Chapter 4
Phenology of the alfalfa weevil in Virginia

An understanding of alfalfa weevil phenology is critical to pest management decision-making in a particular region because the severity of crop injury depends on the timing of larval infestation (DeGooyer et al. 1996). The earlier the plant is attacked in the spring, the greater the damage potential from alfalfa weevil (Hintz et al. 1976, Shade and Hintz 1983).

The phenology of alfalfa weevil is also important to biological control. The success of a biological control agent requires synchrony between the parasitoid (or predator) and its 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). As a result, larval populations peak in 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).

Microctonus aethiopoides is a bivoltine endoparasitoid that oviposits in adult weevils. It overwinters as a 1st instar within its host (Abu and Ellis 1976). Morales and Hower (1981) studied the thermal requirements for *M. aethiopoides* development and determined that 50% of the 1st generation adults emerge at 242 DD (base 8.4˚C) after 1 January. This generally occurs in April or May in the northeastern U.S. and coincides with the peak oviposition period of alfalfa weevil. Parasitization rates of 70 to 90% are not uncommon in the Northeast (Brunson and Coles 1968, Abu and Ellis 1976, van Driesche and Gyrisco 1979). Also, because parasitized hosts become sterilized (Drea 1968), alfalfa weevil reproductive potential can be reduced substantially by *M. aethiopoides* parasitism (van Driesche and Gyrisco 1979). Second generation *M. aethiopoides* adults take flight approximately 251 DD after the peak flight of their

parents (Morales and Hower 1981). In the northeastern states, this generally occurs in late May or June and coincides with the emergence of the next generation of alfalfa weevil adults.

Bathyplectes anurus is considered second only to *M. aethiopoides* in efficacy of control against alfalfa weevils in eastern North America (Day 1981, Radcliffe and Flanders 1998). *B. anurus* is a univoltine, solitary, endoparasitoid of alfalfa weevil larvae. The parasitoid prefers to oviposit in 2nd to 3rd instars (Dowell and Horn 1977). Los (1982) and Kingsley et al. (1993) showed that the adult activity period of *B. anurus* adults occurs from 177 to 260 DD (base 9°C) after 1 January. Giles et al. (1994) reported similar results in Iowa. This period overlaps the occurrence of alfalfa weevil larvae in the northeastern U.S. (Kingsley et al. 1993).

In the southern U.S. (below 40° latitude) climatic conditions are warmer and alfalfa weevil phenology varies from that in the North. Oviposition begins soon after weevil adults return to alfalfa fields in the fall and continues throughout the winter and spring (Woodside et al. 1968, Campbell et al. 1975, Whitford and Quisenberry 1990, Stark et al. 1993). Temperature during the winter months influences the rate of oviposition, embryogenesis, and egg survival, and dictates the timing of larval populations in the spring (Shade and Hintz 1983, Stark et al. 1993).

Winter climate in Virginia is greatly influenced by the Appalachian Mountains, which create a considerable range of vertical relief in the state (Hoffman 1969). Alfalfa is an important crop in the low-lying Piedmont plateau (elevation = 60–160 m) as well as in the cooler mountain and valleys of western Virginia (elevation = 300-1500 m) (Virginia Agricultural Statistics Service 1998). Woodside et al. (1968) studied alfalfa weevil oviposition in the different regions of Virginia and reported that more eggs were laid earlier in the Piedmont compared with the mountain and valleys. They did not report how this regional difference in oviposition altered alfalfa weevil phenology with its host crop or with its natural enemies.

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 some other southern states.

Materials and methods

Alfalfa weevil populations were sampled in nine Virginia alfalfa fields in 1997-98 and 1998-99 (Chapter 3). Three of the fields were located near the town of Rustburg (79°10'W 37°20'N; elevation ≈ 200 m) and represented the central Piedmont region. Another 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 every 10-15 days from November to May. Twenty 0.02-m^2 samples of plant material (live and dead stems) were collected from each field. Alfalfa weevil eggs were extracted from the plant material using a blender-flotation method (Pass and VanMeter 1966). Total density of eggs deposited over time was estimated by summing the sample means of egg density at approximately 100 degree-day intervals (base 9°C) before and after the population peak (Stark et al. 1994). Eggs were assumed to have hatched before the accumulation of 100 DD (Litsinger and Apple 1973).

Alfalfa weevil larval density was estimated every 10-15 days from mid-February to late-May (first harvest). Twenty samples of 10 alfalfa stems were shaken into a bucket to expose larvae for counting (Luna and Ravlin 1992). 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 (1^{st} and 2^{nd} instars) or period-two (3^{rd} and 4^{th} 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 square meter. Alfalfa stem height and developmental stage were recorded from 20 randomly-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 treatments. Means were separated using Fisher's Protected LSD at the 0.05 level of significance.

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 1st and 2nd generations of *M. aethiopoides* were calculated using a minimum developmental temperature of 8.4˚C (Morales and Hower 1981). The activity period of *B. anurus* adults was estimated to occur between 170 and 260 DD base 9˚C, after 1 January (Los 1982, Kingsley et al. 1993).

Results

Alfalfa weevil egg and larval phenology. In both years, alfalfa weevil eggs were deposited from November to May at all three Virginia locations (Figs 4.1 and 4.2). A high percentage of eggs were deposited by 1 January and egg-laying was nearly 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. Phenological area graphs depicting life-stage composition of the alfalfa weevil populations over time are presented for 1997-98 (Fig. 4.3) and 1998-99 (Fig. 4.4). These graphs indicate that virtually all eggs had hatched and most period-one larvae had completed their development by 1 May in both years.

There were strong phenological differences in alfalfa weevil larval populations among the locations. In 1998, 50% of the total larval population occurred by 29 March at Rustburg, which was more than 2 wk earlier than Fairfield and Blacksburg (Fig. 4.5a). 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 (Fig. 4.5b). Although the actual date of 50% larval occurrence was different

among the locations, the accumulated degree-days was similar (± 1 SEM) at each of the locations, and ranged from 150 to 160 DD after 1 January.

By using the developmental requirement for alfalfa weevil egg hatch, ≈156 DD base 9°C (Roberts et al. 1970), and extrapolating backwards from the time of 50% larval emergence, an estimate of the timing of oviposition was calculated for each location (Table 4.1). This indicated that infestations of alfalfa weevil larvae resulted from eggs deposited during December and January at all three locations.

Alfalfa weevil phenology with its host crop. Spring growth of alfalfa commenced in March and increased in a nonlinear fashion at all locations (Fig. 4.6). There was a significant year by 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 in March (F = 1.441; df = 2, 6; P = 0.3082), early April (F = 2.984; df = 2, 6; P = 0.1260), and late April (F = 3.232; df = 2, 6; P = 0.1115). In 1999, alfalfa growth was again similar at all three locations in March (F = 1.953; df = 2, 6; P = 0.2222) and early April (F = 0.272; df = 2, 6; P = 0.7705), but differed in late April (F = 71.251; df = 2, 6; P < 0.001). Alfalfa growth in late-April averaged 2.18 ± 0.15 cm/day at Blacksburg, which was higher than Fairfield (1.1 ± 0.03 cm/day) and Rustburg (0.43 ± 0.1 cm/day).

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 by 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 4.2).

Phenology with *M. aethiopoides*. Using the degree-day model of Morales and Hower (1981), the predicted dates of *M. aethiopoides* adult emergence were calculated for each of the Virginia locations (Table 4.3). In both years, predicted emergence of 1st generation *M. aethiopoides* adults occurred in early April at Rustburg, and late-April to early May at Fairfield and Blacksburg. Results from Chapter 3 showed that by mid-March, overwintering alfalfa

weevil adult populations had declined by ≈70% at all locations. Thus, by April, it was likely that few alfalfa weevil adults were present in fields to be parasitized by *M. aethiopoides*. Most of those still remaining in fields in April also probably would not survive long enough to support *M. aethiopoides* through development. This relative asynchrony between alfalfa weevil and *M. aethiopoides* would explain the low levels of parasitization (1.8 to 24.0%) found at all three Virginia locations in 1997-98 and 1998-99 (Chapter 3). Also, because most alfalfa weevil egg-laying in Virginia was completed by April, *M. aethiopoides* parasitism likely had very little impact on reducing alfalfa weevil abundance.

Emergence of 2nd generation *M. aethiopoides* occurred from May to early June and did overlap the emergence of next-generation alfalfa weevil adults in Virginia (Table 4.3). However, in both years at all locations, alfalfa fields were harvested in May, which likely interfered with 2nd generation *M. aethiopoides* parasitism. Harvesting the field creates an unfavorable environment for alfalfa weevil adults, triggering them to migrate out of alfalfa and initiate summer aestivation (Prokopy and Gyrisco 1965a, Manglitz 1976). Again, this causes a condition where *M. aethiopoides* adults would be active when few hosts are available.

Phenology with *B. anurus*. Based on the reported adult activity period of 177 and 260 DD, for *B. anurus*, this parasitoid was well synchronized with the occurrence of alfalfa weevil larval populations in 1998 (Fig. 4.7) and 1999 (Fig. 4.8). Parasitoids were active from late March to mid-April at Rustburg, mid-April to early May at Fairfield, and mid-April to mid-May at Blacksburg. These activity periods overlapped the peak occurrence of alfalfa weevil larvae at all three locations. As would be expected, *B. anurus* parasitization rates were relatively high (36 to 92%) in all fields (chapter 3).

Discussion

In the northeastern U.S., very few alfalfa weevil eggs laid during the fall and winter survive in large enough numbers to contribute to spring larval populations (Townsend and Yendol 1968,

Blickenstaff et al. 1972). In Virginia, this does not appear to be the case. Eggs deposited in December and January resulted in alfalfa weevil larval populations emerging in March and April at the three Virginia locations studied in 1998 and 1999. Because of warmer winter climates, 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 major role in reducing weevil populations below damaging levels (van Driesche and Gyrisco 1979, Day 1981, Radcliffe and Flanders 1998). In Virginia, however, it appears that *M. aethiopoides* is not well-synchronized with the alfalfa weevil. Adult emergence of the 1st generation of *M. aethiopoides* occurred in April and early May, when few overwintering alfalfa weevils adults were present in fields. Moreover, emergence of the 2nd generation of the parasitoid occurred in late May to June after many of the fields had been harvested. Harvesting alfalfa causes adult alfalfa weevils to migrate out of the crop to seek shelter (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 less than 5% of adult alfalfa weevils were parasitized in Tennessee. Low levels of adult parasitism also occur in Oklahoma (R. C. Berberet, *personal communication*).

Good host-parasitoid synchrony between alfalfa weevil and *B. anurus* has been reported in several states, including Pennsylvania (Smilowitz et al. 1972), Kentucky (Parr et al. 1993), Iowa (Giles et al. 1994) and Oklahoma (Berberet and Bisges 1998). My results showed that *B. anurus* is well synchronized with its host in Virginia. The activity period of the parasitoid overlapped the peak occurrence of alfalfa weevil larvae at all locations. Day (1981) suggested that

successful establishment of one parasitoid species is not enough to reduce alfalfa weevil populations below damaging levels. The occurrence of *M. aethiopoides* with at least one of the *Bathyplectes* species is needed for successful biological control of the alfalfa weevil (Kingsley et al. 1993, Radcliffe and Flanders 1998).

Table. 4.1. Occurrence of 50% of the total larval population of alfalfa weevil at three locations in Virginia

Location	Year	Occurrence of 50% of the total larval population: Date	DD after 1 January	Estimated date of oviposition
Rustburg	1998	28 March	148.2	25 December
	1999	18 March	151.8	21 December
			x ± SE = 150.0 ± 2.5	
Fairfield	1998	14 April	152.6	19 December
	1999	11 April	164.4	20 January
			x ± SE = 158.5 ± 8.3	
Blacksburg	1998	20 April	170.0	2 January
	1999	7 April	130.1	6 December
			x ± SE = 150.0 ± 28.2	

[1]The estimated date of oviposition was calculated by extrapolating backward 156 DD base 9°C from the date of 50% larval emergence.

Table 4.2. Alfalfa stem height at peak infestation of alfalfa weevil larvae in three locations of Virginia

	Alfalfa stem height (cm) mean ± SEM	
Location	1998	1999
Rustburg (*n* = 3 fields)	19.6 ± 0.5a	12.3 ± 5.9a
Fairfield (*n* = 3 fields)	59.6 ± 6.5c	38.4 ± 3.2c
Blacksburg (*n* = 3 fields)	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. 4.3. Predicted adult emergence of *Microctonus aethiopoides* in Virginia

Location	Year	50% emergence of 1st generation *M. aethiopoides*[1] Date (DD after 1 January)	50% emergence of 2nd generation *M. aethiopoides*[2] Date (DD after 1 January)	Estimated date of alfalfa weevil adult eclosion[3] Date (DD after 1 January)
Rustburg	1998	6 April (210)	14 May (460)	11 May (433)
	1999	3 April (195)	11 May (412)	9 May (396)
Fairfield	1998	24 April (196)	26 May (477)	21 May (423)
	1999	23 April (206)	27 May (439)	23 May (409)
Blacksburg	1998	24 April (172)	30 May (434)	27 May (400)
	1999	7 May (223)	9 June (466)	1 June (382)

[1]Emergence of 1st generation *M. aethiopoides* was predicted to occur after the accumulation of 242 DD base 8.4°C after 1 January (Morales and Hower 1981).

[2]Emergence of 2nd generation *M. aethiopoides* was predicted to occur after the accumulation of 493 DD after 1 January (Morales and Hower 1981).

[3]Eclosion of alfalfa weevil adults occurred ≈213 DD base 9°C after the population peak of Period-one larvae (Hsieh et al. 1974).

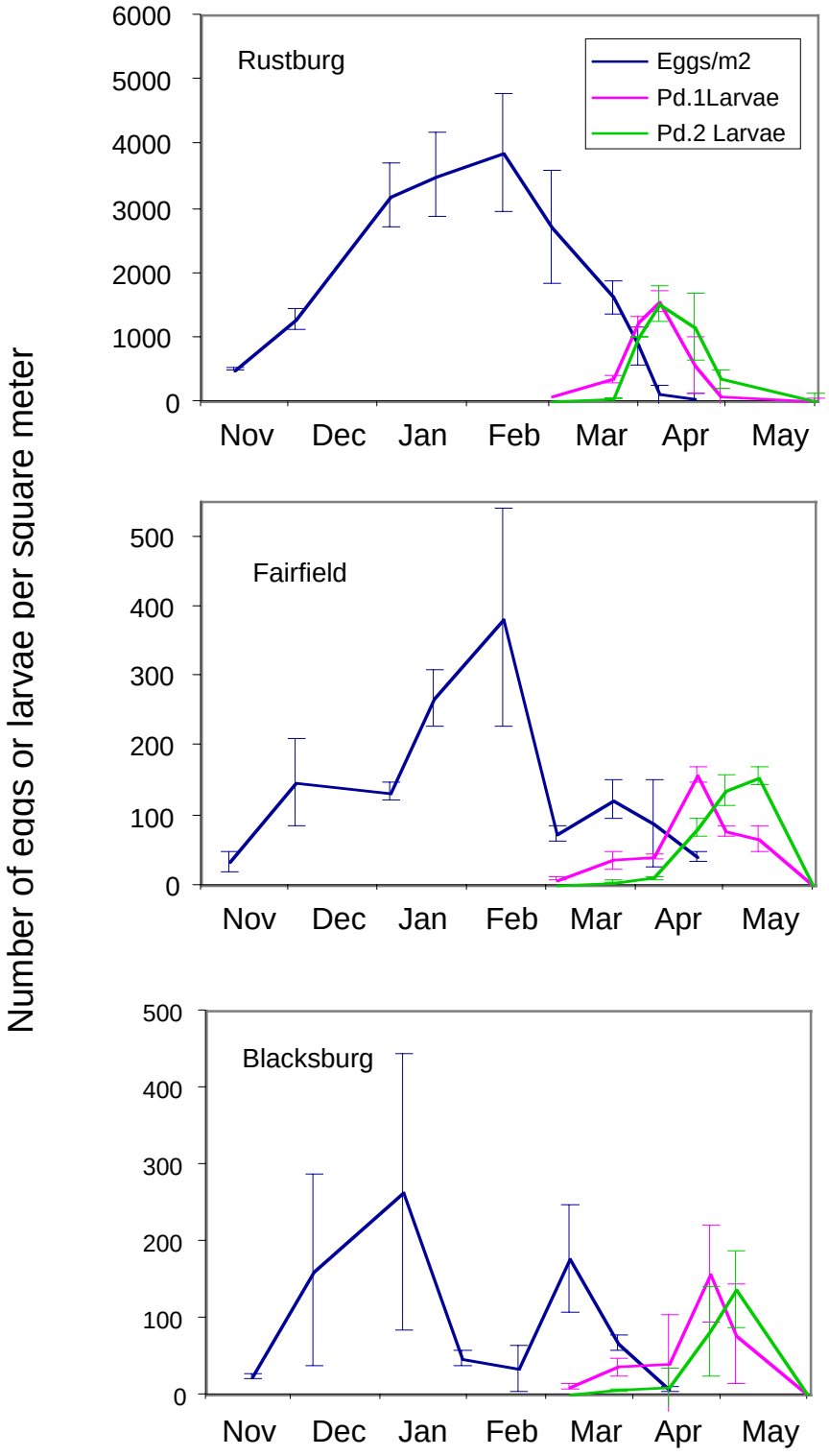

Fig. 4.1. Population dynamics of alfalfa weevil eggs, period-one larvae (instars 1 and 2), and period-two larvae (instars 3 and 4) at three locations in Virginia; 1997-98. Data points represent the mean ± SEM of three fields.

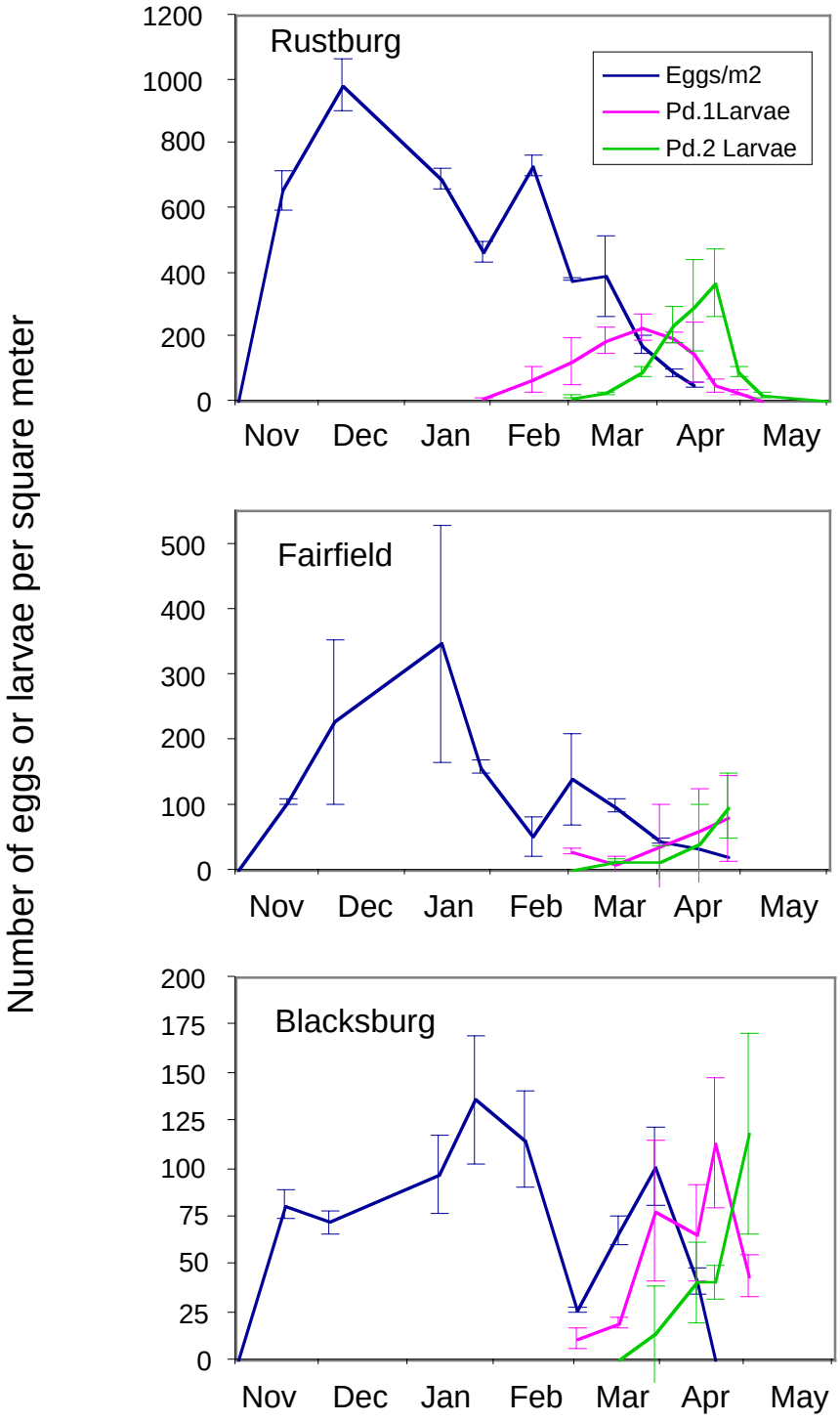

Fig. 4.2. Population dynamics of alfalfa weevil eggs, period-one larvae (instars 1 and 2), and period-two larvae (instars 3 and 4) at three locations in Virginia; 1998-99. Data points represent the mean ± SEM of three fields.

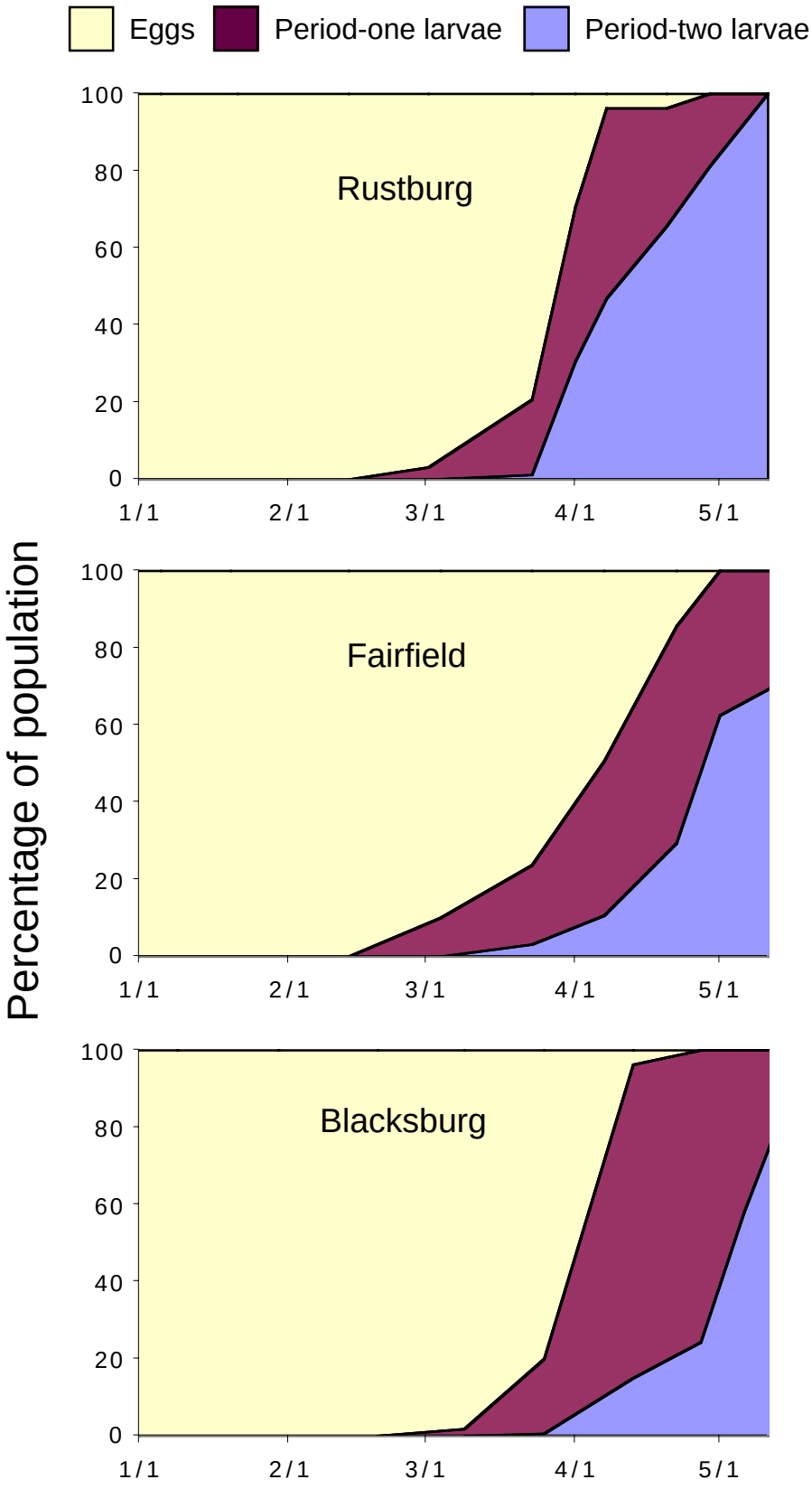

Fig. 4.3. Phenological area graphs of alfalfa weevil populations at three locations of Virginia; 1997-98. Each graph represents a mean of 3 fields.

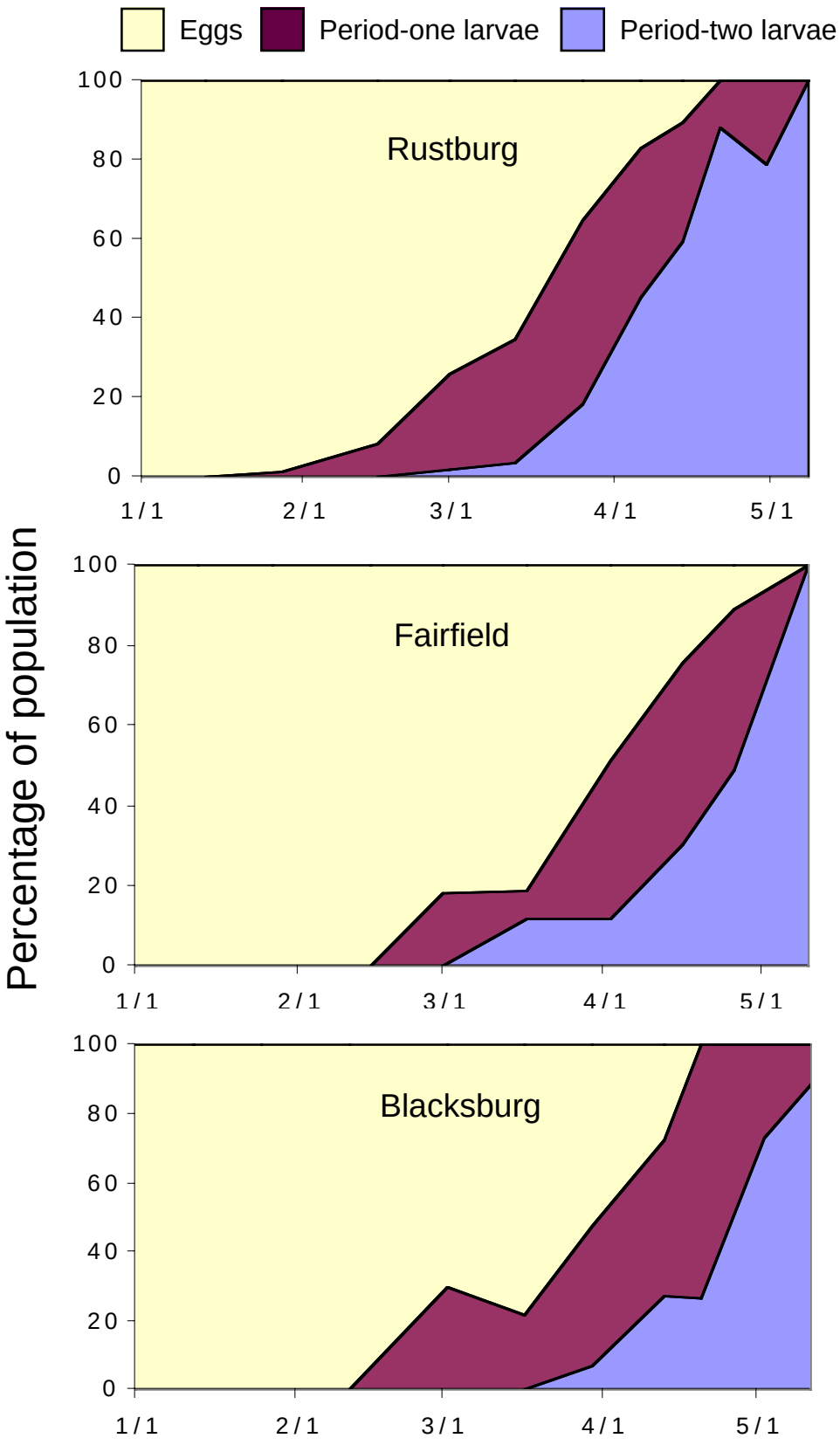

Fig. 4.4. Phenological area graphs of alfalfa weevil populations at three locations of Virginia; 1998-99. Each graph represents a mean of 3 fields.

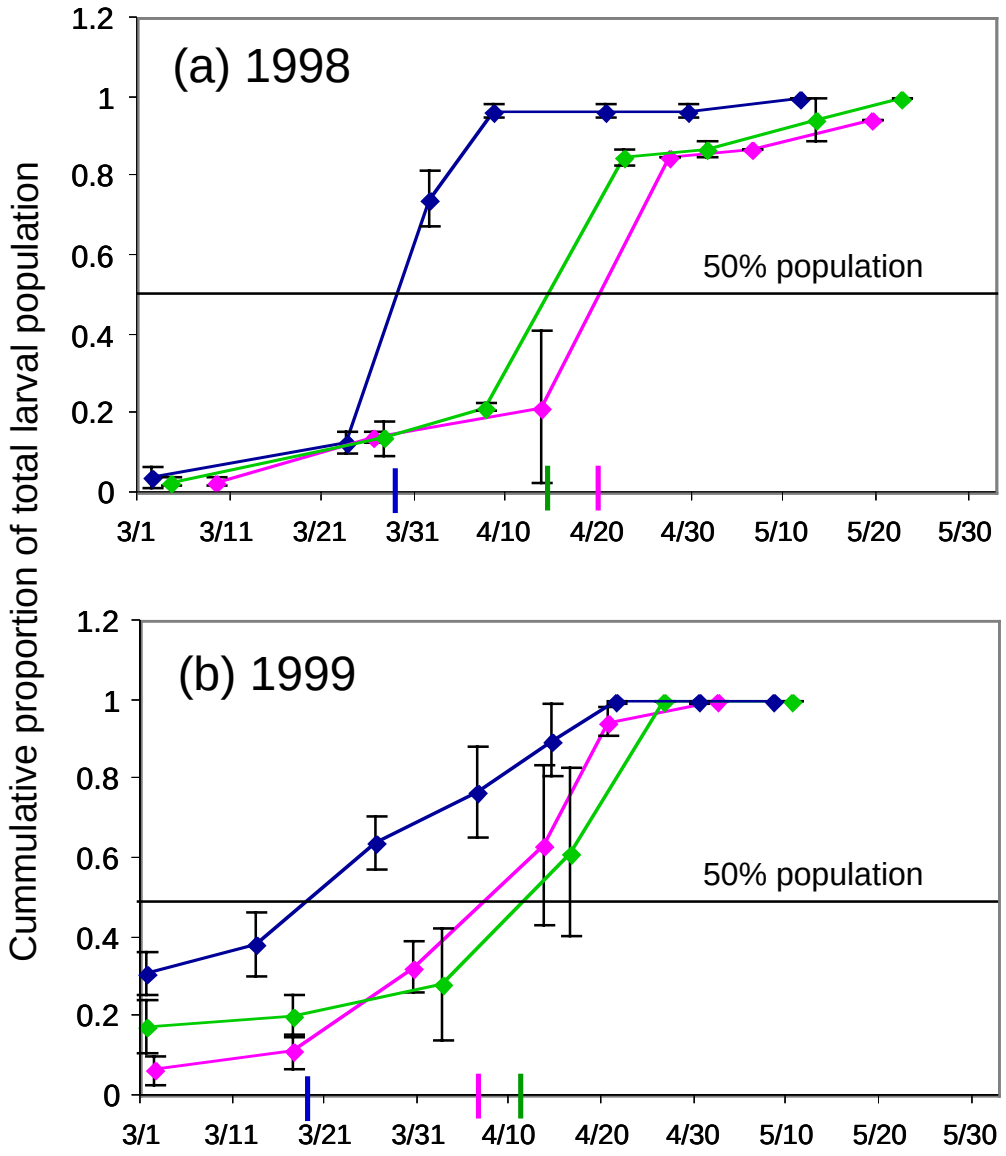

Fig. 4.5. Seasonal occurrence of alfalfa weevil larvae over time. Data represent the mean ± SEM of three fields at each of three locations in 1998 (a) and (1999 (b); Blue = Rustburg, Green = Fairfield, Pink = Blacksburg. Colored tick marks on the x-axis refer to the date of 50% occurrence of the population.

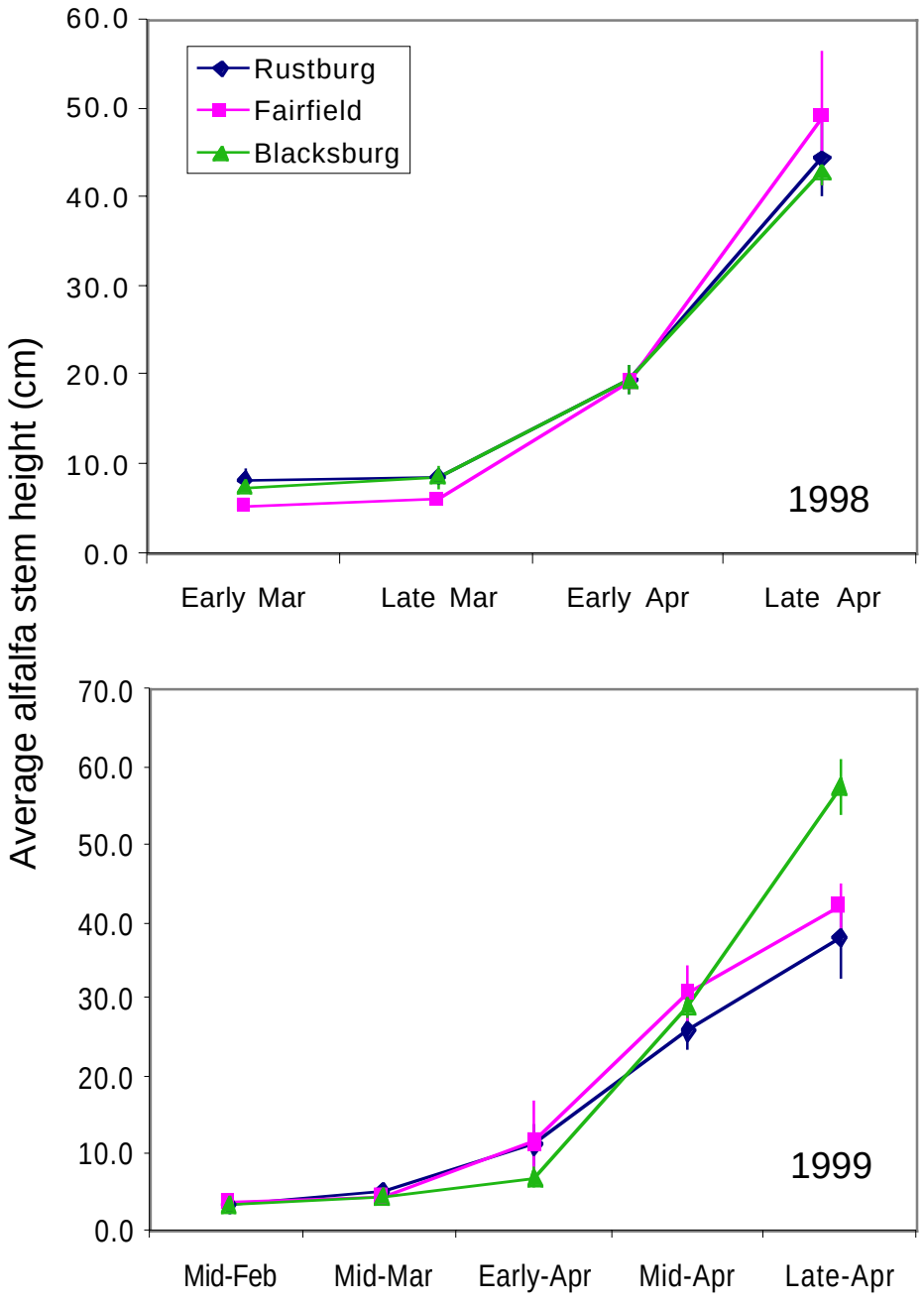

Fig. 4.6 Spring growth of alfalfa at three Virginia locations in 1998 and 1999. Data points represent the mean ± SEM of three fields.

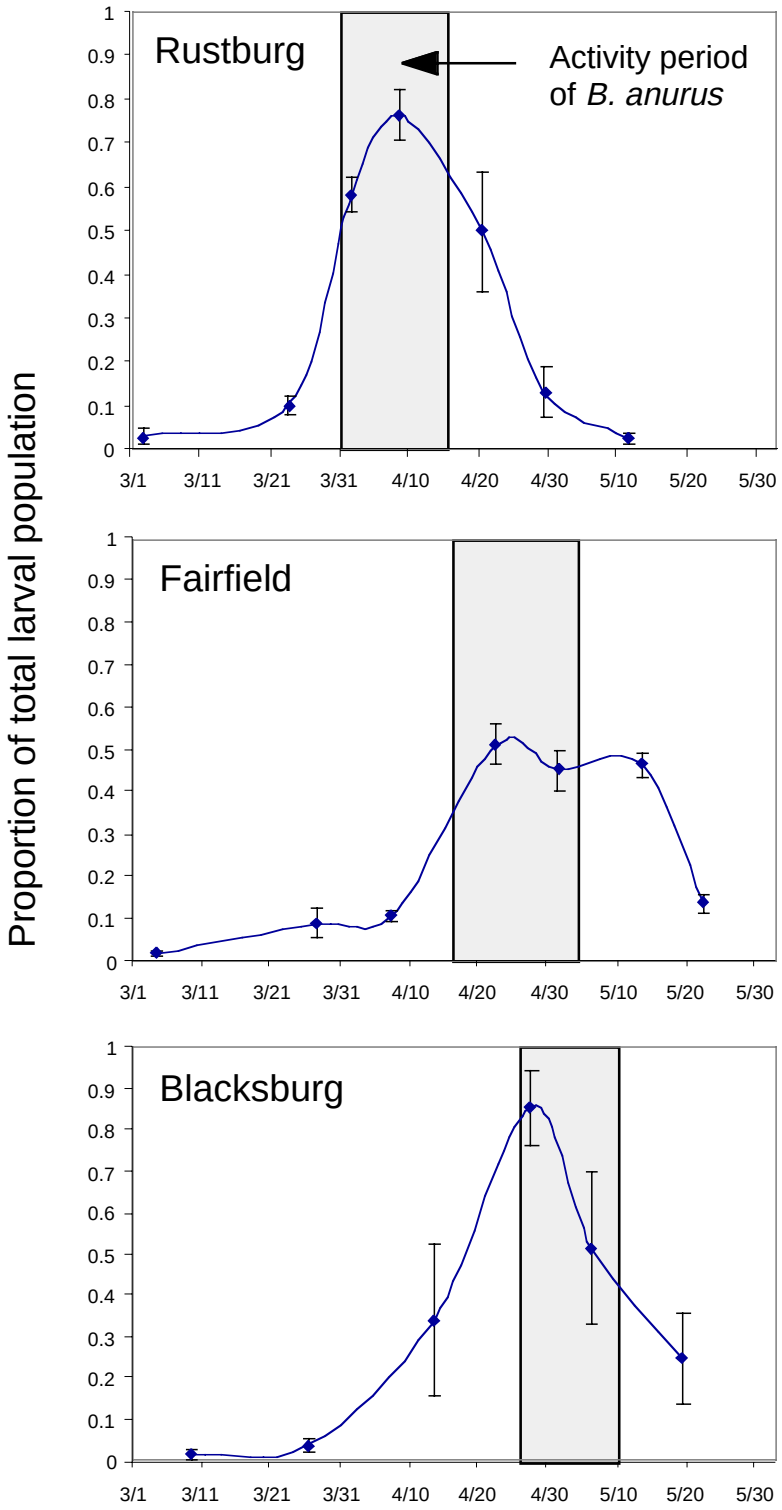

Fig. 4.7. Phenological synchrony of the parasitoid, *B. anurus* with alfalfa weevil larval populations at three locations in Virginia, 1998. Data points represent the mean ± SEM of three fields. Hatched areas indicate the activity period of B. anurus adults based on a degree-day range of 177 to 260 DD base 9°C.

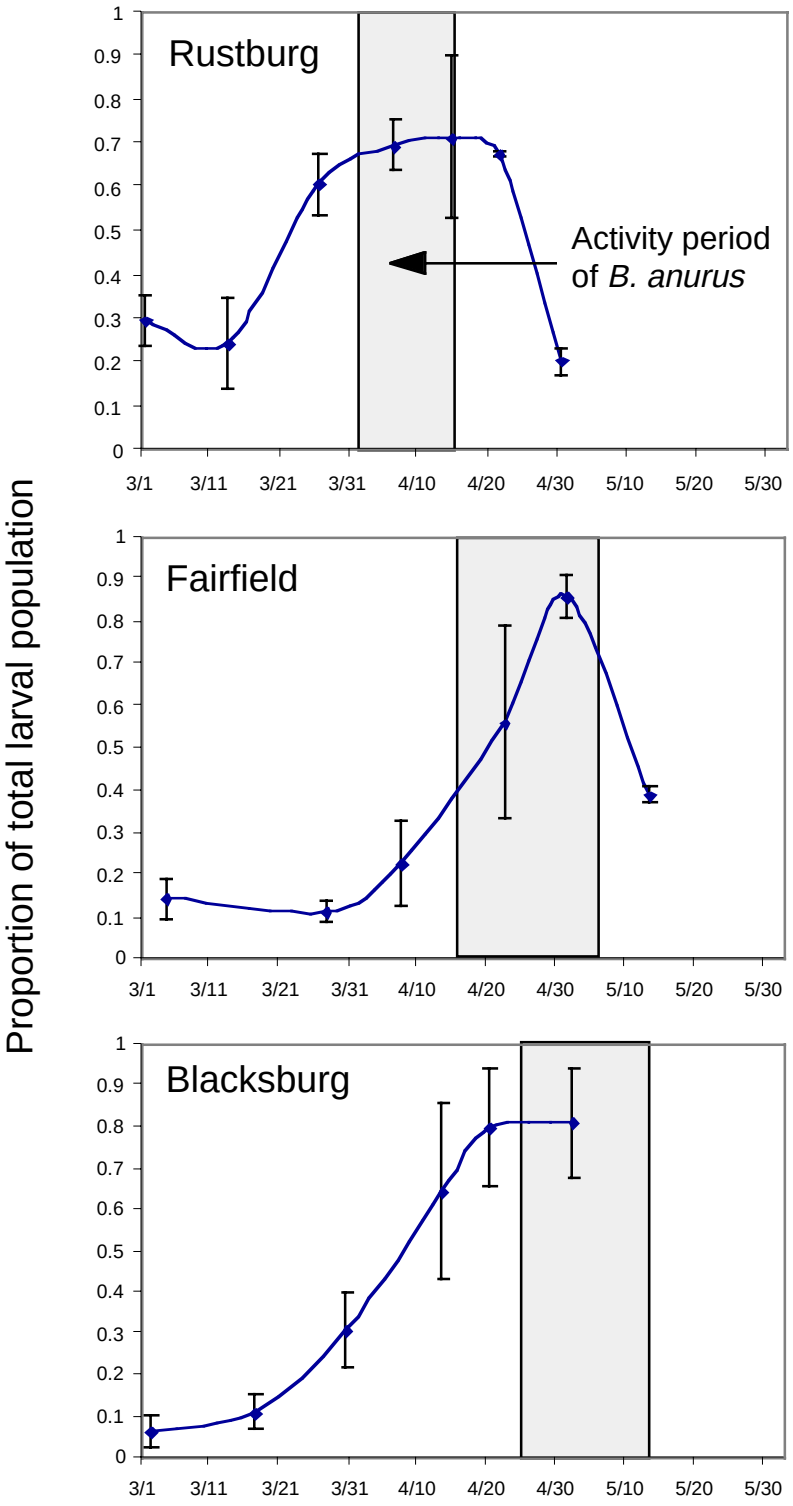

Fig. 4.8. Phenological synchrony of the parasitoid, *B. anurus* with alfalfa weevil larval populations at three locations in Virginia, 1999. Data points represent the mean ± SEM of three fields. Hatched areas indicate the activity period of *B. anurus* adults based on a degree-day range of 177 to 260 DD base 9°C.