.4	85.4 ± 9.8
	20	70.0	85.0	77.5 ± 10.6
	27	80.0	90.0	85.0 ± 7.1
August	3	95.0	90.0	92.5 ± 3.5
	10	90.0	--	90.0 ± 0.0

Figure 8. Numbers of adults of Hypera postica, numbers of adults of adults of Microctonus aethiopoides, and percentage parasitism at Aylmer, Ontario, in 1975.

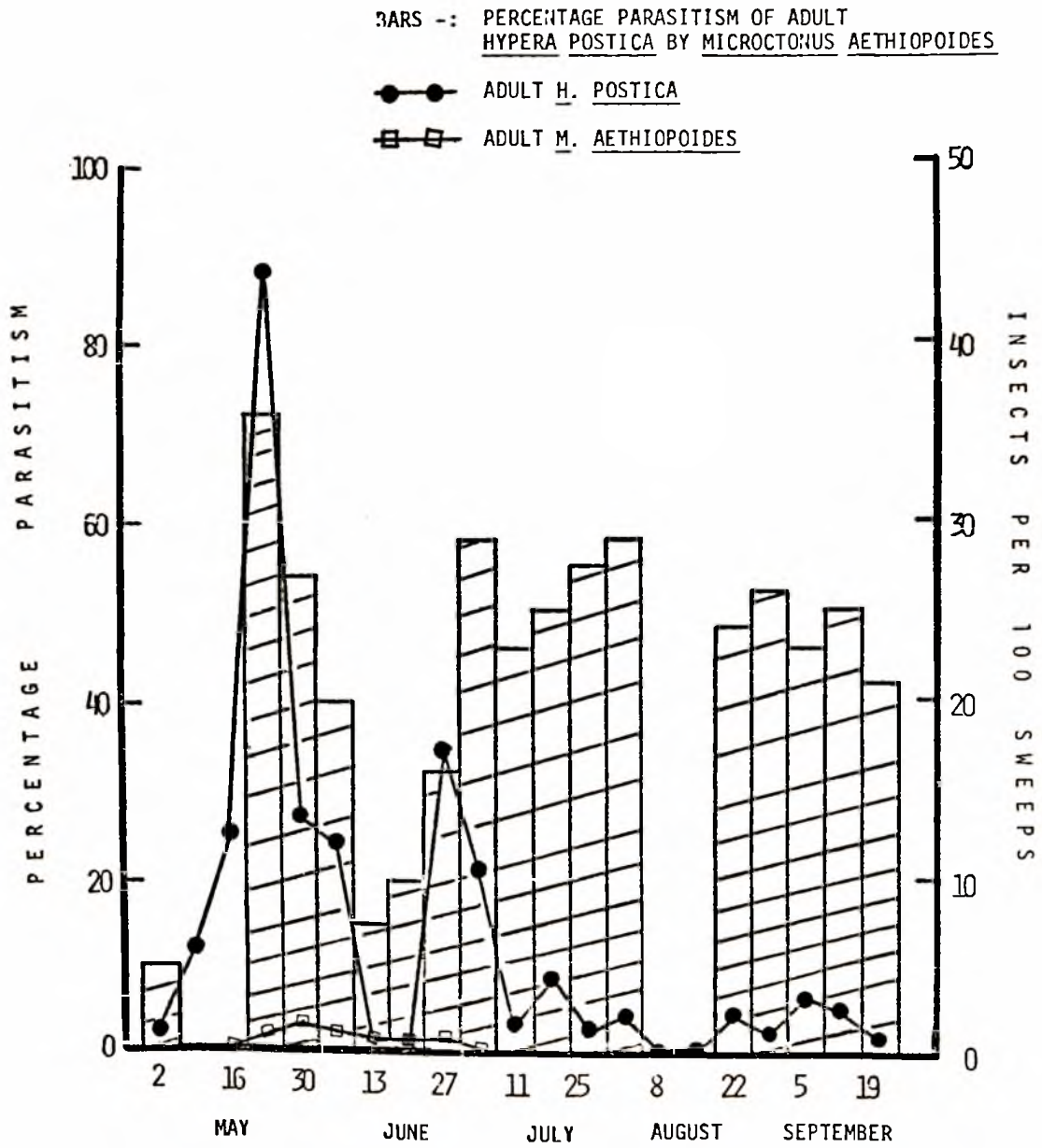
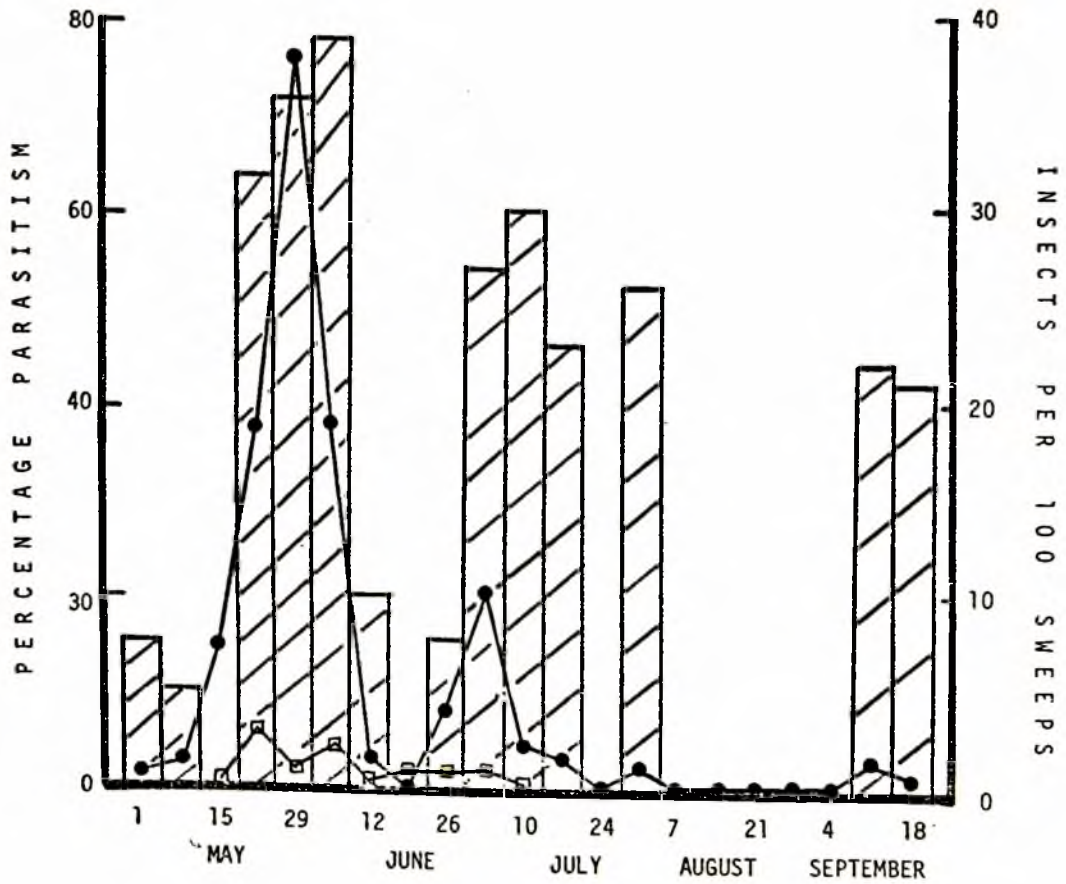


Figure 9. Numbers of adults of Hypera postica, numbers of adults of Microctonus aethiopoidea, and percentage parasitism at South Bay, Ontario, in 1975.

BARS :- PERCENTAGE PARASITISM OF ADULT HYPERA POSTICA BY MICROCTONUS AETHIOPOIDES

●● ADULT H. POSTICA

□□ ADULT M. AETHIOPOIDES



noted in New York State that in areas where parasitism by M. aethiopoides was high, overwintered H. postica had virtually disappeared from the alfalfa fields by mid-June. In the absence of this parasitoid, 50% of these weevils persisted until August 1.

The second increase in population of weevils was due to emergence of new adults. The changes in the population of these summer adults were erratic, varying from 0 to 18 insects per 100 sweeps. These fluctuations were believed to be related to the aestivation habits of this insect and also the weather conditions when samples were taken. Blickenstaff et al. (1972), Manglitz (1958), and Tysowsky and Dorsey (1970) observed that as temperatures increased in the summer, most of the new adults aestivated under the vegetation, either in the alfalfa fields or nearby wooded areas.

Adults of M. aethiopoides appeared in the weekly samples from late May to early July. During this period, their numbers varied from 0.2 to 3.0 per 100 sweeps. Only 10-15% (Figs. 8 and 9) of the overwintered adult weevils, obtained in the first week of May, contained larvae of M. aethiopoides. These parasitoids had already developed to the fourth larval instars, as determined by dissection. In New York State, first-instar larva of M. aethiopoides resumed development in March and emerged from the overwintered weevils during mid to late April (Van Driesche, 1975). It can be deduced from these observations that most of the larvae of M. aethiopoides developed, emerged from their host, and pupated in the soil before the non-parasitized adult weevils became active. Hence, there were periods (May 9 - May 16 at Aylmer and May 15 at South Bay) during which no parasitoid larvae were in the adult weevils; all parasitized weevils

had died, following the emergence of the parasitoids. Consequently, while more non-parasitized weevils resumed activity, adult M. aethioides emerged from pupation to oviposit in them. This good, host-parasite synchrony was responsible for the high parasitism of 72.0% at Aylmer and 78.0% at South Bay. The percentage parasitism was underestimated because parasitoid eggs were difficult to detect within the dissected weevils and some of them were probably missed. Rearing of adult weevils collected on May 23 at Aylmer and June 5 at South Bay showed parasitism of 76.6% and 82.0%, respectively; 4.0% and 4.6% higher, compared to the parasitism determined from dissection.

Parasitism of the overwintered adult weevils gave rise to a second generation of adult M. aethioides. This led to an overlap of the populations of the adult parasitoids and the new adult weevils for about two weeks (Figs. 8 and 9). At the time sampling was terminated in September, parasitism of the new adult weevils at Aylmer and South Bay was 41.7% and 42.9%, respectively. These figures are substantially higher than the 27.3% (Van Driesche, 1975) and 30.0% (Brunson and Coles, 1968) reported in the States of New York and New Jersey, respectively. In some parts of eastern United States, most of the new adult weevils moved from the field to aestivate soon after emergence (Blickenstaff et al., 1972; Manglitz, 1958; Tysowsky and Dorsey, 1970), but in Ontario the cooler summer climate delayed aestivation (Surgeoner, 1973). Thus, in Ontario, it is more likely that there would be many active new adult weevils in the fields when the second brood of adults of M. aethioides emerge. This could be the reason for the higher percentage parasitism of summer-emerged

adult weevils in Ontario.

Loan (1963) studied the relationship between Sitona scissifrons Say (Coleoptera: Curculionidae) and its parasitoid, Microctonus sitonae Mason and observed that, in the summer, most of the aestivating adult weevils were inaccessible to the parasitoid. Consequently, parasitism of the subsequent overwintering population was only 6%. Based on the results of the present investigation and Loan's (1963) finding, it can be concluded that unless adults of the second brood of M. aethiopoides emerge at a time when non-aestivating summer adults of H. postica are available, parasitism of overwintering weevils will be minimal. Since M. aethiopoides overwinters as first-instar larvae within these summer adult weevils, the overlap of populations of the two species is critical to the survival of the parasitoid.

#### Overwintering Mortality of B. curculionis

Data could not be obtained on the overwintering mortality of B. curculionis which entered diapause prior to June 20 because sufficient numbers of parasitoid cocoons were not available. This was due to the lower percentage parasitism of weevil larvae by B. curculionis during early spring. Mortalities of overwintered parasitoids were 30.0-83.3% (mean =  $56.9 \pm 15.6$ ) for 1973-74 (Table VII) and 25.0-61.0% (mean =  $42.3 \pm 14.0$ ) for 1974-75 (Table VIII). Irrespective of the date of collection, emergence of adult parasitoids began 5-7 days after the cocoons had been returned to the laboratory and ceased by the 20th day. Most of the emergence occurred between the 7th and 10th day. Mortality was highest among parasitoids that entered diapause in late June and

Table VII. Dates when larvae of Hypera postica were collected from Elora, Ontario, in 1973 and reared, number of cocoons of Bathyplectes curculionis obtained and overwintered, and percentage survival and mortality of B. curculionis during overwintering.

Date of collecting larvae of <u>H. postica</u>	No. of cocoons of <u>B. curculionis</u> overwintered*	No. of adults of <u>B. curculionis</u> emerging	Survival (%)	Mortality (%)
June 20	24	4	16.7	83.3
27	43	13	30.2	69.8
July 4	31	12	38.7	61.3
11	16	7	43.8	56.2
18	32	15	46.9	53.1
25	33	16	48.5	51.5
August 1	105	55	52.4	47.6
8	60	42	70.0	30.0
TOTAL	344	164	Mean 43.4 ± 15.8	56.9 ± 15.6

\* Cocoons were overwintered in field cages from October 1, 1973 to May 1, 1974.

Table VIII. Dates when larvae of Hypera postica were collected from Elora, Ontario, in 1974 and reared, number of cocoons of Bathyplectes curculionis obtained and overwintered, and percentage survival and mortality of B. curculionis during overwintering.

Date of collecting larvae of <u>H. postica</u>	No. of cocoons of <u>B. curculionis</u> overwintered*	No. of adults of <u>B. curculionis</u> emerging	Survival (%)	Mortality (%)
June 21	100	40	40.0	60.0
28	100	39	39.0	61.0
July 5	100	51	51.0	49.0
12	100	48	48.0	52.0
19	100	56	56.0	44.0
26	100	63	63.0	36.0
August 2	100	73	73.0	27.0
9	87	65	74.7	25.3
16	36	27	75.0	25.0
TOTAL	823	462	Mean 57.7 ± 14.4	42.3 ± 14.0

\* Cocoons were overwintered in field cages from October 1, 1974 to May 1, 1975.

decreased as the summer progressed (Tables VII and VIII). These observations, generally, agreed with those of Armbrust *et al.* (1972). In late June (June 20-30), 76.9-89.1% of the cocoons of B. curculionis remained in diapause (Table V). This high incidence of diapause was a disadvantage to the survival of B. curculionis since 60.0-83.3% (Tables VII and VIII) of these parasitoids failed to emerge after the winter. It was evident that diapause prior to August 1 was detrimental to the parasitoid's survival. Under field conditions, early diapause also led to prolonged exposure of the cocoons to high summer temperatures which caused mortality (Casagrande and Stehr, 1973). The higher percentage parasitism by B. curculionis, during early to mid-August (Figs. 6 and 7), when the host population was low, was actually of survival value to the parasitoid. Incidence of diapause was high in August (Tables V and VI), and a high proportion of the parasitoids that were in diapause at this period survived the winter (Tables VII and VIII).

#### Effect of Parasitism by M. aethiopoides on the Overwintering Mortality of H. postica

The result of the field experiment to test the overwintering mortality of parasitized and non-parasitized adult weevils are presented in Table IX. The mean percentage mortalities,  $48.7 \pm 8.6$  for the non-parasitized weevils and  $53.5 \pm 7.6$  for the parasitized weevils, were not significantly different. Parasitism by M. aethiopoides did not, therefore, affect the host's ability to survive under the field conditions of winter. When the surviving weevils of the parasitized group were dissected, 46.8% (87 out of 186) were found to contain

Table IX. Percentage mortality of non-parasitized and parasitized adults of Hypera postica overwintered in field cages at Elora, Ontario, from October 1, 1974 to May 1, 1975.

	No. of adults of <u>H. postica</u> overwintered	No. alive	No. dead	Mortality* (%)
Non-parasitized	400	205	195	48.7 ± 8.6
Parasitized by <u>Microctonus</u> <u>aethiopoides</u>	400 <sup>†</sup>	186 <sup>†</sup>	214	53.5 ± 7.6
TOTAL	800	391	409	---

\* Twenty replicates of 20 weevils each,  $t = 1.84 < t_{.05} = 2.02$

† Parasitism at beginning of experiment = 61.0; parasitism at end of experiment = 46.8%.

larvae of M. aethiopoulos in various stages of development; 57.5% (50 out of 87) were second- and third-instar larvae and 42.5% (32 out of 87) were fourth-instar larvae. No first-instar larva was found.

Effect of Parasitism by M. aethiopoulos on Mortality of H. postica  
During Low Temperature Storage

Mean percentage mortalities among non-parasitized and parasitized weevils were  $15.7 \pm 3.0$  and  $18.1 \pm 3.5$ , respectively (Table X). These means were not significantly different. Dissection revealed that, out of 474 parasitoid larvae, 284 (59.9%) were first-instar larvae and 190 (40.1%) were second-instar larvae, indicating that some of the parasitoid larvae were no longer in diapause.

The results of the two experiments were complementary. Diapausing larvae of M. aethiopoulos did not affect the mortality of the hosts either under field conditions in the winter or at 1.5°C. Since M. aethiopoulos overwinters within the host, survival of the latter during the winter, is of advantage to the parasitoid itself. Equal mortality rates in the parasitized and non-parasitized weevils will favour a constant host:parasitoid ratio until spring, when emergence of the parasitoids and the consequent death of the parasitized weevils will lead to a lower host:parasitoid ratio. In the spring, such a situation can give rise to high percentage parasitism of the remaining overwintered weevils when the first brood of adult parasitoids emerge. Parasitized weevils, stored in the growth cabinet for 30 weeks, suffered only 18.1% mortality. It is, therefore, feasible to store large quantities of parasitized hosts for subsequent field releases

Table X. Percentage mortality of non-parasitized and parasitized adults of Hypera postica stored at  $1.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  from October 1, 1974 to May 1, 1975.

	No. of adults of <u>H. postica</u> stored	No. alive	No. dead	Mortality* (%)
Non-parasitized	1000	843	157	$15.7 \pm 3.0$
Parasitized by <u>Microctonus</u> <u>aethiopoides</u>	1000 <sup>†</sup>	819 <sup>†</sup>	181	$18.1 \pm 3.5$
TOTAL	2000	1662	338	---

\* Four replicates of 250 weevils each,  $t = 1.03 < t_{.05} = 2.45$

† Parasitism at beginning of experiment = 61.0; parasitism at end of experiment = 57.9%.

of M. aethiopoides.

TOXICITY OF INSECTICIDES TO THE ALFALFA WEEVIL AND ITS PARASITOIDS  
IN THE LABORATORY

It was noted that, with carbaryl, malathion, phosmet, and carbofuran, the two species of parasitoids exhibited similar symptoms of poisoning. Within 15-30 minutes after treatment, the insects became moribund and remained in that state for about 3 hours. However, after 48 hours all survivors were as active as untreated controls. Similarly, with malathion and phosmet, there was rapid knock-down of adult alfalfa weevils but the recovery of those that survived was fast. Treated weevil larvae curled and remained motionless for a while and then most of the eventual survivors resumed activity before the susceptible ones died.

Figs. 10 to 16 show the dosage-mortality curves of the five insecticides to female B. curculionis, female M. aethiopoides, male M. aethiopoides, third-instar larvae of H. postica, third-instar larvae of H. postica parasitized by B. curculionis, adult H. postica and adult H. postica parasitized by M. aethiopoides, respectively. The  $LD_{50}$  values, in micrograms per insect, along with the 95% confidence limits and slopes of the probit lines for these same figures are presented in Tables XI to XVII, respectively. The  $LD_{50}$ 's were converted to micrograms per gram of insect weight to permit comparisons between the two species of parasitoids and the hosts. These  $LD_{50}$ 's in micrograms per gram weight are shown in the last columns of Tables XI to XVII.

Bathyplectes curculionis

Carbofuran ( $LD_{50} = 3.47 \mu\text{g/g}$ ) and malathion ( $LD_{50} = 3.75 \mu\text{g/g}$ ) were highly toxic to B. curculionis (Table XI). As compared to carbofuran and malathion, the other insecticides, phosmet, carbaryl, and methoxychlor were, respectively, about 3, 5, and 25 times less toxic to the parasitoid. The regression line for phosmet (Fig. 10) had the steepest slope. A t-test showed that the slope for phosmet was significantly different only from that of carbofuran (Table XI), indicating that a change in dose of the former insecticide had greater effect on mortality than did the latter insecticide (Finney, 1971).

The  $LD_{50}$  values of malathion ( $0.0054 \mu\text{g/insect}$ ) and carbaryl ( $0.0296 \mu\text{g/insect}$ ) were similar to the results obtained by Lingren et al. (1972) for the ichneumonid parasitoid, Camponotus perdistinctus (Viereck). Using topical application, they reported  $LD_{50}$ 's of  $0.0064 \mu\text{g/insect}$  and  $0.0126 \mu\text{g/insect}$  for malathion and carbaryl, respectively.

Microctonus aethioides

Comparisons of the  $LD_{50}$ 's for female B. curculionis (Table XI) and female M. aethioides (Table XII) indicated that the latter parasitoid was more susceptible to each of the insecticides. The differences in the  $LD_{50}$ 's of identical insecticides were significant at the 5% level.

In terms of  $\mu\text{g/insect}$ , the  $LD_{50}$ 's for female and male M. aethioides for identical insecticides, were virtually the same (Tables XII and XIII). However, owing to differences in body weights, the conversion to  $\mu\text{g/g}$  revealed that the female was slightly more tolerant

Figure 10. Dosage-mortality lines for five insecticides applied topically to adult females of Bathyplectes curculionis.

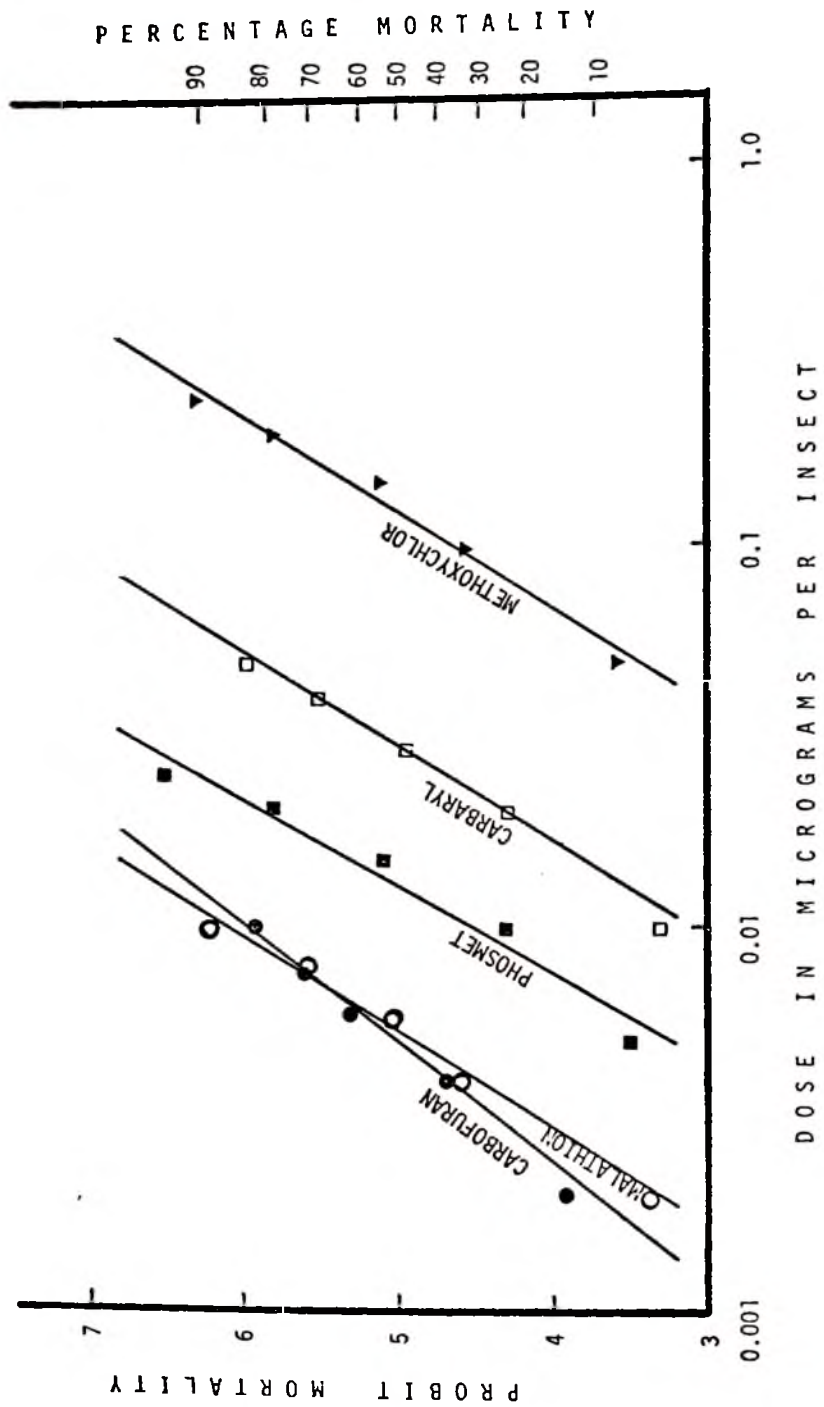


Table XI. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to adult females of Bathyplectes curculionis.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.0050	.0046-.0054	3.18 ± 0.31 A	3.47 A
malathion	.0054	.0050-.0058	4.01 ± 0.37 AB	3.75 A
phosmet	.0131	.0122-.0140	4.93 ± 0.42 BC	9.10 B
carbaryl	.0296	.0275-.0319	4.12 ± 0.37 AB	20.55 C
methoxychlor	.1246	.1144-.1356	3.93 ± 0.37 AB	86.53 D

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

Figure 11. Dosage-mortality lines for five insecticides applied topically to adult females of Microctonus aethioides.

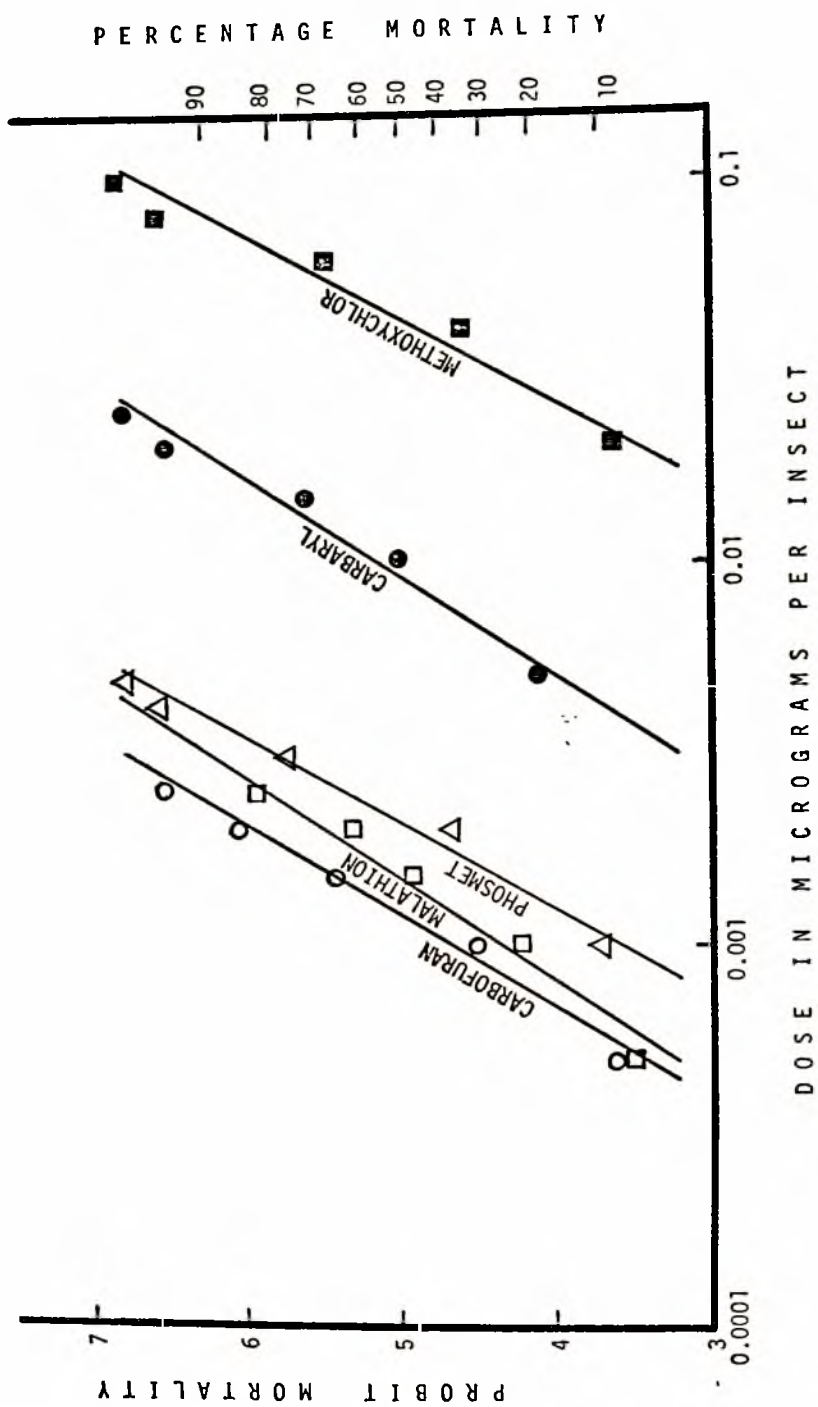


Table XII. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to adult females of Microctonus aethiopoides.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.0012	.0011-.0013	4.32 ± 0.42 A	1.97 A
malathion	.0015	.0013-.0016	3.80 ± 0.38 A	2.46 AB
phosmet	.0021	.0019-.0023	4.48 ± 0.37 A	3.44 B
carbaryl	.0093	.0084-.0102	3.86 ± 0.34 A	15.65 C
methoxychlor	.0443	.0410-.0480	4.81 ± 0.42 A	72.62 D

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

Figure 12. Dosage-mortality lines for five insecticides applied topically to adult males of Microctonus aethioides.

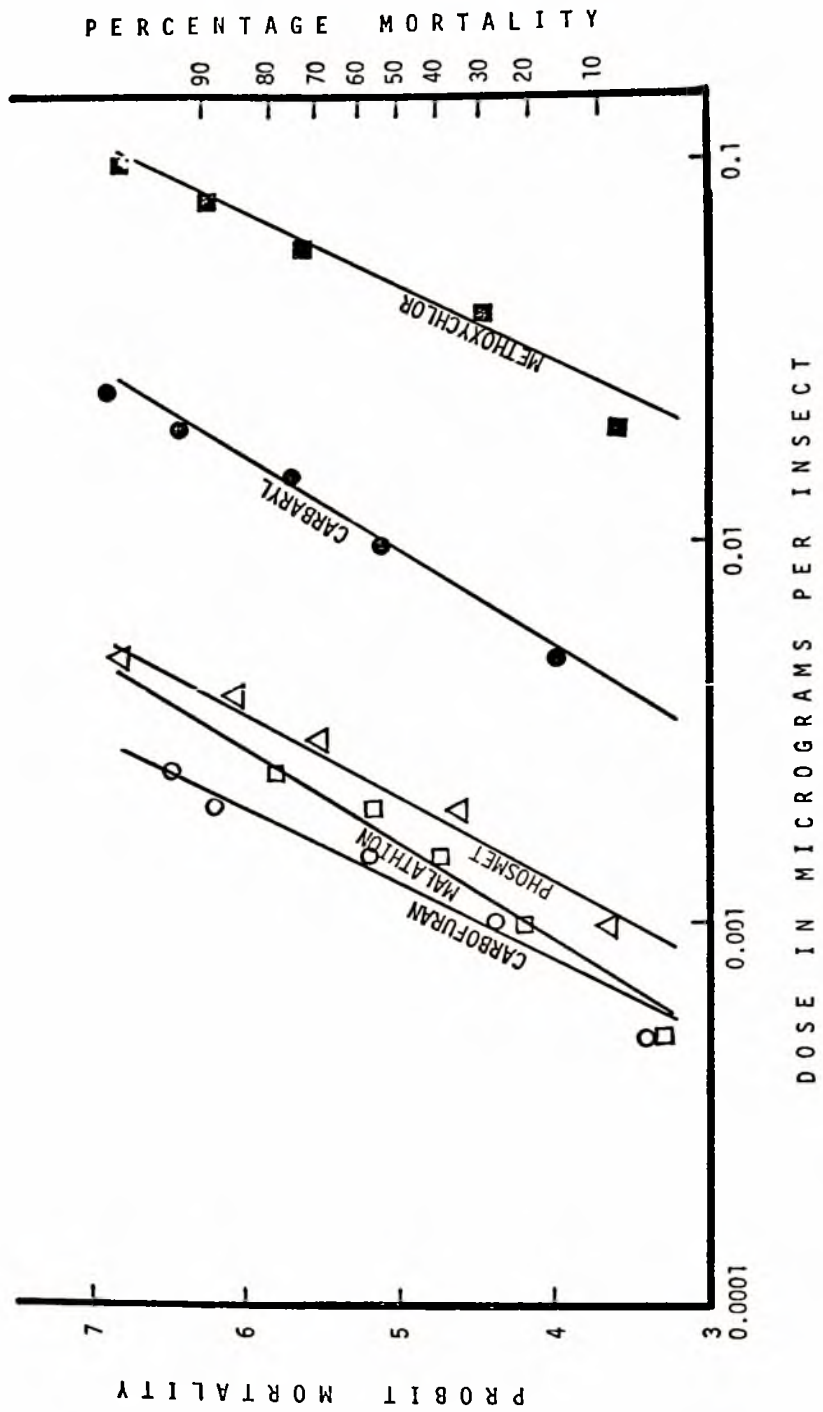


Table XIII. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to adult males of Microctonus aethiopoies.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.0012	.0011-.0013	4.81 ± 0.43 A	1.85 A
malathion	.0016	.0015-.0018	4.01 ± 0.37 A	2.46 AB
phosmet	.0021	.0019-.0023	4.60 ± 0.47 A	3.23 B
carbaryl	.0093	.0084-.0101	3.99 ± 0.33 A	14.31 C
methoxychlor	.0464	.0431-.0499	5.12 ± 0.42 A	71.38 D

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

to each of the insecticides except malathion which had equal toxicity to both the female and male. The  $LD_{50}$ 's of identical insecticides for the two sexes were not significantly different. Hence, the comparison of the toxicities of the five insecticides was based on the female parasitoid (Table XII).

Carbofuran ( $LD_{50} = 1.97 \mu\text{g/g}$ ), malathion ( $LD_{50} = 2.46 \mu\text{g/g}$ ), and phosmet ( $LD_{50} = 3.44 \mu\text{g/g}$ ) were very toxic to M. aethiopoides, whereas carbaryl ( $LD_{50} = 15.65$ ) and methoxychlor ( $LD_{50} = 72.62$ ) were less potent. Compared to carbofuran, the other four insecticides, malathion, phosmet, carbaryl, and methoxychlor were, respectively, 1.3, 1.7, 7.2, and 36 times less toxic. There were no significant differences between the  $LD_{50}$ 's of carbofuran and malathion, nor between malathion and phosmet.

The results of a few investigations on toxicity of topically applied insecticides to parasitic insects, demonstrate that carbaryl is very lethal to braconid parasitoids. For the first generation of non-resistant Bracon mellitor Say, Adams and Cross (1967) reported that the  $LD_{50}$  was  $0.00373 \mu\text{g/insect}$  and Tomlin and Forgash (1972) found that on Apanteles melanoscelus (Ratzeburg) the  $LD_{50}$  was between 0.01 and  $0.05 \mu\text{g/insect}$ .

#### Non-parasitized and Parasitized Larvae of H. postica

The  $LD_{50}$ 's for non-parasitized third-instar larvae of H. postica are presented in Table XIV. The slope of the probit line for phosmet (Fig. 13) was significantly steeper than those of malathion, carbaryl, and methoxychlor. Thus, a change in the dose of phosmet had greater effect on mortality of the larvae than any other insecticide, except

Figure 13. Dosage-mortality lines of five insecticides applied topically to third-instar larvae of Hypera postica.

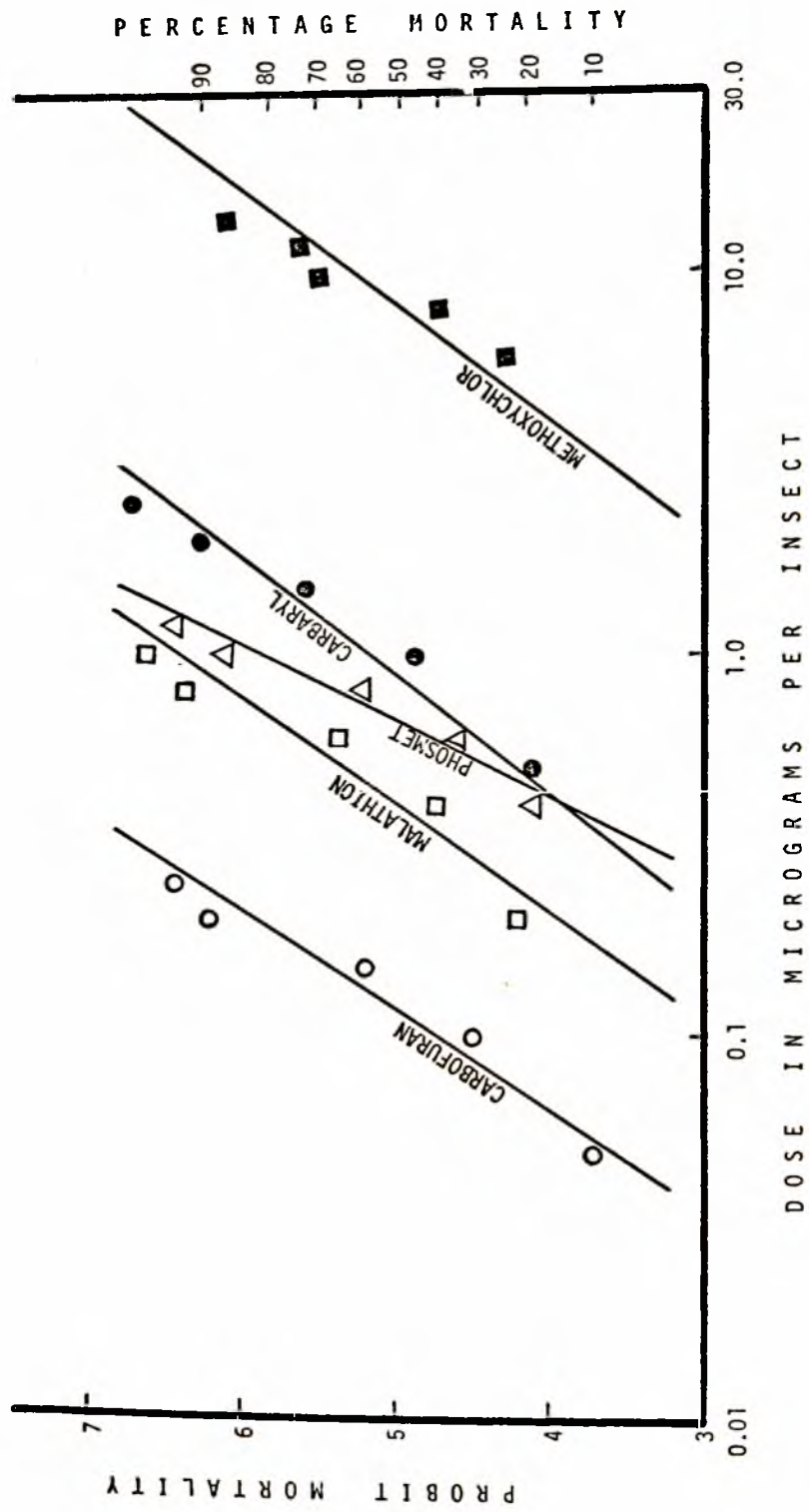


Table XIV. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to third-instar larvae of Hypera postica.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.1149	.1057- .1249	3.95 ± 0.34 AB	14.2 A
malathion	.4013	.3651- .4411	3.59 ± 0.31 A	49.7 B
phosmet	.6608	.6213- .7029	5.13 ± 0.44 B	81.8 C
carbaryl	.8709	.7751- .9785	3.20 ± 0.32 A	107.8 D
methoxychlor	8.2433	7.8119-8.6986	3.45 ± 0.56 A	1020.2 E

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

carbofuran. Carbofuran ( $LD_{50} = 14.22 \mu\text{g/g}$ ) was the most toxic insecticide to the larvae whereas methoxychlor ( $LD_{50} = 1020.2 \mu\text{g/g}$ ) was the least toxic. Carbofuran was approximately 4, 6, 8, and 72 times more toxic to the larvae than was malathion, phosmet, carbaryl, and methoxychlor, respectively (Table XIV).

The  $LD_{50}$  values for malathion, carbaryl, and methoxychlor in Table XIV were lower compared to the results of Bass and Blake (1964) (malathion =  $44 \mu\text{g/g}$ , carbaryl =  $160 \mu\text{g/g}$ , methoxychlor =  $1200 \mu\text{g/g}$ ) and Harrendorf *et al.* (1967) (malathion =  $0.47 \mu\text{g/insect}$ ; methoxychlor =  $9.44 \mu\text{g/insect}$ ). Both investigators obtained these  $LD_{50}$ 's on fourth-instar larvae. It is therefore not surprising that they reported greater  $LD_{50}$  values because, generally, latter instars of the same species of insect are more tolerant to pesticides (Busvine, 1971).

The toxicity of the five insecticides to third-instar larvae parasitized by *B. curculionis* (Fig. 14, Table XV) was similar to those of the non-parasitized larvae but in every instance the parasitized larvae were significantly more susceptible at the 5% level. Dissection of the larvae after treatment showed that, irrespective of the concentration and the insecticide, 70-100% (mean =  $79.9 \pm 18.2$ ) of the parasitoid larvae died within dead hosts. In contrast, mortality of larval parasitoids within the surviving host was only 0-40% (mean =  $36.8 \pm 14.9$ ). These differences were significant at the 1% level, indicating that the mortality of the larval parasitoids depended on the mortality of the hosts; most of the parasitoid larvae died within the dead hosts and most survived within the surviving hosts. Also, there were no significant differences between the mortalities of the

Figure 14. Dosage-mortality lines for five insecticides applied topically to third-instar larvae of Hypera postica parasitized by Bathyplectes curculionis.

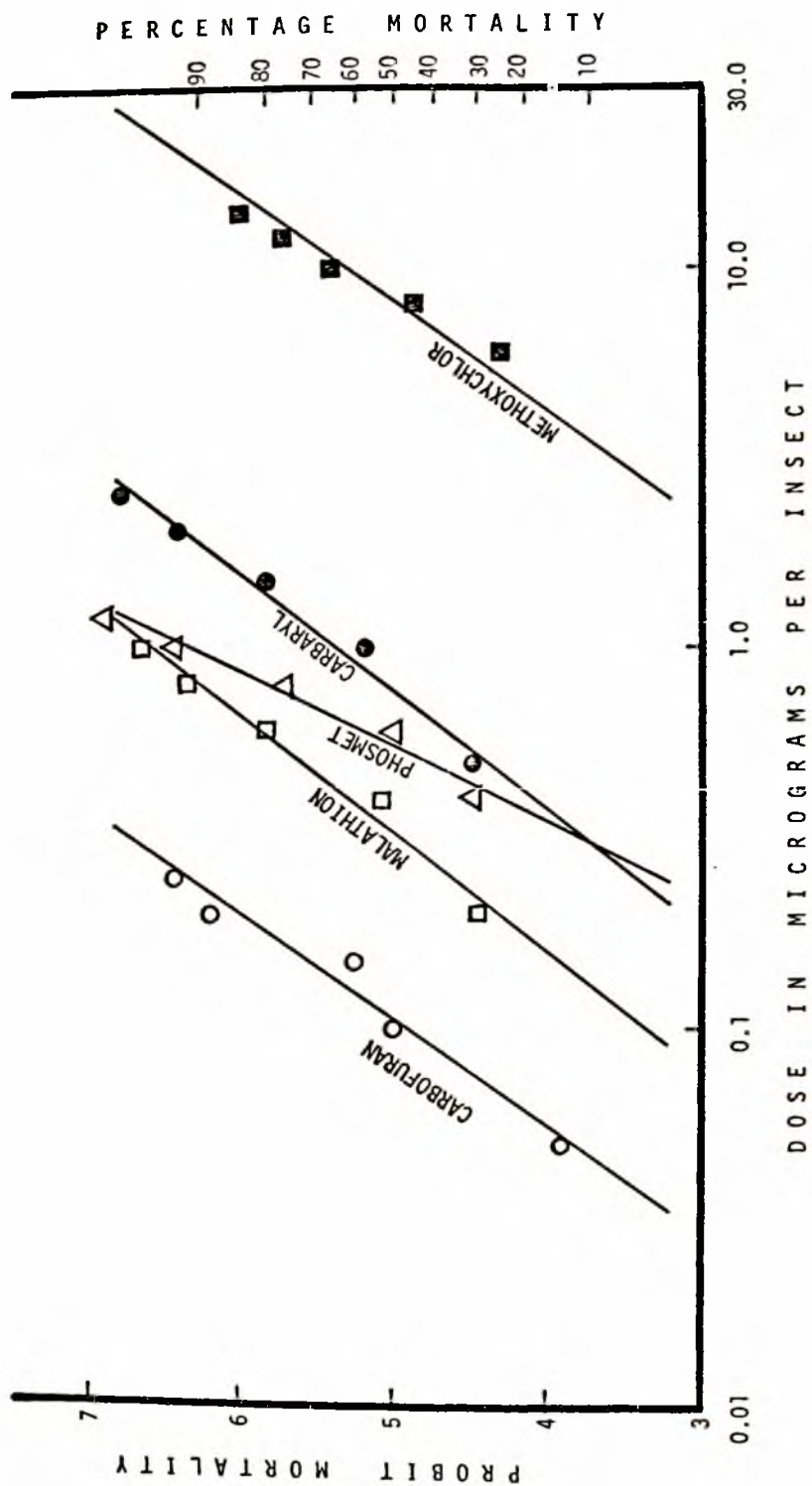


Table XV. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to third-instar larvae of Hypera postica parasitized by Bathyplectes curculionis.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.1082	.0986- .1188	3.56 ± 0.32 AB	13.7 A
malathion	.3333	.2971- .3739	3.20 ± 0.30 A	42.2 B
phosmet	.5506	.4955- .6122	5.20 ± 0.67 B	69.7 C
carbaryl	.7844	.6944- .8861	3.20 ± 0.31 A	99.3 D
methoxychlor	8.0039	7.7823-8.8992	3.41 ± 0.80 AB	1013.4 E

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

parasitoid larvae at different concentrations of any of the insecticides. Dissection of the survivors of the acetone-treated control failed to show any mortality of parasitoid larvae. Thus, the 36.8% mortality of parasitoid larvae within the surviving hosts could have resulted from the insecticides.

#### Non-parasitized and Parasitized Adults of H. postica

The response of non-parasitized adults of H. postica to the five insecticides is presented in Fig. 15 and Table XVI. As compared to the larvae (Table XIV), adults were more susceptible to the insecticides. Carbaryl, methoxychlor, malathion, phosmet, and carbofuran were, respectively, approximately 5, 10, 13, 14, and 15 times more toxic to the adult weevils than to the larvae. In some insect species, the adults are more susceptible to pesticides than are the larvae (Busvine, 1971). Comparison of Tables XVI and XVII revealed that the insecticides were consistently more toxic to weevils parasitized by M. aethiopoulos but these differences were not significant at the 5% level except for methoxychlor. When the parasitized hosts were dissected it was observed that 60-100% (mean =  $81.7 \pm 10.0$ ) of parasitoid larvae died within the susceptible hosts but parasitoid mortality within the surviving hosts was 0-20% (mean =  $17.2 \pm 8.0$ ). These means were significantly different at the 1% level. Similar to the observations made in the dissection of treated host larvae, no parasitoid mortality was detected in any survivors of the acetone-treated control.

The greater susceptibility of parasitized larvae, as compared to non-parasitized ones (Table XIV vs. Table XV), might have resulted from weakening of the host through reduced feeding (Armbrust et al.,

Figure 15. Dosage-mortality lines for five insecticides applied topically to adults of Hypera postica.

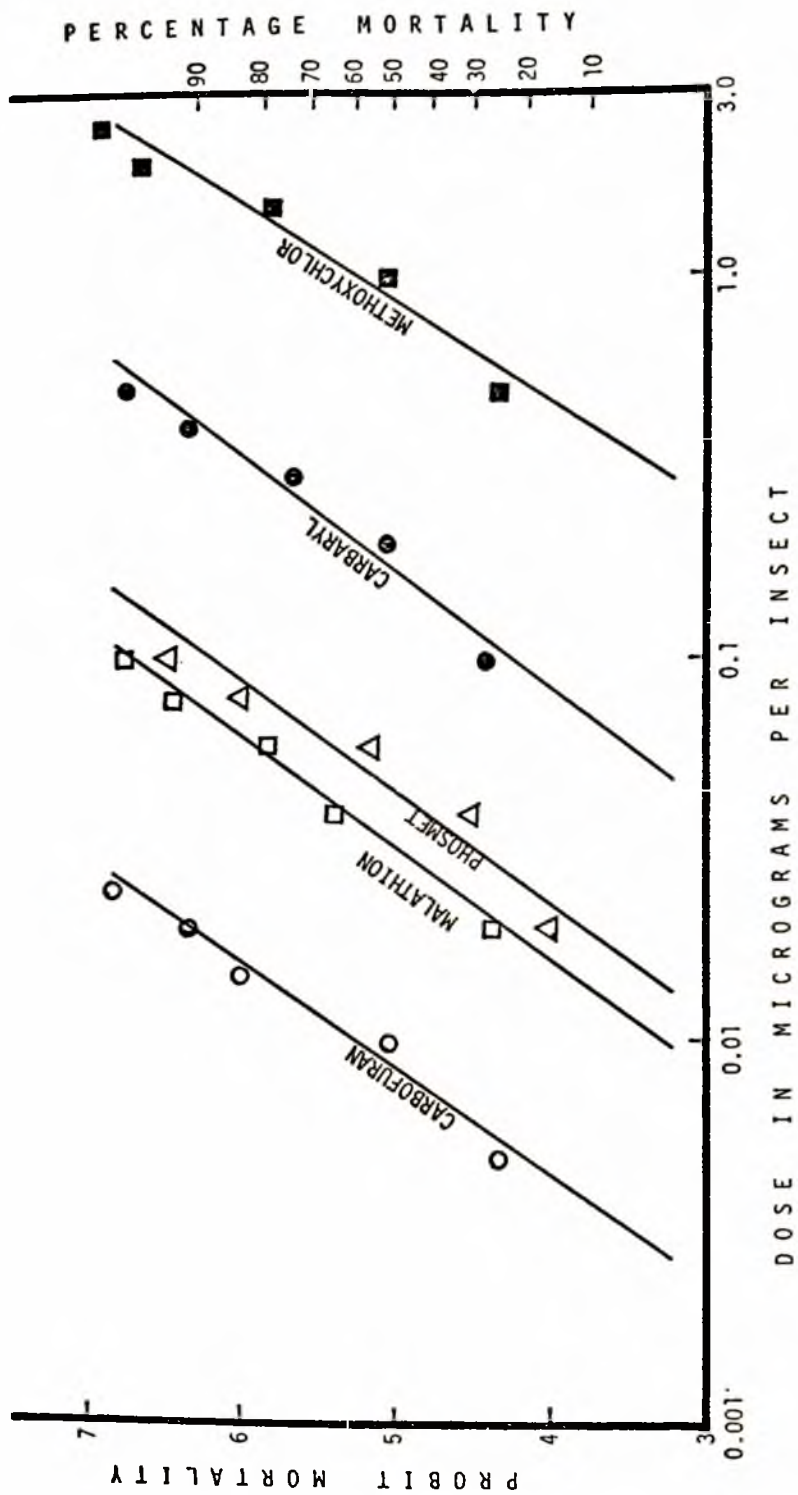


Table XVI. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to adults of Hypera postica.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.0089	.0076-.0094	3.53 ± 0.31 A	1.00 A
malathion	.0334	.0294-.0365	3.42 ± 0.30 A	3.90 B
phosmet	.0477	.0415-.0498	3.43 ± 0.31 A	5.42 C
carbaryl	.1816	.1544-.1929	3.29 ± 0.30 A	20.55 D
methoxychlor	.8943	.7682-.9400	3.73 ± 0.33 A	101.17 E

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

Figure 16. Dosage-mortality lines for five insecticides applied topically to adults of Hypera postica parasitized by Microctonus aethiopoides.

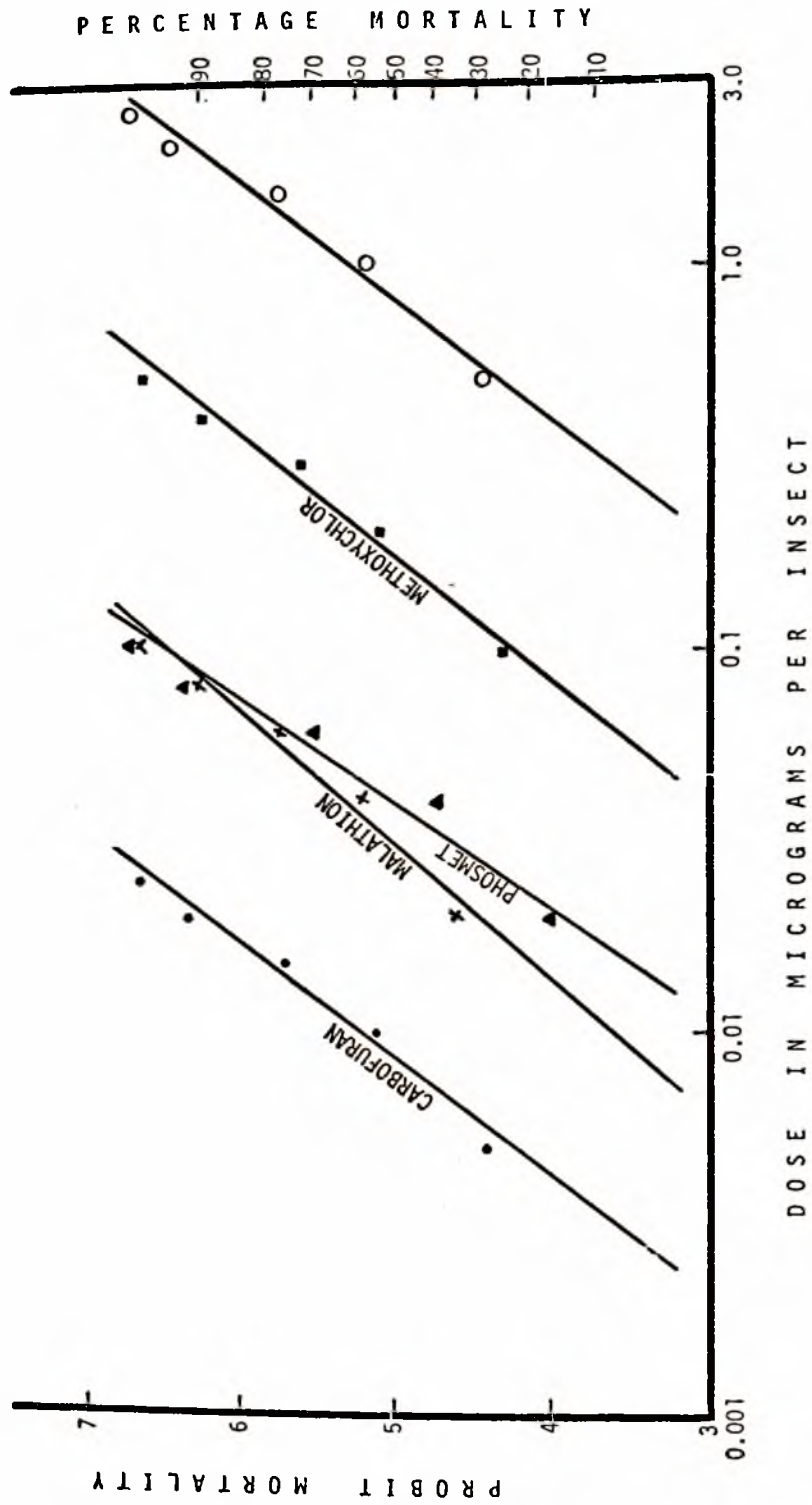


Table XVII. LD<sub>50</sub> values, 95% confidence limits of the LD<sub>50</sub> values, and slopes of dosage-mortality lines for five insecticides applied topically to adults of Hypera postica parasitized by Microctonus aethiopoides.

Insecticide	LD <sub>50</sub> µg/insect	95% confidence limits of LD <sub>50</sub>	Slope of dosage mortality line ± standard error*	LD <sub>50</sub> µg/g*
carbofuran	.0082	.0076-.0094	3.31 ± 0.29 A	0.97 A
malathion	.0306	.0269-.0348	2.84 ± 0.27 A	3.62 B
phosmet	.0398	.0362-.0437	3.62 ± 0.31 A	4.71 C
carbaryl	.1732	.1549-.1937	3.14 ± 0.29 A	20.50 D
methoxychlor	.8000	.7149-.8954	3.25 ± 0.29 A	94.67 E

\* Slopes or LD<sub>50</sub>'s followed by the same letter were not significantly different at the 5% level by the t-test.

1974; Duodu and Davis, 1974c) and physiological changes (Duodu and Davis, 1974c). Reasons similar to these could be responsible for the reduced tolerance of parasitized adult weevils, as compared to the non-parasitized ones (Table XVI vs. Table XVII).

The effect of insecticides on the larvae of B. curculionis and M. aethiopoides depended mainly on the response of their hosts, since these internal parasitoids were protected from direct contact with the pesticides. Consequently, most parasitoid larvae survived if the hosts were not susceptible to the insecticide. Citing examples from several investigations, conducted mainly under field conditions, Croft and Brown (1975) concluded that internal parasitoids in early developmental stages usually died after their hosts had been killed because the parasitoids were deprived of the proper requisites to complete their life cycles. They attributed the death of parasitoids within surviving hosts to the ability of a chemical to penetrate the host to reach the parasitoids. In the present investigation, there was evidence of parasitoid mortality, though not significant, within surviving hosts that had been treated with insecticide. It was not evident, from this study, whether the mortality of larval parasitoids within the treated host resulted from direct contact with the insecticides or through any indirect effect.

#### Comparative Susceptibility of Hosts and Parasitoids

The relative toxicities of the insecticides to the alfalfa weevil and the two parasitoids were compared by deriving selectivity ratios, defined by Metcalf (1972) as the  $LD_{50}$  of the non-target organism divided by the  $LD_{50}$  of the pest. Hence, using the  $LD_{50}$

values of female parasitoids and non-parasitized weevils in the equation:

$$\text{Selectivity Ratio (SR)} = \frac{\text{LD}_{50} \text{ of Parasitoid}}{\text{LD}_{50} \text{ of Weevil Larva or LD}_{50} \text{ of Adult Weevil}}$$

The following four sets of selectivity ratios were obtained:

- 1) adults of B. curculionis vs. third-instar larvae of H. postica (Table XVIII);
- 2) adults of M. aethiopoides vs. third-instar larvae of H. postica (Table XIX);
- 3) adults of B. curculionis vs. adults of H. postica (Table XX);
- 4) adults of M. aethiopoides vs. adults of H. postica (Table XXI).

An SR value of less than 1 indicates selectivity favouring the pest and a value of more than 1 represents selectivity favouring the parasitoid. In each of the four Tables as listed above, the insecticide with the lowest SR was used as the point of reference for comparison. The SR of any insecticide in each set was divided by the lowest SR. This permitted a direct rating and comparison of the selectivity ratios of the five insecticides.

Tables XVIII and XIX indicate that, with regard to third-instar larvae of H. postica, each of the insecticides was more toxic to both B. curculionis and M. aethiopoides. Insecticides with the lowest selectivity ratings were malathion, phosmet, and methoxychlor (Tables XVIII and XIX). Carbofuran and carbaryl had higher ratings for both parasitoids, and therefore were safer to the parasitoids than were malathion, phosmet, and methoxychlor. Comparison of the LD<sub>50</sub> values of B. curculionis and adult H. postica (Table XX) revealed that carbofuran and phosmet, with SR values exceeding 1, were more toxic

Table XVIII. LD<sub>50</sub> values, selectivity ratios, and selectivity ratings of five insecticides applied topically to adult females of Bathyplectes curculionis and third-instar larvae of Hypera postica.

Insecticide	LD <sub>50</sub> µg/g		Selectivity ratio	Rating*
	Adult females of <u>B. curculionis</u>	Third-instar larvae of <u>H. postica</u>		
carbofuran	3.47	14.22	0.24	3.00
malathion	3.75	49.67	0.08	1.00
phosmet	9.10	81.78	0.11	1.38
carbaryl	20.55	107.78	0.19	2.38
methoxychlor	86.53	1020.21	0.09	1.13

\* Rating based on selectivity ratio of test insecticide divided by selectivity ratio of malathion.

Table XIX. LD<sub>50</sub> values, selectivity ratios, and selectivity ratings of five insecticides applied topically to adult females of Microctonus aethiopoidea and third-instar larvae of Hypera postica.

Insecticide	LD <sub>50</sub> µg/g		Selectivity ratio	Rating*
	Adult females of <u>M. aethiopoidea</u>	Third-instar larvae of <u>H. postica</u>		
carbofuran	1.97	14.22	0.14	3.50
malathion	2.46	49.67	0.05	1.25
phosmet	3.44	81.78	0.04	1.00
carbaryl	15.25	107.78	0.15	3.75
methoxychlor	72.62	1020.21	0.07	1.75

\* Rating based on selectivity ratio of test insecticide divided by selectivity ratio of phosmet.

Table XX. LD<sub>50</sub> values, selectivity ratios, and selectivity ratings of five insecticides applied topically to adult females of Bathyplectes curculionis and adults of Hypera postica.

Insecticide	LD <sub>50</sub> µg/g		Selectivity ratio	Rating*
	Adult females of <u>B. curculionis</u>	Adults of <u>H. postica</u>		
carbofuran	3.47	1.00	3.47	3.99
malathion	3.75	3.90	0.96	1.10
phosmet	9.10	5.42	1.68	1.93
carbaryl	20.55	20.55	1.00	1.14
methoxychlor	86.53	101.17	0.87	1.00

\* Ratings based on selectivity ratio of test insecticide divided by selectivity ratio of methoxychlor.

Table XXI. LD<sub>50</sub> values, selectivity ratios, and selectivity ratings of five insecticides applied topically to adult females of Microctonus aethioides and adults of Hypera postica.

Insecticide	LD <sub>50</sub> µg/g		Selectivity ratio	Rating*
	Adult females of <u>M. aethioides</u>	Adults of <u>H. postica</u>		
carbofuran	1.97	1.00	1.97	3.13
malathion	2.46	3.90	0.63	1.00
phosmet	3.44	5.42	0.64	1.02
carbaryl	15.25	20.55	0.74	1.17
methoxychlor	72.62	101.17	0.72	1.14

\* Rating based on selectivity ratio of test insecticide divided by selectivity ratio of malathion.

to adult weevils than to the parasitoids. The ratings indicated that carbofuran was about 2 times more selective than phosmet. Carbaryl (SR = 1) had equal toxicity to the host and parasitoid, whereas malathion and methoxychlor were more toxic to the parasitoid than the host. Only carbofuran had selectivity favouring M. aethionoides (Table XXI).

In terms of LD<sub>50</sub> values, carbofuran was the most toxic compound to all the insects, followed in descending order of toxicity by malathion, phosmet, carbaryl, and methoxychlor. The higher toxicity of these chemicals to the two parasitoids agrees with the general observations made by several investigators that parasitoids are often very susceptible to the pesticides applied against their hosts. Croft and Brown (1975) reviewed the literature in which parasitoids had been compared with their hosts for susceptibility to insecticides. They reported that in 11 of 15 instances the parasitoids were more susceptible. Thus, among the present synthetic pesticides, insecticides that favour parasitoids in relation to the hosts are rare.

None of the five insecticides tested in the present investigation was highly selective but, when rated in relative selectivity, the carbamates, carbofuran, and carbaryl, had the highest selectivity ratings. The organophosphates, malathion, and phosmet, had ratings very close to methoxychlor, an organochlorine. Metcalf (1964) reviewed the selective action of various groups of insecticides to different species of insects. He concluded that the carbamates are often species-specific and therefore show differential toxicity. The higher selectivity ratings of carbofuran and carbaryl might have

resulted from the species-specificity characteristic of carbamates.

## CONCLUSIONS

The two-year sampling at Arkell and Elora indicated that the emergence of the first brood of adult B. curculionis from overwintered cocoons coincided with the initial increase in the populations of weevil larvae. However, the populations of the adult parasitoids were low throughout the season and there was no marked increase in the percentage parasitism of the pest prior to the third or fourth week of June when infestation by the weevil was highest. The percentage parasitism was higher after the last week of June when the host population was low. The high percentage parasitism in the late season helps to reduce the population of the summer adults of the weevil and is of advantage to B. curculionis because the investigation of overwintering mortality demonstrated that survival was higher for parasitoids that went into diapause late in the season. Apart from the high overwintering mortality, B. curculionis also suffers from hyperparasitism. In a preliminary observation during 1973, 22.4% of the parasitoid was parasitized by four species of hyperparasitoids (Appendix 1).

The appearance of the first generation adults of M. aethiopoides in the field was synchronized well with the population of the overwintered weevils. Hence, parasitism was high (76.6-82.0%) when the host population was high. Since parasitism by M. aethiopoides reduced oviposition in the alfalfa weevil within 48 hours (Van Driesche, 1975), the quantity of eggs laid in the field was greatly reduced. The second generation of adult parasitoids emerged earlier than the summer-adult weevils. Consequently, there was a period (June 13-20 at Aylmer, and

June 12-19 at South Bay) when virtually no summer-adult weevils could be found in the field while there were adult parasitoids.

Possibly, some of the parasitoids died without finding any host to parasitize. Parasitism of these summer adults was 41.6-60.0%. The subsequent year's population of M. aethiopoides depends on the synchrony between the second generation adults of the host and parasitoids. To achieve continuous carry over of M. aethiopoides from year to year, it is important that many of the summer-adult weevils are parasitized. However, as pointed out by Loan (1963), lower percentage parasitism of the summer adults ensures that there will be adequate non-parasitized, overwintered hosts when the adult parasitoids emerge in the following spring. Since parasitism by M. aethiopoides does not significantly affect the overwintering mortality of the host, it can be concluded that the percentage parasitism of the summer-adult weevils is the major factor that determines the survival of the parasitoid. Extremely low or high percentage parasitism of the new adult weevils is detrimental to the survival of M. aethiopoides. This may account for the failure of the parasitoid to establish at many release sites in Ontario because poor synchrony with the summer-adult weevils can be more devastating when parasitoid numbers are low.

Laboratory tests on the five insecticides recommended for weevil control in Ontario showed that, compared to the weevil larvae, none of the pesticides had selectivity favouring the adults of the two parasitoids. Also, parasitized larvae were more susceptible to the insecticides than their non-parasitized counterparts. Because the parasitoid larvae perished with the susceptible hosts, field applications of any of the five insecticides against the larvae of

the alfalfa weevil will be unfavourable to the parasitoids.

Frequently, weather conditions and farm operations will permit growers to achieve weevil control by cutting early as recommended. However, if infestation reaches the economic threshold prior to the first-flower stage of the crop and cutting is not possible, only pesticides that have comparatively high selectivity in favour of the parasitoids should be used. It was pointed out by Ripper (1956), Van den Bosch and Stern (1962), and Croft and Brown (1975) that the pupae of most species of parasitic insects are more tolerant to insecticides than are the adults. It can be inferred from the present investigation that by the time a grower decides to spray the alfalfa, the majority of the two parasitoids, B. curculionis and M. aethiopoides, will be at the adult stage which is very susceptible to the insecticides. It was found that carbofuran and carbaryl were 2 to 3 times safer to the parasitoids than malathion, phosmet, and methoxychlor. Therefore, if a grower has to spray for weevil larvae, carbofuran and carbaryl are preferable. The choice between carbofuran and carbaryl should be based on the probable time interval between treatment and harvesting of the crop. The minimum time interval from treatment to cutting is 7 days and 1 day for carbofuran and carbaryl, respectively (Ontario Ministry of Agriculture and Food, 1975). It is expected that the insecticides will be applied at the first-flower stage of the crop and therefore mostly third- and fourth-instar larvae will be killed because they feed on the leaves. Most of the first- and second-instar larvae will be missed since they are usually concealed in the unopened florets (Miller and Guppy, 1971).

Since adult weevils are more susceptible to the insecticides

than the larvae, it would be preferable in an integrated control program to spray for adult weevils rather than for larvae. Presently, chemical control is aimed at the larvae, because the decision to treat the crop cannot be taken until feeding damage is high and cutting is not feasible.

Niemczyk and Flessel (1969; 1970) and Dorsey (1970) demonstrated that chemical control was feasible and more economical on adult weevils than on the larvae. Based on the crop growth, relative to the population of overwintered weevils in early spring a damaging infestation can be predicted (Blickenstaff *et al.*, 1972). Thus, for growers who produce alfalfa seed and therefore cannot cut the crop early it may be more appropriate to initiate chemical control when overwintered weevils resume activity in the spring. Bartlett (1958) pointed out that where low dosages of an insecticide suffice to kill the pest, the adult parasitoid would be less drastically affected. Comparatively low dosages will be required to kill adult weevils and, consequently, survival of the parasitoids will be enhanced. The selectivity ratios indicate that carbofuran is more toxic to the adult weevil than to B. curculionis or M. aethiopoides. This implies that the use of carbofuran, in particular, to control adult H. postica will not be so detrimental to the two parasitoids.

There was a period (May 9-16 at Aylmer and May 15 at South Bay) when no parasitized adult weevil or adult M. aethiopoides were found in the field while there were non-parasitized adult weevils. Hence, insecticidal application against the overwintered adult weevils during that period would miss most of the larvae and adults of M. aethiopoides. Observations at Arkell and Elora showed that adult B.

curculionis emerged from overwintered cocoons about the last week of May. Thus, spraying the crop against adult weevils will have a minimal effect on B. curculionis. The height of the alfalfa crop is usually low when overwintered weevils are abundant and active in the field. Hence, sprayed insecticides can easily reach the target insect. Despite its advantages, spraying against overwintered weevils is advisable only if the population of the pest is high when the adults of B. curculionis and M. aethiopoides are not abundant in the field.

It is expected that under various field conditions and with different formulations of the insecticides, the susceptibilities of the alfalfa weevil and the two parasitoids may not follow the same trend as found in the present investigations. The data should serve as reference points for future investigations and insecticide recommendations.

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Appendix 1. Species of hyperparasitoids reared from cocoons of Bathylectes curculionis collected from Elora, Ontario and percentage hyperparasitism by the hyperparasitoids.

Hyperparasitoids <sup>+</sup>	No. of cocoons of <u>B. curculionis</u> parasitized	As percentage of no. of cocoons of <u>B. curculionis</u> reared*	As percentage of no. of cocoons of <u>B. curculionis</u> parasitized
<u>Gelis</u> spp.	46	12.7	56.8
<u>Eupteromalus viridescens</u> (Walsh.)	25	6.9	30.8
<u>Pteromalus</u> sp.	8	2.2	9.9
<u>Eupelmella vesicularis</u> (Retzius)	2	0.6	2.5
TOTAL	81	22.4	100.0

<sup>+</sup> Determined by C.M. Yoshimoto, Entomology Research Institute, Agriculture Canada, Ottawa, Ontario.

\* 361 cocoons were hand-picked from alfalfa foliage during July 11-19, 1973.