Phenology of the Alfalfa Weevil (Coleoptera: Curculionidae) and its Associated Parasitoids in Minnesota[1]

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Abstract Field surveys were conducted in Minnesota to determine the seasonal incidence of parasitism of alfalfa weevil, *Hypera postica* (Gyllenhal), by *Bathyplectes curculionis* (Thomson), *B. anurus* (Thomson), *Oomyzus* (=*Tetrastichus*) *incertus* (Ratzeburg), and *Microctonus aethiopoides* (Loan). During the 1991-1993 survey, alfalfa weevil population density was typically below that required to cause economic damage. In Minnesota, peak density of alfalfa weevil larvae tended to occur around 300 dd (base 9°C). Typically, first cutting of alfalfa in Minnesota occurs early enough to remove most alfalfa weevil before they have completed larval development. In our earliest collected larvae, parasitism by *Bathyplectes* spp. approached 30%. A low incidence of parasitism by *O. incertus* also was detected in these larvae. With rapidly declining numbers of host larvae available after first cutting, parasitism rates by *B. curculionis* and *O. incertus* increase greatly, with *Oomyzus* assuming greater importance as *Bathyplectes* declines. The greatest incidence of *O. incertus* parasitism occurred ~500 to 600 dd, but the proportion of the hosts that were parasitized peaked at ~750 to 900 dd. Insecticide sprays for potato leafhopper, *Empoasca fabae* (Harris), usually between 300 to 900 dd, could adversely effect *B. curculionis* and *O. incertus.* Second-generation alfalfa weevil larvae were found each year, but were most common during an unusually cool summer (1992), and in the southern half of the transect. Density of second-generation larvae peaked after mid-August (~1000 dd). Each year, peak parasitism by *M. aethiopoides* was observed during its first generation, around 300 dd, in overwintering alfalfa weevils. There may have been three generations of *M. aethiopoides* in Minnesota in 1992 and in 1993. In Minnesota, *M. aethiopoides* apparently overwinters as eggs, first-instar larvae, or advanced stage larvae.

Key Words Alfalfa weevil, *Hypera postica, Microctonus aethiopoides, Bathyplectes curculionis, Oomyzus incertus, Bathyplectes anurus*

Field surveys were conducted in Minnesota in 1991-1993 to determine the seasonal incidence of parasitism of alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). Alfalfa is grown extensively in Minnesota, primarily for on-farm use as feed for dairy cows. Of the state's 596,000 ha of alfalfa (USDA 1998), 60% is grown in 34 contiguous counties in eastern and east central Minnesota, an area known locally as 'The Dairy Belt.'

Establishment of alfalfa weevil in the eastern United States in the early 1950s necessitated greatly increased insecticide use on alfalfa, which was once ranked sixth among U.S. crops in insecticide treated hectarage (National Academy of Sciences

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1975). Beginning in 1957, USDA ARS implemented a program of introduction and establishment of alfalfa weevil parasitoids (Brunson and Coles 1968). That program focused first (1957-1979) on the eastern U.S. then subsequently became a national effort by USDA-APHIS (1980-1988) (Bryan et al. 1993). This effort is now recognized as one of the most successful and economically beneficial biological control programs ever undertaken (Day 1981, Kingsley et al. 1993, Radcliffe and Flanders 1998).

The alfalfa weevil (eastern strain) reached Minnesota in 1970 (Radcliffe and Chiang 1972). Spread was rapid and within three years alfalfa weevil had extended its range west to the South Dakota border and north to Sterns Co. *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) arrived in Minnesota in 1970 with its host. Other parasitoids were intentionally introduced after the weevil was established (Bryan et al. 1993, Radcliffe et al. 1983). At the time of this survey, *B. curculionis, Oomyzus* (=*Tetrastichus*) *incertus* (Ratzeburg) (Hymenoptera: Eulophidae), and *Microctonus aethiopoides* (Loan) (Hymenoptera: Braconidae) were well established throughout the Dairy Belt; whereas, *B. anurus* (Thompson) had just been recovered from southeastern Minnesota (Flanders et al. 1994). *Bathyplectes anurus* was first reported in Minnesota in 1991 (Wabasha Co. and Houston Co.). We recovered *B. anurus* in 4 counties in 1992 and 6 counties in 1993. One other alfalfa weevil biological control agent, *Zoophthora phytonomi* (Arthur) (Zygomycetes: Entomophthoraceae), a fungal pathogen of larvae, is also established throughout the state (Flanders et al. 1994). The parasitoid *Microctonus colesii* Drea, which attacks alfalfa weevil larvae, then develops in alfalfa weevil adults, was not found during the survey (Flanders and Radcliffe, unpubl. data).

A more detailed understanding of the phenology of alfalfa weevil parasitoids was needed to make informed insect pest management decisions because alfalfa weevil is not the only insect pest of alfalfa in Minnesota. Potato leafhopper, *Empoasca fabae* (Harris), densities frequently exceed economic thresholds, and management of this insect by means of insecticide or altered cutting practices is intensifying (Flora 1998). Poorly-time insecticide applications could interfere with biological control of alfalfa weevil.

Materials and Methods

Each year (1991-1993) of the study, alfalfa weevil adults and larvae were collected at 6 or 7 field sites. Alfalfa fields were sampled along a 455-km transect from Houston Co. in southeastern Minnesota to Wadena Co. in north central Minnesota (Fig. 1). This transect passes through the Dairy Belt. Regular sampling was taken every 3 to 4 days (~35 dd, base 9°C) until ~700 dd (1991-1993). Additional sampling was continued in 1992-1993 after ~700 dd, but was done less frequently, typically every 10 to 14 days. Each sampling date, 30 to 50 alfalfa weevil adults and larvae were collected with a 38-cm diam sweep-net. An estimate of density of alfalfa weevil larvae and adults was made, based on total number collected and total number of sweeps. Sometimes, alfalfa weevil densities were so low that 30 to 50 adults or larvae could not be easily collected. On these dates, we stopped sampling after 5,000 sweeps per field. Larvae collected by sweep net were mostly third and fourth instars (98%).

Alfalfa fields in second to fourth year of production after the year of establishment were chosen since these were most likely to have a well-established fauna of alfalfa weevil natural enemies. When in the course of the study, research sites were lost because the fields were taken out of production or winter-killed, a site nearby was

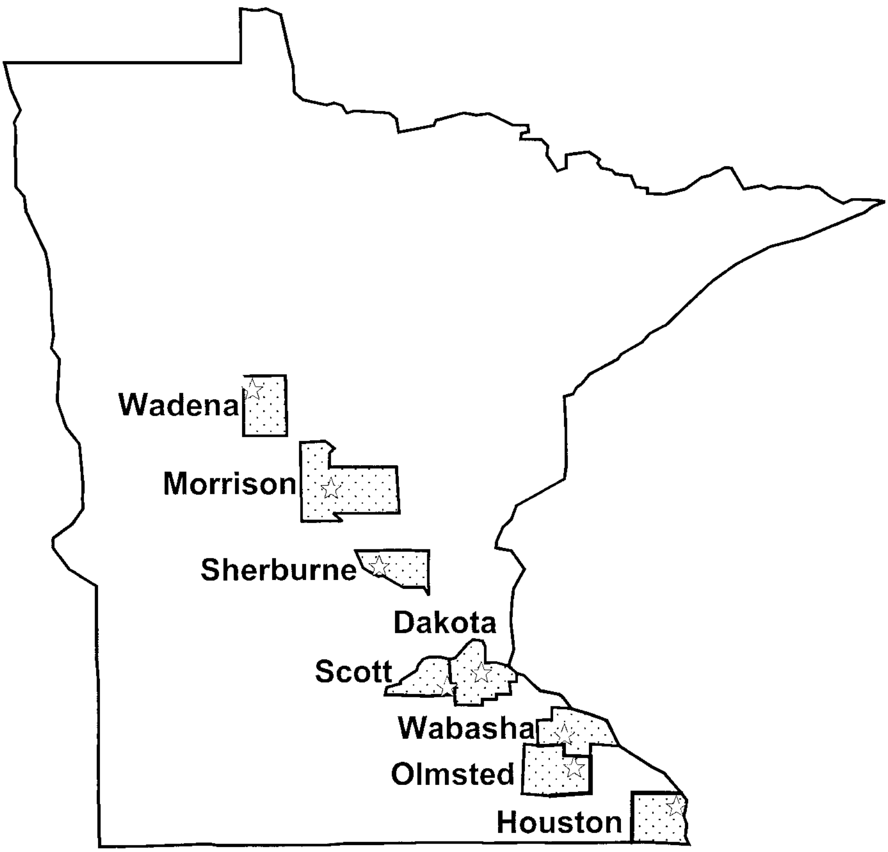

Fig. 1. Location of sample sites in Minnesota, 1991-1993.

substituted. Selected fields were all within 16 km of a weather station (operated by the National Oceanic and Atmospheric Administration or the University of Minnesota).

In 1993 a leaf vacuum was modified to collect alfalfa weevil adults in the leaf litter. Fields in Dakota Co., Houston Co., and Scott Co. were sampled intensively in April with the vacuum sampler to determine early-season phenology of *M. aethiopoides* within overwintering alfalfa weevil adults. The vacuum sampler was also used in June and July to collect new generation adults that hide in the leaf litter during the day.

Weather monitoring. Several temperature thresholds have been proposed for development of alfalfa weevil. Roberts et al. (1970) developed a model predicting alfalfa weevil egg hatch using 7°C as a base temperature. In this paper, 9°C is used as a base temperature for presenting results, as this temperature accurately predicted alfalfa weevil development events in Ontario (Harcourt 1981), and was calculated as a theoretical minimum for alfalfa weevil larval development (Koehler and Gyrisco 1961). The base temperature 9°C was also closer to the lower developmental threshold (8.4°C) determined for *M. aethiopoides* (Morales and Hower 1981).

In 1991-1992, maximum and minimum temperatures from the weather station closest to each sampling site were used to calculate cumulative degree-days (Table 1) using the double sinewave method (Frazer and Gilbert 1976). In 1993, temperatures were monitored at 30-min intervals using Davis Instrument Weather Wizard II weather stations placed on each farm. Cumulative degree-days were calculated by summing the difference between the average temperature and the base temperature for each of the 48 half-hour intervals in each day. The weather station at Scott Co. malfunctioned so the nearest NOAA weather station was used to determine cumulative degree-days.

Sample processing and data analysis. Insect samples were frozen until they could be dissected. Alfalfa weevil larvae and adults were dissected in 0.7% saline solution under a stereomicroscope. Larvae were sorted to instar, and a stratified proportion of parasitism calculated (Cochran 1977). Parasitoid immatures were identified using materials provided at a training workshop at the USDA APHIS PPQ Niles Plant Protection Laboratory, Niles, MI. Encapsulated parasitoid immatures were not classified as parasitized. In total, 1,720 alfalfa weevil larvae were dissected in 1991; 4,259 in 1992; and 3,894 in 1993. An average of 57 larvae were dissected per sample per site in 1991; 35 larvae in 1992; and 45 larvae in 1993. In total, 893 adults were dissected in 1991; 2,991 in 1992; and 2,958 in 1993. An average of 23 adults was dissected in 1991 per sample per site; 28 adults in 1992; and 25 adults in 1993.

Average proportion of parasitized alfalfa weevil larvae was determined by averaging across sites and years for each 50 dd interval from 0 to 1650 dd. Samples of larvae in which fewer than 10 specimens were dissected were not included in the estimates of parasitism reported in this paper. Proportion of parasitized adults was calculated from data pooled across sites and years for each 50 dd interval from 0-1500 dd. Data were pooled rather than averaged, because so few adults were collected from the northern half of the transect. In 1992-1993, presence of well-developed eggs in dissected specimens was used to indicate the reproductive maturity of healthy female weevils.

Results and Discussion

Heat unit accumulations varied greatly across study sites and years (Table 1). Phenologically, the most southern site, Houston Co., was generally 1 to 2 wks ahead of the most northern site, Wadena Co. The warmest growing season was 1991. The summers of 1992 and 1993 were both cooler than average (U.S. Dept. of Commerce, 1992-1993). Of the 3 yrs, the coolest spring was in 1993, but the growing season with least total heat units was 1992.

Highest seasonal densities of weevil larvae were 0.8 per sweep in 1991, 2.0 per sweep in 1992, and 2.3 per sweep in 1993. During the 1991-1993 survey, alfalfa weevil population density (Fig. 2b) was typically below that capable of causing economic damage. In Minnesota, action thresholds are set at 30 to 35% of stems showing feeding damage, with larvae still active (preharvest), or 8 larvae per ft^2 (0.93 m^2) (postharvest) (Ives and Radcliffe 1986).

Alfalfa weevil larvae were first consistently collected at ~150 to 200 dd. Peak larval densities in Minnesota tended to occur around 300 dd (Fig. 2b). The phenology of alfalfa weevil larvae in Minnesota appears to be similar to that reported for Ontario, where third instar larvae peak in abundance at 260 dd, and fourth instar peak at 306 dd (Harcourt 1981).

Table 1. Phenologies of alfalfa weevil larvae and parasitoids, in relation to degree-day accumulation and calendar date.

DD (base 9°C)	event	Earliest and latest date of occurrence: Houston Co.	Wadena Co.
200	First collection of alfalfa weevil larvae	15 May, 1991 21 May, 1993	22 May, 1991 7 Jun., 1993
300	Alfalfa bud stage, beginning of first alfalfa harvest, peak parasitism by first-generation *M. aethiopoides* in overwintered weevils	26 May, 1991 8 Jun., 1993	1 Jun., 1991 19 Jun., 1993
400	Peak numbers of alfalfa weevil if not previously removed by harvest	2 Jun., 1991 16 Jun., 1993	12 Jun., 1991 1 Jul., 1993
500	Peak proportion of larvae parasitized by *Bathyplectes*	12 Jun., 1991 24 Jun., 1992 and 1993	22 Jun., 1991 13 Jul., 1993
600	Larvae parasitized by *Bathyplectes* and *Oomyzus incertus* in near equal proportions, second alfalfa harvest, most overwintering alfalfa weevil adults have died	19 Jun., 1991 5 Jul., 1992	1 Jul., 1991 25 Jul., 1992
700	*O. incertus* predominant larval parasitoid	27 Jun., 1991 14 Jul., 1992	12 Jul., 1991 7 Aug., 1992
900	Advanced stages of *M. aethiopoides* present in first-generation alfalfa weevil adults	11 Jul., 1991 5 Aug., 1992	31 Jul., 1991 3 Sept., 1992
1000	First collection of second-generation alfalfa weevil larvae	18 Jul., 1991 16 Aug., 1992	11 Aug., 1991 23 Sept., 1992
1100	Partial third-generation of *M. aethiopoides*	26 Jul., 1991 25 Aug., 1992	20 Aug., 1991 after 1 Oct., 1992
1400	Second-generation alfalfa weevil larvae present, and parasitized by *Bathyplectes* and *O. incertus*	23 Aug., 1991 11 Oct., 1992	- -

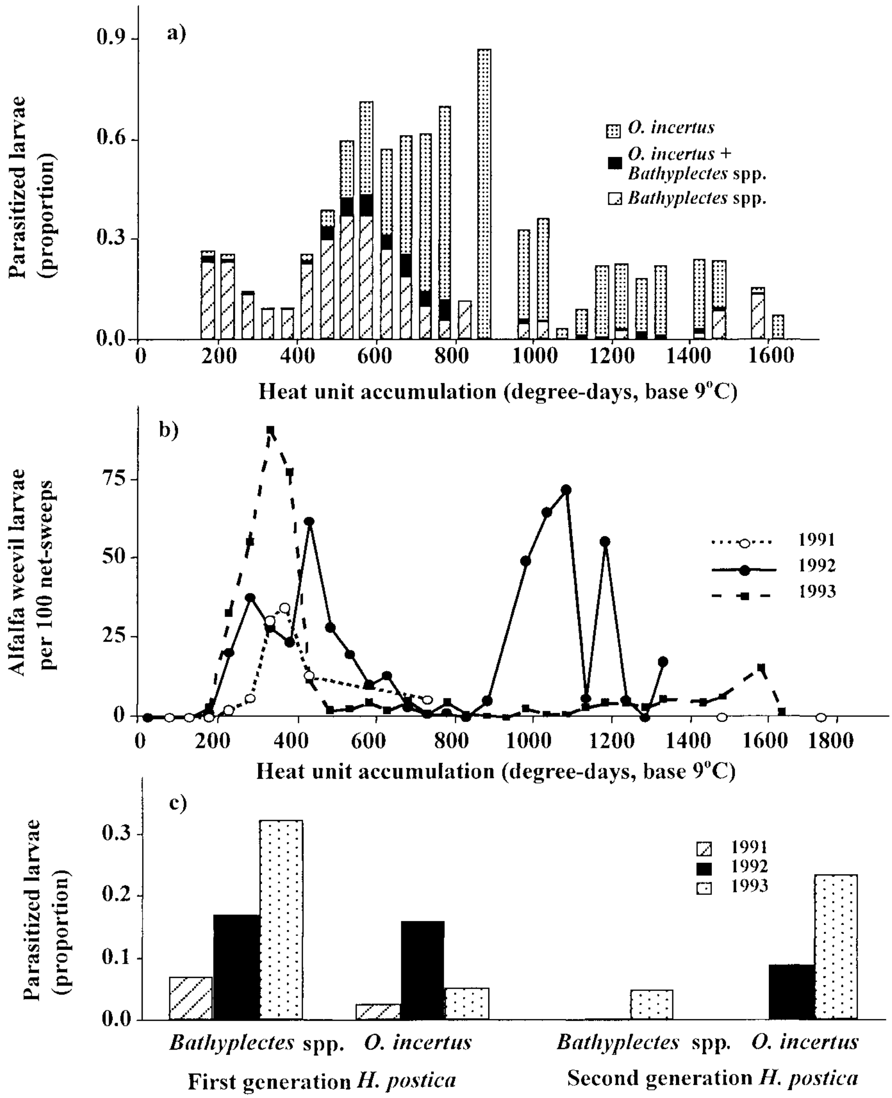

Fig. 2. Phenology of alfalfa weevil larva and its parasitoids in Minnesota, 1991-1993: (a) parasitism by *Bathyplectes* species and *Oomyzus incertus,* where proportion parasitism was averaged across sites and years for each 50 dd interval from 0 to 1650 dd; (b) average density of alfalfa weevil larvae for each 50 dd interval from 0 to 1750 dd; and (c) changes in rates of parasitism from 1991-1993.

Typically, the first cutting of alfalfa in Minnesota occurs between ~300 to 400 dd, early enough to remove most weevil larvae before they have completed their development. When fields are clean cut and postharvest conditions are sunny and hot, most larvae are killed. When a green stubble is left and post harvest conditions are cool, many alfalfa weevil larvae may survive (Chamberlin 1924, Cuperus et al. 1986). In 1991, weather conditions were hot and dry following harvest, and few larvae were collected after first harvest. Weather conditions tended to be cooler at first harvest in 1992-1993, and alfalfa weevil larvae were abundant on the regrowth.

Alfalfa weevil density was lower in the northern half of our transect than in the southern half, perhaps because alfalfa develops at a lower base temperature (~5°C [Onstad and Fick 1983]) than alfalfa weevil. This favors the crops in locations and years with less rapid heat unit accumulations, such as would be found in northern Minnesota. The typically milder winters in southern Minnesota may also contribute to the higher weevil populations that are observed there (Flanders et al. 1994).

Second-generation alfalfa weevil larvae were observed in Minnesota in 1991-1993. This inference is supported by the timing of occurrence of reproductively mature female weevils. Early in the season, the high proportion of reproductively mature females indicates the main egg-laying period of the overwintered adults (Fig. 3c). The sharp decrease in the proportion of mature females, around 400 dd in 1992 and 600 dd in 1993, is most likely an indication that the overwintering adults have died off, and that the remaining, reproductively immature females are first-generation adults. In 1992-1993, first-generation adults reached reproductive maturity by 800 dd (2 to 3 August) in Dakota Co. Then, by 900 to 950 dd, an increase in density of alfalfa weevil larvae occurred (Fig. 2b), indicating a second generation. This fall generation was most common in fields in the southern half of the transect, and in 1992. Alfalfa weevil is usually reported to have a single generation per year. However, a second generation was reported in Ontario (Loan et al. 1983). The cool summer in 1992 may have shortened the time alfalfa weevil adults spent in aestivation and caused a large proportion of females to oviposit in the late summer. Shortened aestivation due to cooler mean temperatures has been previously reported (Cothran 1967). In early spring, 1993, almost half of the non-parasitzed adult females were not reproductively mature. This may indicate that some of the second-generation alfalfa weevil may have developed into adults by late fall 1992.

Phenology of *Microtonus aethiopoides*. In Minnesota, *M. aethiopoides* is believed to be the key biotic agent responsible for maintaining alfalfa weevil population levels below the economic injury level (Flanders et al. 1994). *Microctonus aethiopoides* begins to oviposit in overwintered alfalfa weevil adults between 150 to 200 dd (Fig. 3a). Timing was such that *M. aethiopoides* prevented some, but not all egg laying by overwintering weevils. Peak parasitism from this generation averaged 35% and occurred around 200 to 300 dd. The next generation of *M. aethiopoides* begins to oviposit between 400 to 450 dd. At this time, the parasitoid is probably still attacking overwintered females, but later individuals of this generation will attack new alfalfa weevil adults, as most overwintering weevil adults are dead by 600 dd. Percent parasitism by second-generation *M. aethiopoides* averaged 15% in 1991-1993. Parasitism rates as high as 80% have been reported for this second generation of *M. aethiopoides* in Wisconsin (Hogg et al. 1990).

The development of *M. aethiopoides* from the first-instar larva to more advanced stages, beginning around 800 dd, differs from the typical phenology reported for *M. aethiopoides* in new-generation alfalfa weevils. Abu and Ellis (1976) and van Driesche and Gyrisco (1979) observed that *M. aethiopoides* develops to the first instar in new-generation weevils and then remains in that stage through the winter. The unusual development of this generation of *M. aethiopoides* may be due to the tendency of the alfalfa weevil itself to have a partial second generation in Minnesota. Loan and Holdaway (1961) found that *M. aethiopoides* larval development in the

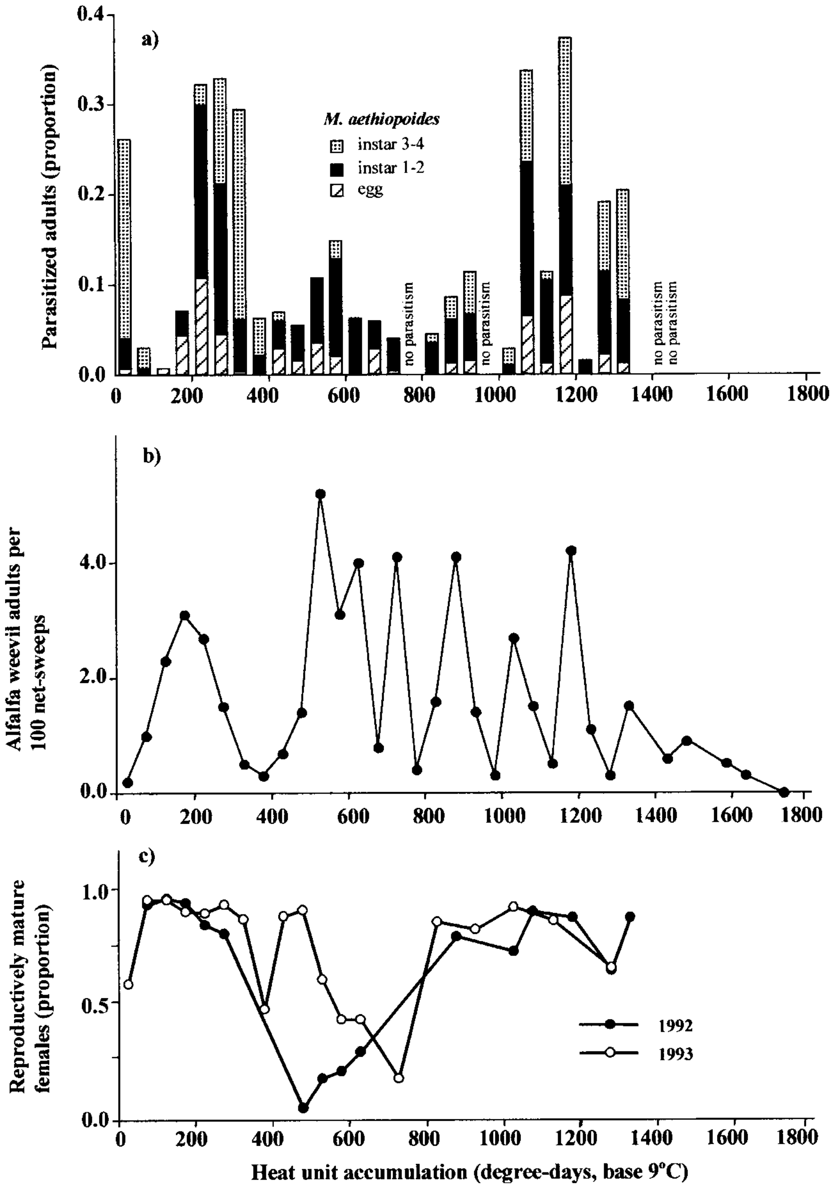

Fig. 3. Phenology of *Microctonus aethiopoides*, parasitoid of alfalfa weevil adults in Minnesota, 1991-1993: (a) parasitism by *M. aethiopoides*, where proportion of parasitized adults was calculated from data pooled across sites and years for each 50 dd interval from 0 to 1500 dd; (b) average density of alfalfa weevil adults collected in net-sweeps for each 50 dd interval from 0 to 1750 dd, and (c) proportion of alfalfa weevil females with well developed eggs in their reproductive tracts, 1992-1993, Dakota Co.

diapausing stage of *Hypera* sp. and *Sitona* spp. is usually arrested until the following spring. However, parasitoid development continued in nondiapausing weevils. There is evidence, therefore, that development of *M. aethiopoides* development is closely linked to the reproductive condition of its adult hosts.

The increase in parasitism between 1050 to 1200 dd may have been due to a third generation of *M. aethiopoides.* Eggs, young larvae, and old larvae of the parasitoid were found within alfalfa weevil adults in the late fall and early spring sampling dates.

Phenology of parasitoids of alfalfa weevil larvae. Early-stages of *B. curculionis* (egg and first-instar larvae) can be distinguished from early stages of *B. anurus,* but later stages cannot (USDA APHIS PPQ Niles Plant Protection Laboratory training manual, unpublished). Therefore, dissection data are presented as *Bathyplectes* spp. (Fig. 2). However, nearly all parasitism by *Bathyplectes* detected in 1991-1993 was *B. curculionis. Bathyplectes anurus* was not present or accounted for less than 1% of parasitized alfalfa weevil larvae with the exception of one site (Houston Co. in 1993). There, parasitism by first-instar *B. anurus* was 5% on 22 May, 6% on 25 May, 2% on 28 May, 2% on 1 Jun, 0% on 4 June and 4% on 9 Jun. At the same site, parasitism by first-instar *B. curculionis* was 9% on 22 May, 22% on 25 May, 7% on 28 May, 15% on 1 Jun, 0% on 4 Jun, and 6% on 9 Jun. Rearing data confirm the percentage relationship between *B. anurus* and *B. curculionis* (Flanders et al. 1994).

Bathyplectes curculionis eggs and young larvae were found during two discrete intervals in 1991-1992, and at three intervals in 1993, indicating that more than one generation occurred each year. A partial second generation is typical of *B. curculionis* (Miller 1970). In Utah, Parrish and Davis (1978) reported that a third generation of *B. curculionis* can occur, but that this was rare. *Oomyzus incertus* was found from 150 dd through 1650 dd. It has been reported to have multiple generations per year (Streams and Fuester 1967).

In our earliest collected larvae, parasitism by *Bathyplectes* approached 30% (Fig. 2a). A low incidence of parasitism by *O. incertus* was also detected in these first larvae. Parasitism rates declined through 300 to 400 dd while densities of alfalfa weevil larvae increased. After 500 dd, more than 60% of alfalfa weevil larvae were parasitized. This increase is due to further generations of the parasitoids occurring at a time when host larvae are rapidly declining. The highest proportion of parasitism by *Bathyplectes* occurred 500 to 600 dd. The greatest incidence of *O. incertus* parasitism also occurred ~500 to 600 dd, but the greatest proportion of parasitized larvae occurred 750 to 900 dd. From 450 to 800 dd, 5 to 10% of individual weevil larvae were parasitized by both *Bathyplectes* and *O. incertus.* Summer application of insecticide for potato leafhopper control could be detrimental to these later generations of *B. curculionis* and *O. incertus.*

On second-generation alfalfa weevil larvae, rates of parasitism by *O. incertus* were around 25% from 950 to 1450 dd. Parasitism by *Bathyplectes* peaked after 1450 dd. When alfalfa weevil is abundant during the fall, as it was at some sites in 1992 and 1993, the mortality caused by these two parasitoids can be substantial.

Epizootics of the *Zoophthota phytonomi* were observed in Houston Co. in 1991 and in Wabasha Co. and Dakota Co. in 1993. Conditions favorable for *Z. phytonomi* epizootics are densities of >1.7 alfalfa weevil larvae per stem (Brown and Nordin 1982), or >0.9 per stem when rainfall is abundant (Harcourt and Guppy 1991). Epizootics of *Z. phytonomi* were observed in Iowa where alfalfa weevil populations were as low as 0.6 larvae per stem (Giles et al. 1994). In Minnesota, alfalfa weevil densities seldom exceed this critical density. *Zoophthora phytonomi* can effectively suppress weevil larvae to subeconomic levels (Harcourt et al. 1984, DeGooyer et al. 1995). The fungus tends to over suppress alfalfa weevil populations and because it competes with parasitoids for the same hosts epizootics can destabilize biological control. There is evidence that *B. anurus* is less likely to be adversely affected than *B. curculionis,* and that therefore, *Z. phytonomi* may be a factor in the displacement of *B. curculionis* by *B. anurus* (Harcourt and Ellis 1991).

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