Alfalfa Weevil (Coleoptera: Curculionidae) Phenology with its Host Crop and Parasitoids in Virginia[1]

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Abstract Alfalfa weevil, *Hypera postica* (Gyllenhal), phenology is influenced by winter climate. In 1997 we initiated a 2-yr study of alfalfa weevil phenology with respect to its host crop and parasitoids in three geographically distinct locations of Virginia: the central Piedmont, Shenandoah Valley, and southwestern region. Alfalfa weevil populations from nine fields were sampled regularly from November until first harvest in each season. Eggs laid in December and January resulted in alfalfa weevil larval infestations in March and April at all locations. Because of warmer winter temperatures, eggs developed faster in the Piedmont compared with the higher elevations, and resulted in larval populations attacking alfalfa earlier in the season, when the crop was at an earlier growth stage. The adult parasitoid, *Microctonus aethiopoides* Loan, was synchronized poorly with alfalfa weevil populations in Virginia. At all locations studied, adult emergence of first generation *M. aethiopoides* occurred in April and early May, when few overwintering alfalfa weevil adults were present in fields. Emergence of the second generation of the parasitoid occurred in late May to June after many of the fields had been harvested. The larval parasitoid, *Bathyplectes anurus* (Thomson), was well synchronized with its host in Virginia. The activity period of the parasitoid overlapped the peak occurrence of alfalfa weevil larvae at all locations.

Key Words *Hypera postica,* biological control, ecology

Alfalfa weevil, *Hypera postica* (Gyllenhal), is an important early-season defoliator of alfalfa, *Medicago sativa* L. In the United States, pest pressure varies with latitude (DeGooyer et al. 1996). In the northern states above 40°N latitude, biological control agents keep populations of *H. postica* below economically-damaging levels (Day 1981, Kingsley et al. 1993). In the southern states, however, alfalfa weevil remains a serious problem despite numerous parasitoid releases (Bryan et al. 1993) and the presence of the entomopathogenic fungus, *Zoophthora phytonomi* (Arthur) (Zygomycetes: Entomophthorales) (Los and Allen 1983, Nordin et al. 1983, Goh et al. 1989). In Virginia, Kuhar et al. (1999) found that alfalfa weevil populations exceeded the economic threshold on approximately 50% of the alfalfa hectarage.

Warm temperatures during the winter months play an important role in the persistence of alfalfa weevil as a major pest in the southern U.S. Winter temperature influences alfalfa weevil oviposition, embryogenesis, and egg survival, and dictates the phenology of populations in the spring (Shade and Hintz 1983, Stark et al. 1993).

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The timing of alfalfa weevil larval infestations is crucial to the severity of injury because the earlier alfalfa is attacked in the spring, the greater the damage to the crop (Hintz et al. 1976).

Alfalfa weevil phenology is also important to biological control. The success of a biological control agent requires synchrony between the parasitoid (or predator) and the preferred host stage (Van Driesche and Bellows 1996). In the northern U.S., alfalfa weevils oviposit primarily in the spring and rarely during the fall and winter months (Armbrust et al. 1966, Casagrande and Stehr 1973, Litsinger and Apple 1973). Thus, larval populations do not peak until late spring. Two species of parasitoids, *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) and *Bathyplectes anurus* Thomson (Hymenoptera: Ichneumonidae), are well synchronized with their respective alfalfa weevil host stages in the northern states and play a major role in alfalfa weevil biological control (Day 1981, Radcliffe and Flanders 1998).

In the southern U.S. (below 40° latitude) climatic conditions are warmer and alfalfa weevil phenology varies from that in the North (Woodside et al. 1968, Campbell et al. 1975, Whitford and Quisenberry 1990, Stark et al. 1993). Oviposition begins in the fall and continues throughout the winter and early spring. Moreover, eggs develop more rapidly in the warmer southern latitudes, resulting in earlier larval infestations (Stark et al. 1994).

In Virginia, the Appalachian Mountains present a considerable range of vertical relief (Hoffman 1969). Alfalfa is an important crop in the low-lying Piedmont plateau (elevation = 60 to 160 m) as well as in the cooler mountain and valleys of western Virginia (elevation = 300 to 1500 m). The objectives of this study were to compare alfalfa weevil phenology with its host crop and primary parasitoids, *M. aethiopoides* and *B. anurus,* in three distinct geographic locations of Virginia: the central Piedmont, Shenandoah Valley, and southwestern region. This information should prove useful to alfalfa pest management decision-making in the different regions of Virginia and explain why alfalfa weevil biological control has not been fully achieved in Virginia and other southern states.

Materials and Methods

Alfalfa weevil populations were sampled in nine Virginia alfalfa fields in 1997-98 and 1998-99 in conjunction with another study (Kuhar et al. 2000). Three fields were located near the town of Rustburg (79°10′W 37°20′N; elevation ≈200 m) and represented the central Piedmont region. Three fields were located near Fairfield (79°31′W 37°78′N; elevation ≈500 m) in the Shenandoah Valley. The remaining three fields were located in Blacksburg (80°25′W 37°14′N; elevation ≈640 m) in the New River Valley of southwestern Virginia.

Alfalfa weevil egg density was estimated in all nine fields every 10 to 15 days from November to May by collecting twenty 0.02-m^2 samples of plant material (live and dead stems) from each field. Alfalfa weevil eggs were extracted from the plant material using a blender-flotation method (Pass and VanMeter 1966). Eggs were categorized as yellow or brown according to embryonic development (Roberts et al. 1970). Total density of eggs deposited over time was estimated by summing the sample means of "brown" eggs at approximately 100 degree-day (DD) intervals (minimum developmental temperature 9°C) before and after the population peak (Stark et al. 1994). Brown eggs were assumed to have hatched before the accumulation of 100 DD.

Alfalfa weevil larval density was estimated every 10 to 15 days from mid-February to late-May (first harvest). Twenty samples of 10 alfalfa stems were shaken into a 11.4-liter bucket to expose larvae for counting (Legg et al. 1985). A sub-sample of 20 shakened stems was placed in a Berlese funnel to estimate the proportion of larvae remaining on stems after the bucket-shake method (Higgins et al. 1991). Larvae were classified as period-one (first and second instars) or period-two (third and fourth instars) according to their size (Harcourt et al. 1977). Alfalfa stem density was measured in each field to convert larvae per stem values to larvae per unit area. Alfalfa stem height and developmental stage were recorded from 20 arbitrarily chosen plants per field on each sample date. Differences in alfalfa stem height at the time of peak larval infestation were compared using two-way ANOVA procedures with years and locations as factors (Abacus Concepts, Inc. 1989). Means were separated using Fisher's Protected LSD at the 0.05 level of significance.

Subsamples of 50 or more period-two larvae were collected from each field in early to mid-May and reared in the laboratory on alfalfa bouquets at room temperature (21 ± 5°C; ≈20% RH). Parasitism by *B. anurus, B. curculionis,* or other species was determined by identifying the parasitoid cocoons and any emerged adults (Brunson and Coles 1968, Bryan et al. 1993). Alfalfa weevil adults were collected from sweep net samples in March and April and assessed for parasitism by rearing the insects on alfalfa until a parasitoid emerged, or by dissecting the insects under water (Brunson and Coles 1968, Hilburn 1985).

Daily max-min temperature data were recorded hourly at each of the field sites using hygrothermograph recorders (Omega Engineering, Inc.) in 1997-98 and Hobo Pro Series® data loggers (Onset Computer Corp., Pocasset, MA) in 1998-99. Degree-days for alfalfa weevil egg and larval development were calculated using a minimum developmental temperature of 9°C (Harcourt 1981). Degree-days for adult emergence of the first and second generations of *M. aethiopoides* were calculated using a minimum developmental temperature of 8.4°C (Morales and Hower 1981).

Results and Discussion

Alfalfa weevil egg and larval phenology. In both years, alfalfa weevil eggs were deposited from November to May at all three Virginia locations (Figs. 1, 2). In 1998, ≈68%, 34%, and 38% of eggs were deposited by 1 January at Rustburg, Fairfield, and Blacksburg, respectively. In 1999, ≈67%, 73%, and 25% of eggs were deposited by 1 January at the same three locations, respectively. Egg laying was nearly (>90%) complete in all fields by 1 April. Alfalfa weevil larvae were detected from early March to first alfalfa harvest, which typically occurred in mid-May.

There were distinct phenological differences in alfalfa weevil larval populations among the locations. In 1998, 50% of the total larval population occurred by 28 March at Rustburg, which was more than 2 wk earlier than Fairfield and Blacksburg (Table 1). In 1999, 50% of the larval population occurred by 18 March at Rustburg, which was again more than 2 wk earlier than the other locations.

Although the actual date of 50% larval occurrence differed among locations, accumulated degree-days were similar (±1 SEM), and when averaged over years ranged from 150 to 160 DD after 1 January. By using the developmental requirement for alfalfa weevil egg hatch, 156 DD (minimum developmental temperature = 9°C) (Roberts et al. 1970), and extrapolating backwards, the peak larval infestation of

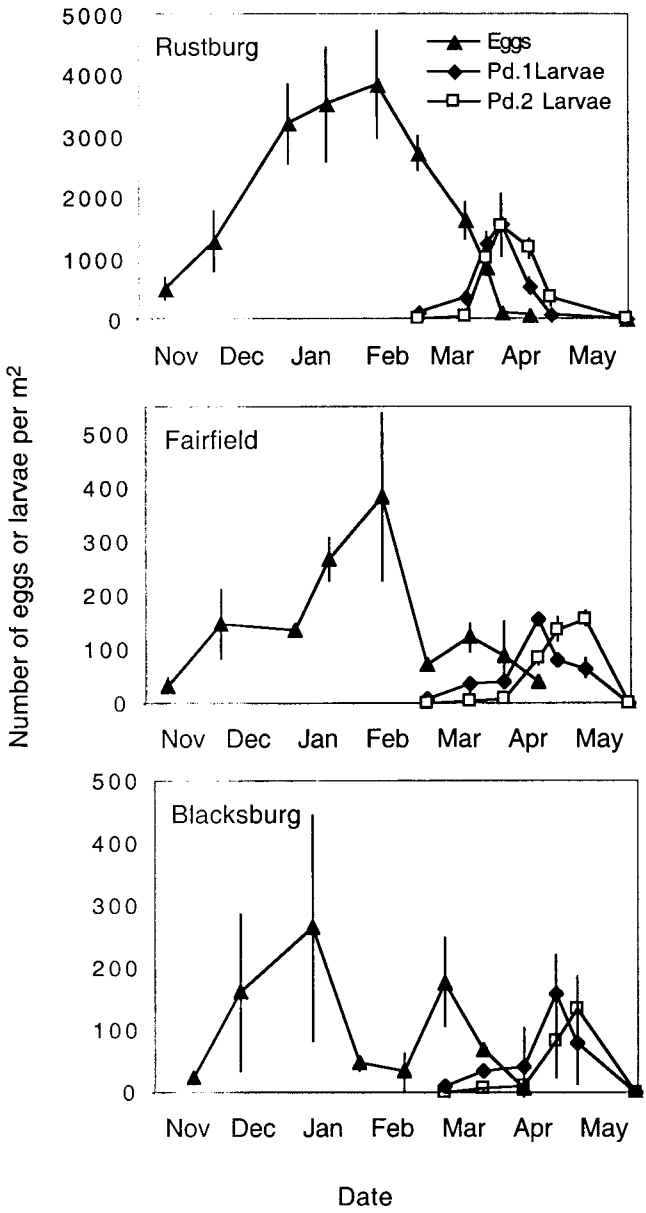

Fig. 1. Seasonal abundance of alfalfa weevil eggs, period-one larvae (instars 1 and 2), and period-two larvae (instars 3 and 4) at three locations in Virginia; 1997-98. Data points represent the mean ± SEM of three fields.

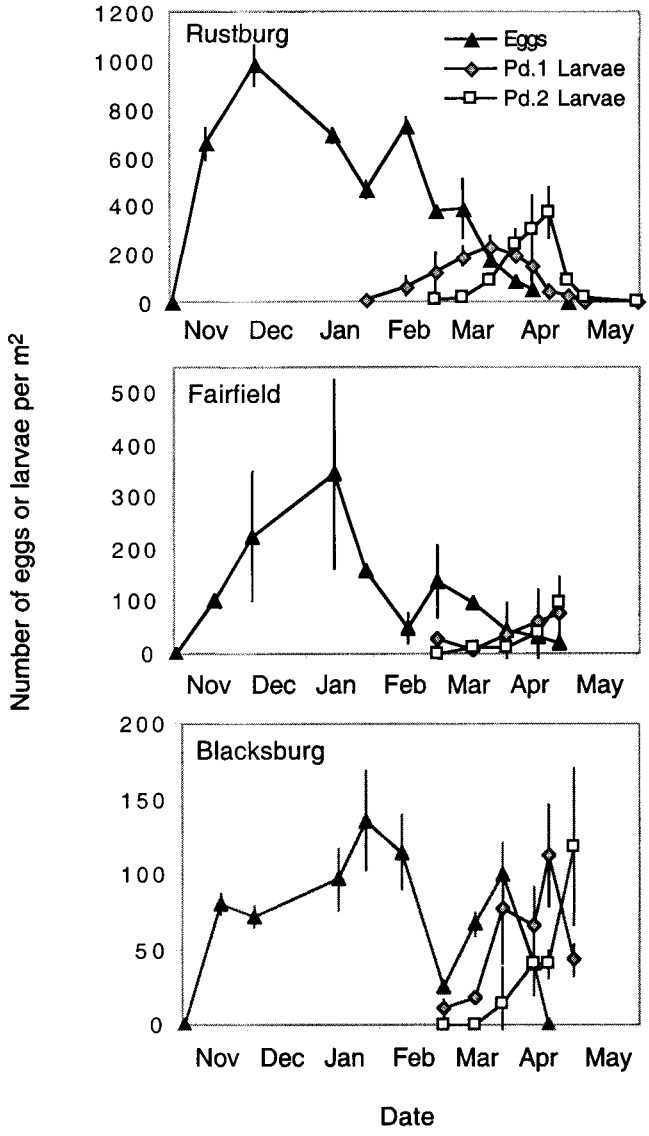

Fig. 2. Seasonal abundance of alfalfa weevil eggs, period-one larvae (instars 1 and 2), and period-two larvae (instars 3 and 4) at three locations in Virginia; 1998-99. Data points represent the mean ± SEM of three fields.

alfalfa weevil occurred as a result of eggs estimated to have been deposited in December or January at all three locations (Table 1).

Alfalfa weevil phenology with its host crop. Spring growth of alfalfa commenced in mid to late March at all locations in 1998 and 1999. There was a significant

Table 1. Occurrence of 50% of the total larval population of alfalfa weevil at three locations in Virginia

Location (n = 3 fields)	Year	Date	DD after 1 January	Estimated date of oviposition*
Rustburg	1998	28 March	148.2	25 December
	1999	18 March	151.8	21 December
			$\bar{X}$ ± SE = 150.0 ± 2.5	
Fairfield	1998	14 April	152.6	19 December
	1999	11 April	164.4	20 January
			$\bar{X}$ ± SE = 158.5 ± 8.3	
Blacksburg	1998	20 April	170.0	2 January
	1999	7 April	130.1	6 December
			$\bar{X}$ ± SE = 150.0 ± 28.2	

* The estimated date of oviposition was calculated by extrapolating backward 156 DD (minimum developmental temperature = 9°C) from the date of 50% larval emergence.

year × location interaction in the growth rate of alfalfa (F = 7.869; df = 2, 12; P < 0.01). In 1998, alfalfa growth was similar at all three locations from early March to late April (Fig. 3). In 1999, alfalfa growth was again similar at all three locations from mid February to mid April, but differed in late April (F = 71.251; df = 2, 6; P < 0.001), when stem height averaged 57.5 ± 3.3 cm at Blacksburg, which was higher than Fairfield (41.9 ± 3.0 cm) and Rustburg (38.1 ± 5.1 cm).

Alfalfa-stem height at the population peak of alfalfa weevil larvae differed by year (F = 19.120; df = 1, 12; P < 0.001) and location (F = 36.985; df = 2, 12; P < 0.001). The year × location interaction was not significant (F = 1.634; df = 2, 12; P = 0.2356). In both years, alfalfa plants at peak weevil infestation were tallest in Fairfield, then Blacksburg, and shortest in Rustburg (Table 2).

Phenology with *M. aethiopoides.* Morales and Hower (1981) studied the thermal requirements for *M. aethiopoides* development and determined that 50% of the first generation adults emerge at 242 DD (minimum developmental temperature = 8.4°C) after 1 January. Second generation adults take flight approximately 251 DD after the peak flight of their parents (Morales and Hower 1981). Using this degree-day model, the emergence of first generation *M. aethiopoides* adults for both years occurred in early April at Rustburg, and late-April to early May at Fairfield and Blacksburg (Table 3). Kuhar et al. (2000) showed that by mid-March in both years, overwintering alfalfa weevil adult populations had declined by ≈70%. By April, few alfalfa weevil adults were available to be parasitized by *M. aethiopoides.* Thus, most alfalfa weevil adults still alive from the previous year would not be expected to survive long enough to support *M. aethiopoides* through development. Emergence of second generation *M. aethiopoides* was predicted to occur in May or early June of both years (Table 3), which would have coincided with the next generation of alfalfa weevil adults, assuming that adult weevil eclosion occurred ≈213 DD after the population peak of period-

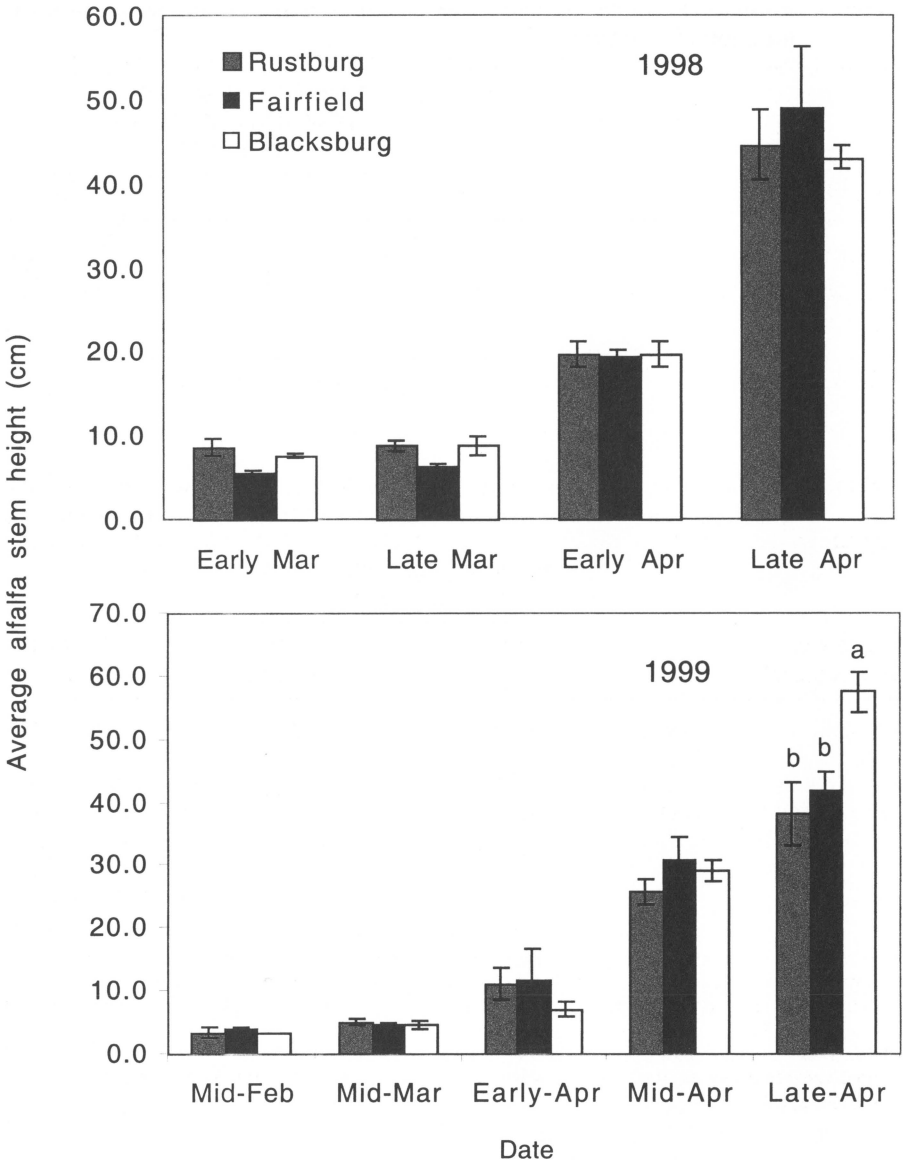

Fig. 3. Spring growth of alfalfa (mean ± SEM) at three Virginia locations in 1998 and 1999. Bars with the same letter within sample dates are not significantly different according to Fisher's Protected LSD at the 0.05 level of significance.

one larvae (Hsieh et al. 1974). However, in both years, alfalfa fields were harvested in May, which likely interfered with second generation *M. aethiopoides* parasitism. Parasitization of overwintering adult weevils ranged from 1.8 to 24% across locations (Table 3).

Table 2. Alfalfa stem height at peak infestation of alfalfa weevil larvae at three locations in Virginia

Location (n = 3 fields)	Alfalfa stem height (cm) at peak larval infestation mean ± SEM	
	1998	1999
Rustburg	19.6 ± 0.5a	12.3 ± 5.9a
Fairfield	59.6 ± 6.5c	38.4 ± 3.2c
Blacksburg	43.0 ± 0.7b	30.0 ± 1.1b

Means within columns followed by the same letter are not significantly different according to Fisher's Protected LSD ($P > 0.05$).

Table 3. Predicted adult emergence dates of *Microctonus aethiopoides* and parasitism levels of alfalfa weevil adults at three locations in Virginia

Location (n = 3 fields)	Year	Predicted peak emergence of *M. aethiopoides**		% Parasitism of adult alfalfa weevils**
		First generation	Second generation	
Rustburg	1998	6 April	14 May	1.8 ± 0.7
	1999	3 April	11 May	5.1 ± 1.1
Fairfield	1998	24 April	26 May	24.0 ± 6.6
	1999	23 April	27 May	8.8 ± 0.3
Blacksburg	1998	24 April	30 May	21.4 ± 9.2
	1999	7 May	9 June	12.4 ± 0.9

* Emergence of first and second generation *M. aethiopoides* were predicted to occur after the accumulation of 242 and 493 DD (minimum developmental temperature = 8.4°C), respectively, after 1 January (Morales and Hower 1981).

** Parasitism of alfalfa weevil adults that overwintered was assessed by individually rearing or dissecting >50 adults collected from the field from early April to May. Kuhar et al. (2000) describes the sampling protocol in more detail.

Phenology with *B. anurus*. The activity period of *B. anurus* adults occurs from 177 to 260 DD (minimum developmental temperature = 9°C) after 1 January (Los 1982, Kingsley et al. 1993, Giles et al. 1994). Using this degree-day interval, *B. anurus* adults were active from late March to mid-April at Rustburg, mid-April to early May at Fairfield, and late-April to mid-May at Blacksburg. These activity periods overlapped the peak occurrence of alfalfa weevil larvae at all three locations in 1998 (Fig. 4) and 1999 (Fig. 5). Parasitism of alfalfa weevil larvae collected in early to mid-May at all three locations averaged 49 to 69% in 1998 and 36 to 92% in 1999 (Kuhar et al. 2000).

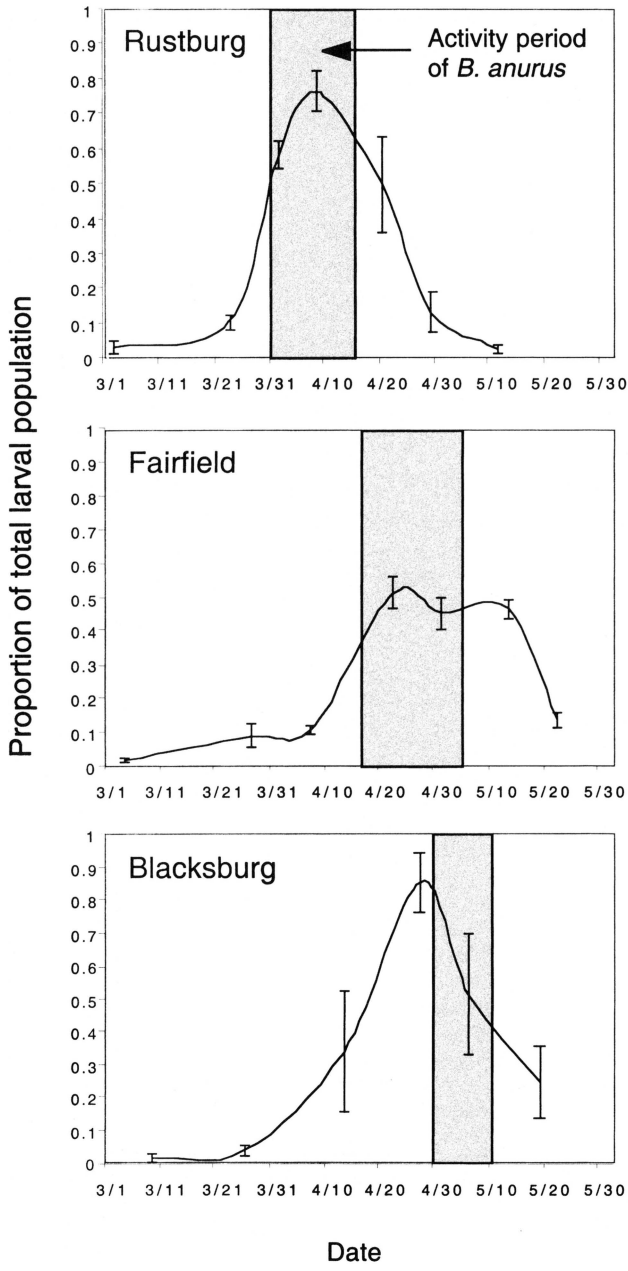

Fig. 4. Phenological synchrony of the parasitoid, *Bathyplectes anurus,* with alfalfa weevil larval populations at three locations in Virginia, 1998. Data points represent the mean ± SEM of three fields. Hatched areas indicate the activity period of *B. anurus* adults based on a degree-day range of 177 to 260 DD (minimum developmental temperature = 9°C).

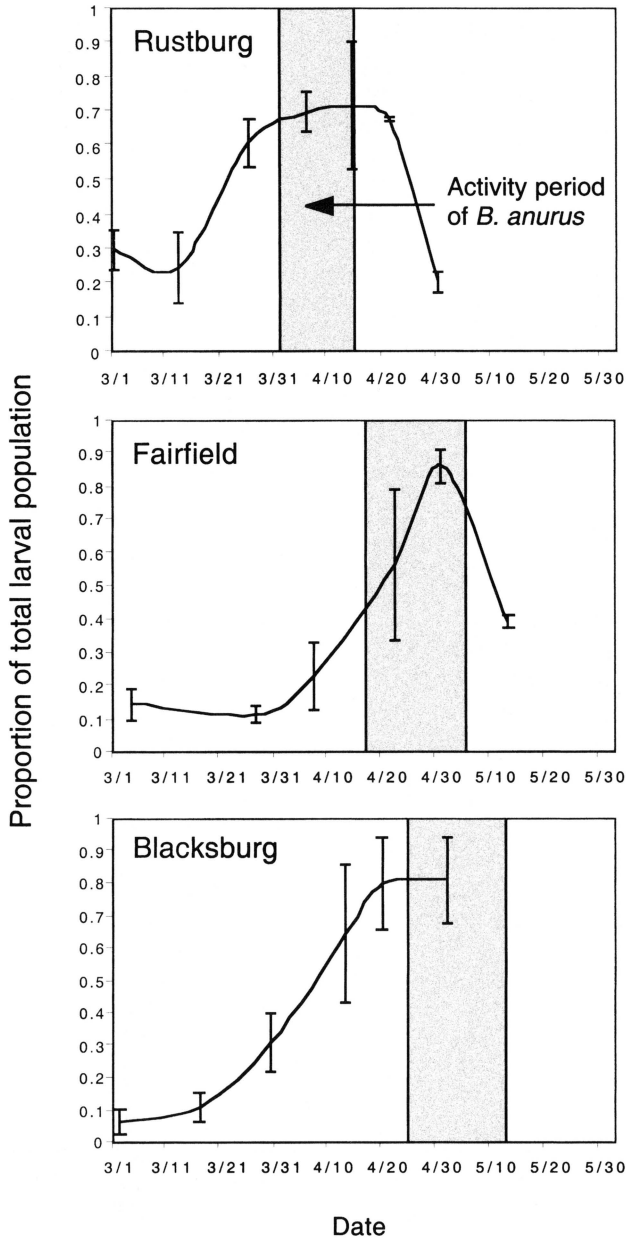

Fig. 5. Phenological synchrony of the parasitoid, *Bathyplectes anurus,* with alfalfa weevil larval populations at three locations in Virginia, 1999. Data points represent the mean ± SEM of three fields. Hatched areas indicate the activity period of *B. anurus* adults based on a degree-day range of 177 to 260 DD (minimum developmental temperature = 9°C).

Summary. In the northeastern U.S., alfalfa weevil eggs laid during the fall and winter do not survive in large enough numbers to contribute to spring larval populations (Townsend and Yendol 1968, Blickenstaff et al. 1972). However, in Virginia eggs deposited in December and January resulted in alfalfa weevil larval infestations in March and April at the three locations studied in 1998 and 1999. Also, because of warmer winter temperatures, eggs developed faster at lower elevations, and resulted in larval populations attacking alfalfa earlier in the season, when the crop was at a shorter growth stage. DeGooyer et al. (1996) found similar phenological differences associated with latitude in Iowa. In the southernmost Iowa fields, alfalfa weevil larval populations peaked earlier and attacked alfalfa at a much shorter stem height compared with the northernmost fields. This phenomenon has important pest management implications because the ability of alfalfa to tolerate feeding damage is related to its growth stage (Hintz et al. 1976).

In the northeastern U.S., the adult parasitoid *M. aethiopoides* plays a larger role in reducing weevil populations below damaging levels (Van Driesche and Gyrisco 1979, Day 1981, Radcliffe and Flanders 1998). Because parasitized hosts become sterilized (Drea 1968), alfalfa weevil reproductive potential can be reduced substantially by *M. aethiopoides* parasitism (Van Driesche and Gyrisco 1979). Parasitization of overwintering weevil adults generally exceeds 50%, and rates of 70 to 90% are not uncommon (Brunson and Coles 1968, Abu and Ellis 1976, Van Driesche and Gyrisco 1979). In Virginia, however, it appears that *M. aethiopoides* is not well synchronized with the alfalfa weevil. At the three locations studied, adult emergence of first generation *M. aethiopoides* was estimated to have occurred in April and early May, when relatively few overwintering alfalfa weevil adults remained in fields. Parasitization of these adults ranged from 1.8 to 24.0%, which is considerably less than rates reported in more northerly states (Radcliffe and Flanders 1998). Also, because alfalfa weevil oviposition was nearly complete by April, *M. aethiopoides* parasitism likely had very little impact on reducing weevil abundance. Furthermore, emergence of the second generation of the parasitoid occurred in late May or June, which was after most of the alfalfa fields had been harvested. Harvesting creates an unfavorable habitat for alfalfa weevil adults, which results in them migrating out of alfalfa and into areas with more vegetative cover to initiate summer aestivation (Prokopy and Gyrisco 1965, Manglitz 1976). Poor synchrony between alfalfa weevil and *M. aethiopoides* may be the case in other southern states as well. Copley and Grant (1998) reported that <5% of adult alfalfa weevils were parasitized in Tennessee.

In contrast, the larval parasitoid, *B. anurus,* was well synchronized with its host in Virginia. The activity period of the parasitoid overlapped the peak occurrence of alfalfa weevil larvae at all locations. As reported in Kuhar et al. (2000), alfalfa weevil larval parasitization by *B. anurus* was relatively high (36 to 92%) at all locations in both years. Good host-parasitoid synchrony between alfalfa weevil and *B. anurus* also has been reported in Pennsylvania (Smilowitz et al. 1972), Kentucky (Parr et al. 1993), Iowa (Giles et al. 1994), and Oklahoma (Berberet and Bisges 1998).

Biological control of alfalfa weevil populations involves a complex of natural enemies (Bryan et al. 1993). Day (1981) suggested that one parasitoid species is not enough to reduce alfalfa weevil populations below damaging levels. The occurrence of *M. aethiopoides* with *B. anurus* or *B. curculionis* is needed for adequate biological control of the alfalfa weevil (Kingsley et al. 1993, Radcliffe and Flanders 1998). However, impact of *M. aethiopoides* appears to be considerably reduced in the southern U.S. because of warmer winter climates.

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