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Effects of the Interaction of the Biocontrol Agent *Agapeta zoegana* L. (Lepidoptera: Cochylidae) and Grass Competition on Spotted Knapweed

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Agapeta zoegana **is a Eurasian root-mining moth introduced for biological control of spotted knapweed,** ***Centaurea maculosa*****, in North America. A study was conducted during 1992 through 1994 to assess the establishment of the moth and to make a preliminary assessment of the combined effects of the moth and grass competition on spotted knapweed plant structure and density at two nearby sites in western Montana. The moth was well established at a release site in 1992 where it had been previously released, and it became comparably well established at a check site, 140 m away, by 1994. Knapweed plants at the** ***A. zoegana*** **release site had less above-ground biomass (43%), fewer stems per plant (29%), and fewer capitula per plant (43%) and were shorter (18%) than knapweed plants at the check site, which may represent impact of the moth on the plant. However, a comparison of infested versus uninfested knapweed plants throughout both sites showed that infested plants had more stems (15%), more capitula (40%), and more above-ground biomass (112%), were taller (7%), had thicker roots (92%), and were older (22%) than uninfested plants, suggesting that the moth preferentially attacked older, larger knapweed plants. Numbers of** ***A. zoegana*** **larvae per root were positively correlated with root diameter. Incidence of attack by** ***A. zoegana*** **was significantly greater in bolted knapweed plants than in rosettes, but larvae showed no preference for bolted plants over rosettes when root diameters were similar.** ***A. zoegana*** **had no effect on knapweed rosette density, but appeared to reduce the number of bolted knapweed plants in plots with low grass density (10% grass cover) by 39% and increase the density of knapweed seedlings in the spring by 65%. Plots with high grass density (50% grass cover) contained fewer bolted knapweed plants (35%), fewer rosettes (38%), and fewer seedlings (50%) than plots with low grass density. The effects of** ***A. zoegana*** **and grass competition on bolted plant density were not additive.**

Key Words: Agapeta zoegana; **biological control; weed; rangeland; grass competition; spotted knapweed;** ***Centaurea maculosa.***

INTRODUCTION

Spotted knapweed, *Centaurea maculosa* Lamarck, is a deeply rooted perennial plant from Eurasia that has become a serious weed on rangelands of the northwestern United States. First reported in North America in 1893 (Groh, 1944), the plant now infests over 3 million ha of rangeland and pasture in 14 states and two Canadian provinces (Lacey, 1989; Sheley *et al.*, 1998). The life history of the plant has been described by Watson and Renney (1974). Seed germination occurs in the fall or early spring, depending upon moisture availability. Seedlings develop into rosettes; plants that overwinter as rosettes usually produce floral stems (bolt) in the following summer. Stem elongation occurs in June followed by flowering in July and seed dispersal in August. A newly bolted plant will usually bolt again in subsequent years. Spotted knapweed plants in western Montana generally have an average maximum life span of 5 to 9 years, although plants may live up to 12 years (Boggs and Story, 1987). Seeds can survive in the soil for 8 or more years (Davis *et al.*, 1993).

A Eurasian root-mining moth, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), was introduced into North America for biological control of the plant. The first United States release of the moth was made in Montana in 1984 and the first field recovery occurred in Montana in 1986 (Story *et al.*, 1991). The moth is now established at numerous sites in Montana (Story, unpublished data). The biology, host specificity, and potential impact of the moth were described by Müller *et al.* (1988) and Müller (1989a). Early instar larvae mine the epidermal tissues of the root crown while older larvae mine the root cortex and endodermis. The moth overwinters as a larva in the root and emerges as an adult between mid-June and mid-September, with peak emergence occurring in early August (Story *et al.*, 1991).

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Females begin mating on the day of emergence and usually begin ovipositing on the second night. Fitzpatrick (1989) reported that each female lays 150–400 eggs. Eggs are laid singly or in groups of 2 to 3 on the leaves and stems of knapweed plants and on adjacent vegetation. The sex ratio of adults is 1:1 throughout most of the season (Story *et al.*, 1991). The moth apparently has only one generation per year in Montana.

Laboratory studies indicate that *A. zoegana* does not immediately reduce fecundity of spotted knapweed and may stimulate compensatory plant growth; however, root-mining tends to reduce the survival rate of immature plants (Müller, 1989b). Competition with a grass species (*Festuca pratensis* Huds.) reduced plant biomass, seed production, and survival of knapweed rosettes (Müller-Schärer, 1991). Knapweed density, grass competition, and soil fertility all appear to interact with the impact of *A. zoegana* on knapweed fitness in the laboratory (Müller-Schärer, 1991). Thus, it may be difficult to quantify the impact of *A. zoegana* on spotted knapweed in the field.

We report on the interaction of the moth and grass competition on spotted knapweed growth characteristics and population density at one location where the moth was well established in 1992.

MATERIALS AND METHODS

Research Area

The study was conducted at two 40 by 40 m sites, approximately 140 m apart, located in a 8-ha, level field on the Teller Wildlife Refuge near Corvallis, Montana (46° 19′ N latitude, 114° 09′ W longitude, elevation 1057 m). The field was an abandoned pasture dominated by spotted knapweed (59% vegetation cover), grass species (35%), and forbs (6%). The knapweed was uniformly distributed, while the grass was clumped. Estimated knapweed density was about 1242 ± 154 (SE) established plants (i.e., not seedlings)/m^2. Primary grass species at the two sites were *Poa compressa* L., *Agropyron repens* (L.) Beauv., and *Festuca* spp.; other grass species included *Poa pratensis* L., *Bromus inermis* Leys., and *Bromus tectorum* L. Soils at the sites are classified as Chamokane gravelly loamy sand. The average annual temperature is 6.8°C, average annual precipitation is 28.6 cm, and average frost-free period is 111 days. Climatological data for the study period are shown in Fig. 1.

Experimental Design

A total of 788 and 2080 *A. zoegana* adults were released at one of the two sites (release site) in 1988 and 1989, respectively, while no moths were released at the second site (check site). Preliminary observations in May 1992 indicated that the moth was well established in the release site but occurred in very low numbers at the check site. The use of true control plots (i.e., plots devoid of moths) was not possible due to the lack of appropriate exclusionary strategies. The use of insecticides was not allowed in the study area, and cages have been found to be ineffective. The moths oviposit on the outside of the cages or on nearby vegetation, and first instar larvae are able to enter the cages and infest the knapweed plants. Thus, the study measured the relationship of the moth and the plant over time in a natural situation as the moth population expanded from the release site.

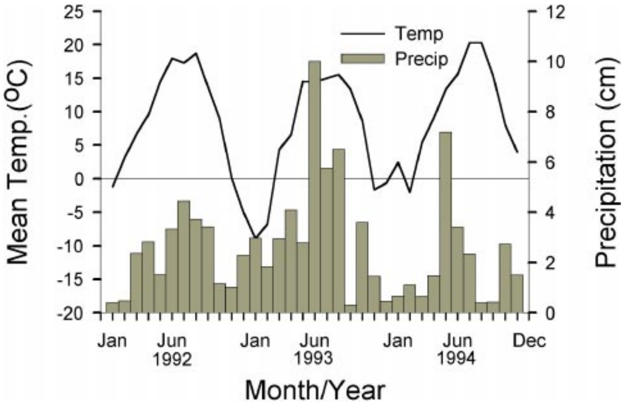

FIG. 1. Temperature and precipitation at Corvallis, Montana during 1992–1994.

In May 1992, eight plots (50 by 50 cm) containing an approximate 90:10 knapweed-to-grass cover ratio (low grass density plots) and eight plots with an approximate 50:50 ratio (high grass density plots) were established at each of the two sites (i.e., 32 total plots) to determine if grass competition affected the moth population or complemented the moth's effect on knapweed. Actual mean percentage of knapweed cover in the low grass density plots was 88.9% ± 1.0 (SE) (range 94–83%), compared to 46.8% ± 1.7 (range 53–41%) in high grass density plots. Foliar cover was determined with the aid of a 50 by 50 cm botanical pin-frame (Levy and Madden, 1933) modified for visual sightings. The pin-frame allowed for 64 observations per plot.

In early June 1992, all of the knapweed plants in four plots in each grass-cover category at the two sites were dug up and taken to the laboratory for analysis. Data were collected on the number of larvae per knapweed plant, above-ground biomass (dry weight) of bolted plants and rosettes, root diameter (measured 2 cm below the root crown), and number of seedlings (i.e., plants with roots less than 0.7 mm in diameter). Notes were kept on visual damage to the roots caused by feeding of the *A. zoegana* larvae.

The procedures on harvested plots were repeated at the end of the growing season (early October 1992) in the remaining four plots in each grass-cover category at the two sites. In addition to the types of data collected in June, data were collected on plant height (height of tallest stem), plant age (determined by counting root xylem rings [Boggs and Story, 1987]), number of stems per plant, number of capitula per plant, and number of bolted plants and rosettes/m^2. Data on plant characteristics (i.e., height, etc.) were only collected from mature knapweed plants.

Following the establishment of eight new plots in May 1993 and four new plots in May 1994, the same types of data collected in 1992 were repeated on four of the plots in each grass-cover category per site in June 1993, October 1993, and June 1994, except that, in June 1993 and 1994, data were also collected on plant age and number of bolted plants and rosettes/m^2.

Comparisons of larval infestation rates on bolted knapweed plants versus rosettes were made only on autumn-collected plants; comparisons were not made on spring-collected plants because of the inability to determine whether a mature plant in the spring had been a rosette or bolted plant during the previous year when oviposition occurred.

Statistical Analysis

Three- to five-way analysis of variance (ANOVA) was performed on most data. Proportion of plants infested was transformed by arcsine of the square root (Y). Plant density data were transformed by square root (Y), and number of larvae and plant morphological data were transformed by $ln(Y)$, or by $ln(Y + 1)$ for variables containing zero values. Plant age and height data did not require transformation. The χ^2 test of independence was used to compare the infestation rate of rosettes and bolted plants. Analysis of covariance (ANCOVA) was used to determine whether larval numbers were affected by knapweed stage (rosettes vs bolted plants) after adjusting for root diameter. Least squares means (LS means) were calculated for plant morphological data because inclusion of all the plants in each plot resulted in unequal numbers of observations for the ANOVA cells.

RESULTS AND DISCUSSION

A. zoegana Infestation (Percentage)

By the first sample date (June 1992), *A. zoegana* had already spread to the check site, 140 m from the release site (Fig. 2). However, infestation levels (i.e., percentage infested plants) per plot were initially much higher at the release site (22.0 ± 2.2% [SE]) than at the check site (5.9 ± 2.3%) ($F_{(1,12)} = 23.7$; $P < 0.001$). This difference in infestation persisted until the June 1994 sample date, when the rate at the check site rose to (22.3 ± 3.4%), a level similar to that at the release site (31.8 ± 4.9%) on the same date. Infestation levels did not significantly increase across years at the release site (linear regression; $F_{(1,22)} = 2.69$; $P = 0.12$), but it did increase at the check site ($F_{(1,22)} = 15.4$; $P = 0.0007$). Three-way analysis of variance (ANOVA) indicated that both *A. zoegana* density (as a plot classification; $F_{(1,36)} = 41.3$; $P < 0.001$) and year ($F_{(2,36)} = 11.3$; $P < 0.001$) had significant effects on the *A. zoegana* infestation rate, while grass density (as a plot classification) and the two-way and three-way interactions were not significant. Therefore, the effects of *A. zoegana* infestation can be studied by comparing the two sites during 1992 and 1993 and by observing temporal changes at the check site.

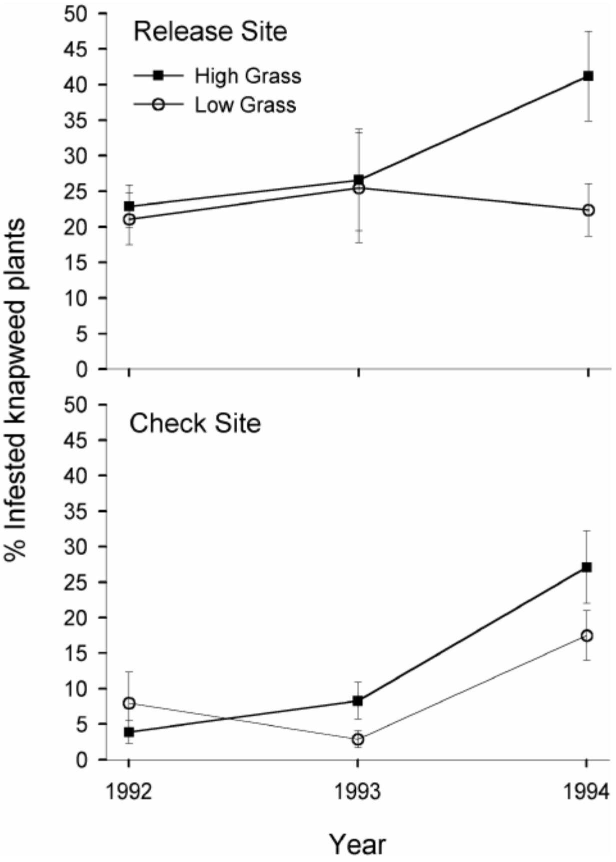

FIG. 2. Percentage of knapweed plants infested by *A. zoegana* in spring samples at the release and check sites in plots with high and low grass density (mean ± SE).

A. zoegana Larval Numbers per Knapweed Plant

The mean number of larvae per knapweed plant (including uninfested plants, but excluding seedlings) was higher at the release site (0.37 ± 0.02 [SE]) than at the check site (0.07 ± 0.01) during the period June 1992 to October 1993 (ANOVA; $F_{(1,7338)} = 293.4$; $P = 0.0001$; Fig. 3). The *A. zoegana* numbers in the release site (i.e., 37 larvae per 100 plants) were compa-

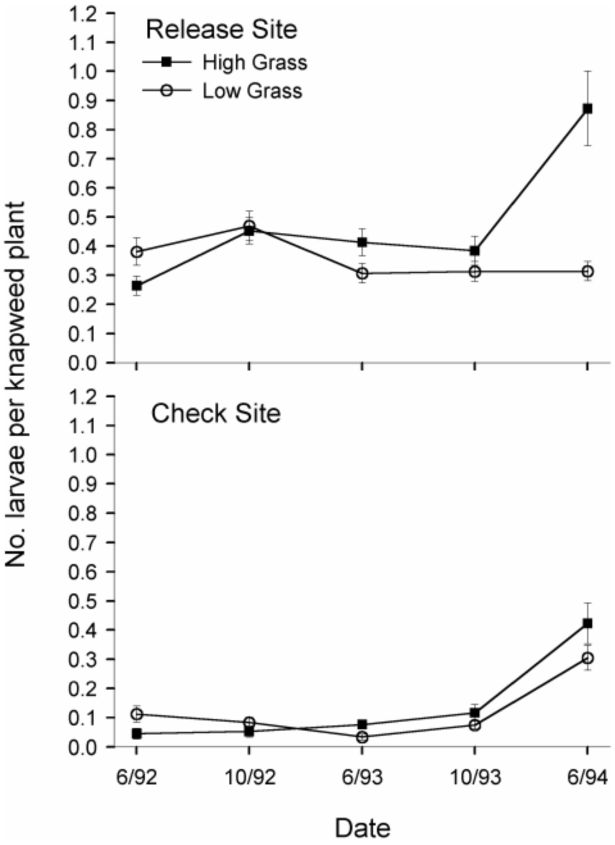

FIG. 3. Number of *A. zoegana* larvae per knapweed plant at the release and check sites in plots with high and low grass density (mean ± SE).

rable to those in Europe, where Müller *et al.* (1988) reported average densities of up to 23.6 larvae per 100 knapweed plants. Larval numbers increased in 1994 at all treatment combinations except at low grass density plots at the release site, a pattern similar to the percentage of plants infested (Fig. 2). Larval numbers in the high grass density plots rose to 0.87 ± 0.13 per plant at the release site and 0.42 ± 0.07 at the check site. Why larval numbers did not increase substantially in the low grass plots at the release site is not clear, as these plots had the highest densities of bolted and rosette plants (see below) in October 1993 and June 1994. Three-way analysis of variance (ANOVA) indicated that *A. zoegana* density (as a plot classification; $F_{(1,7338)} = 293.4$; $P < 0.0001$), grass density ($F_{(1,7338)} = 15.0$; $P < 0.0001$), and sample date ($F_{(4,7338)} = 30.0$; $P < 0.0001$) had significant effects on larval numbers per knapweed plant. All two-way and three-way interactions were significant.

A. zoegana Larval Numbers per Infested Knapweed Plant

Multiple *A. zoegana* larvae were found in many infested spotted knapweed roots (Fig. 4). The observed frequency distribution suggests that there was no tendency for the moth to avoid plants already attacked. The aggregation of larvae was not significantly different from that expected due to random attack (predicted by a simple binomial model assuming the attack rate $P = 1 -$ [number of uninfested plants sampled]/[total number of plants sampled]) (two-sided Kolmogorov Goodness-of-Fit test; $T = 0.028$; $N = 17$ levels of infestation; 4324 plants; $\alpha = 0.05$; Conover, 1980). Although

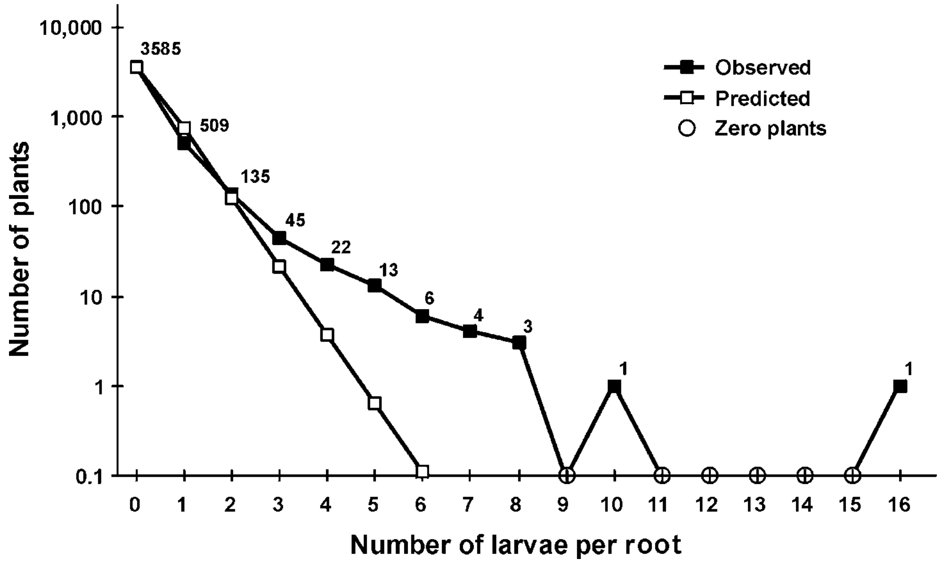

FIG. 4. Frequency distribution of the number of *A. zoegana* larvae per root in infested spotted knapweed plants (numbers next to observed points represent the sample size).

the lines were not significantly different, the consistent divergence of the curves suggests that there is a tendency for nonrandom multiple infestation, but there were insufficient data to confirm this hypothesis.

Three-way ANOVA indicated that *A. zoegana* density (as a main effect; $F_{(1,729)} = 9.00$; $P < 0.003$) and year ($F_{(2,729)} = 5.96$; $P < 0.003$) had a significant effect on larval numbers per infested plant, but grass density (as a main effect) had no effect (Fig. 5). The number of *A. zoegana* larvae per infested plant (sampled in the spring) was higher at the release site (1.65 ± 0.06 (SE) than at the check site (1.43 ± 0.08). The number of larvae per infested plant at both sites was higher in 1994 (1.72 ± 0.09) than in 1992 (1.50 ± 0.08) and 1993 (1.53 ± 0.07) (Fisher's protected LSD of main effect; $\alpha = 0.05$).

Plant Class Preference by A. zoegana

A. zoegana larvae infested proportionately more bolted knapweed plants (25.7 and 23.3%) than rosettes (15.6 and 7.6%) in autumn 1992 and 1993, respectively (Table 1; χ^2 tests of independence, $P < 0.001$). Overall, 60% of the plants containing *A. zoegana* larvae were bolted plants, even though bolted plants ($n = 1243$) comprised only 41% of the total knapweed plants sampled. However, larval attack was not different in bolted plants and rosettes when root diameters were similar (ANCOVA; $F_{(1,3006)} = 0.36$; $P = 0.55$; Fig. 6), suggesting that root diameter, rather than plant stage,

TABLE 1

Proportion of Spotted Knapweed Plants Infested by *Agapeta zoegana* at Two Sites

	Oct. 1992		Oct. 1993	
	Bolted	Rosette	Bolted	Rosette
Infested (%)	25.7	15.6	23.3	7.6
Uninfested (%)	74.3	84.4	76.7	92.4
Total number	467	835	776	965
Plant stage (χ^2)[a]		19.8		85.7
P		<0.001		<0.001
Year (χ^2)[b]			0.9	28.7
P			>0.1	<0.001

[a] χ^2 tests of independence on infestation of bolted versus rosette plants within each year (1 *df*).

[b] χ^2 tests of independence on infestation during 1992 versus 1993 on bolted and rosette plants (1 *df*).

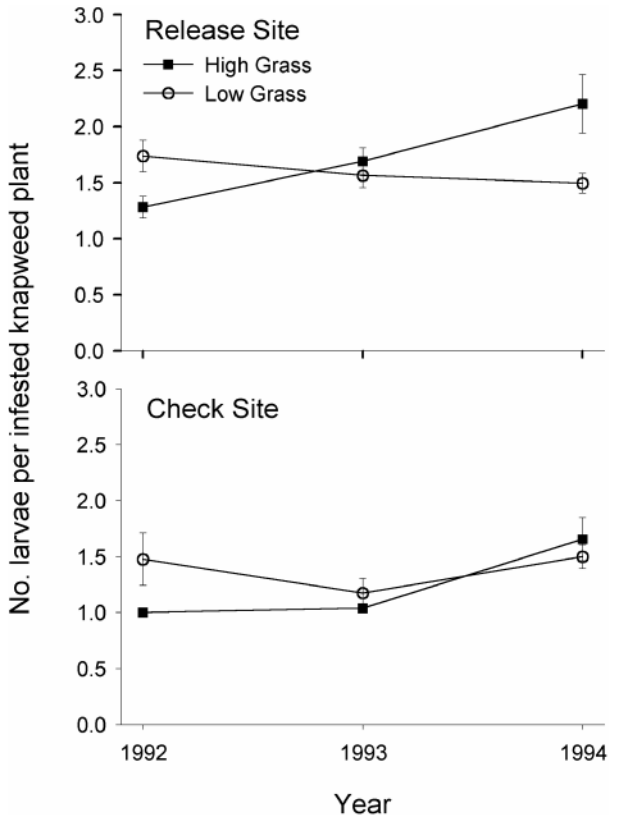

FIG. 5. Number of *A. zoegana* larvae per infested knapweed plant in spring samples at the release and check sites in plots with high and low grass density (mean ± SE).

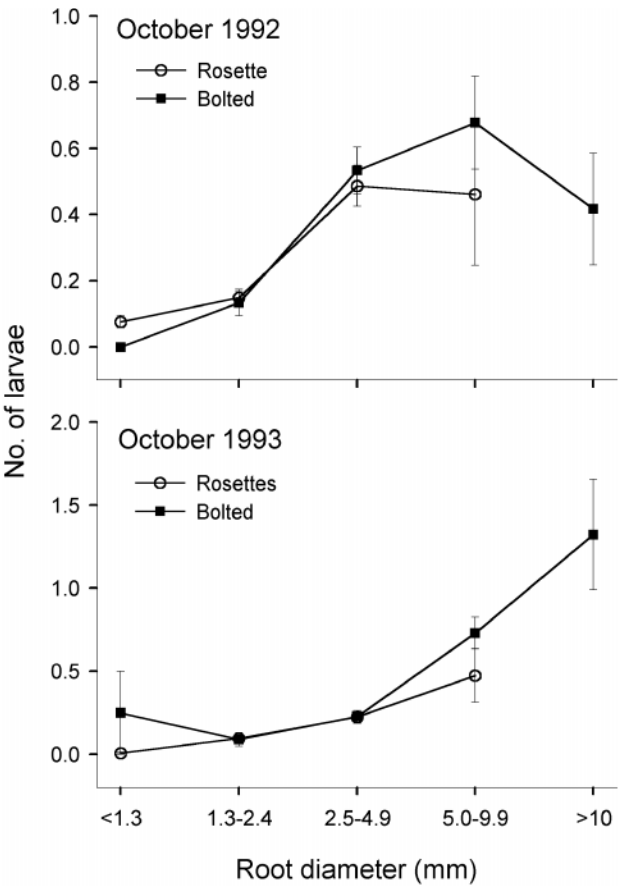

FIG. 6. Number of *A. zoegana* larvae in bolted and rosette plants with respect to root diameter (mean ± SE).

had the greatest influence on the number of larvae found per plant. The number of *A. zoegana* larvae per root was positively correlated with root diameter; for fall: $Y = -0.017$ (± 0.021 [SE]) $+ 0.095$ (± 0.006)X, $R^2 = 0.08l$, $P = 0.001$; for spring: $Y = -0.289$ (± 0.017) $+ 0.203$ (± 0.005)X, $R^2 = 0.30$, $P = 0.001$, where X is root diameter in mm. Mean root diameter of bolted knapweed plants in October 1992 and 1993 was greater (LS mean: 4.47 ± 0.05 [SE] mm) than that of rosettes (1.80 ± 0.04 mm); this difference may explain why, proportionately, more bolted plants were infested than rosettes.

Knapweed Plant Structure

Fall-harvested spotted knapweed plants newly infested with *A. zoegana* tended to have larger root diameters, were older (based on number of root xylem rings), had more above-ground biomass, were taller, had more stems per bolted plant, and had more capitula per bolted plant than uninfested plants (Table 2). In short, *A. zoegana* was strongly associated with large, mature (usually bolted) knapweed plants having large roots. The predominance of larvae on large plants was in contrast to the report by Müller *et al.* (1988), who suggested that *A. zoegana* attack in Europe is generally directed at rosettes. The latter result may be due to a difference in plants; Müller *et al.* (1988) reported that there are distinct differences in the biology of the European *C. maculosa,* which is diploid, and the North American species, which is tetraploid.

Because knapweed plants bolt before oviposition occurs, *A. zoegana's* association with larger, older knapweed plants was probably a result of the insect's oviposition or larval attack behavior rather than a plant response to the moth infestation. *A. zoegana* oviposition on knapweed tends to occur largely on stems and foliage (Müller *et al.*, 1988); thus, large plants may either be more attractive to the moth or, because of their greater foliage, may simply receive more eggs due to moths randomly ovipositing on leaves. In addition, as mentioned above, newly hatched larvae may be attracted to knapweed plants with large roots.

TABLE 2

Comparison of Knapweed Plants Infested and Uninfested with *A. zoegana*[a]

Knapweed characteristics	Uninfested	Infested	Percentage change
Height (cm)	52.86 ± 0.74a	56.37 ± 1.20b	6.6
Stems/plant	1.51 ± 0.04a	1.73 ± 0.09b	14.6
Biomass (g)	0.76 ± 0.03a	1.61 ± 0.07b	111.8
Capitula/plant	8.35 ± 0.60a	11.72 ± 0.89b	40.3
Root diameter (mm)	2.44 ± 0.20a	4.65 ± 0.08b	91.7
Age (years)	2.79 ± 0.03a	3.44 ± 0.06b	22.4

[a] Mean ± SE. Means in the same row followed by the same letter are not significantly different at the $P < 0.05$ level as main effect in ANOVA model.

Evaluation of all plants sampled at the release and check sites showed that knapweed height, above-ground biomass, and number of stems and capitula per bolted plant were significantly lower at the release site than at the check site in October 1992 and 1993 samples (Table 3). Because the moth population was higher at the release site and had been present for a longer time than at the check site, we suggest that the moth may be causing the reduction in knapweed growth and reproduction. Roots of bolted plants often contained *A. zoegana* larval mines from previous years. This observation and the fact that the moth is associated with large, mature plants suggest that the effect of the moth on these plants is probably cumulative; multiple years of attack are probably required before the large plants succumb. Overall, these field results are particularly encouraging because Steinger and Müller (1992) reported that *A. zoegana* failed to reduce stem or root mass in controlled greenhouse studies in Switzerland, and Müller-Schärer (1991) suggested that low levels of herbivory could stimulate compensatory plant growth. Knapweed root diameter and age were higher in the high grass density plots, which suggests that intraspecific competition may be more important than interspecific competition.

Callaway *et al.* (1999) reported that *A. zoegana* infestation had no effect on above-ground biomass of spotted knapweed after 2 years in caged garden plots containing Idaho fescue (*Festuca idahoensis* Elmer) as a grass competitor. While their results appear to be different from those that we observed, it is difficult to compare the two studies. Callaway *et al.* (1999) provided no information on the size of the knapweed plants, percentage infested plants, number of larvae per plant, or number of female moths initially introduced per cage. Thus, if their knapweed plants were relatively large (as suggested by the reported strong effect on Idaho fescue reproduction) and were infested with few larvae, it would not be surprising that they failed to measure any impact. Our study represents a natural field situation with no human manipulation and with realistic *A. zoegana* densities.

Grass density (as a main effect) was not related to number of knapweed stems, but knapweed plants in plots with high grass density were shorter, had less above-ground biomass, and had fewer capitula, compared to plots with low grass density (Table 3). These results were in agreement with those of Müller-Schärer (1991), who reported that competition with grass in controlled greenhouse studies in Switzerland reduced growth, biomass accumulation, and fecundity of spotted knapweed. As mentioned earlier, knapweed plants in high grass density plots had larger root diameters and were older than plants in low grass density plots.

TABLE 3

Comparison of Knapweed Growth Characteristics in Plots with Contrasting *A. zoegana* and Grass Competition Levels During October 1992 and 1993[a]

		A. zoegana sites			Grass plots		
N	Knapweed characteristics	Release site	Check	Percentage reduction	High grass	Low grass	Percentage reduction
1159	Height (cm)	47.84 ± 0.83a	58.73 ± 0.93b	18.5	49.79 ± 0.98a	56.78 ± 0.76b	12.3
1258	Stems/plant	1.32 ± 0.05a	1.86 ± 0.06b	29.0	1.62 ± 0.06a	1.57 ± 0.05a	−3.2
3007	Biomass (g)	0.91 ± 0.09a	1.61 ± 0.10b	43.5	1.12 ± 0.10a	1.41 ± 0.08b	20.6
1251	Capitula/plant	6.69 ± 0.67a	11.70 ± 0.78b	42.8	8.20 ± 0.81a	10.20 ± 0.63b	19.6
3036	Root diameter (mm)	2.95 ± 0.06a	2.87 ± 0.06b	−2.8	3.01 ± 0.07a	2.81 ± 0.05b	−7.1
1728	Age (years)	3.07 ± 0.04a	2.77 ± 0.05b	−10.8	3.14 ± 0.05a	2.70 ± 0.4b	−16.3

[a] Mean ± SE. Means in the same row within a treatment followed by the same letter are not significantly different at the $P < 0.05$ level as main effect in ANOVA model.

These results may simply be a reflection of the competitiveness of established spotted knapweed plants. Grass competition is probably much more effective against knapweed seedlings than mature plants (see below). The possible role played by soil quality or presence of mycorrhizae was not investigated in this study (e.g., Jacobs and Sheley, 1997; Harris and Clapperton, 1997).

Knapweed Density

The overall density of bolted knapweed plants was lower (35%) in the high grass density plots than in the low grass density plots (33.9 ± 3.6 [SE] vs 52.3 ± 4.6 plants/0.25 m^2; $F_{(1,36)} = 15.5$; $P = 0.0004$; Fig. 7). This difference was most evident at the check site during 1992 and early 1993, before *A. zoegana* numbers increased. In late 1993 and 1994, the density of bolted plants at the check site decreased to levels comparable to those at the release site, possibly due to the increased *A. zoegana* population and its effect on the number of bolted plants at the low grass density plots. The density of bolted plants at the check site (where *A. zoegana* was rare) was 51% lower in high grass density plots (28.7 ± 4.9 plants/0.25 m^2) than in low grass density plots (58.1 ± 7.3; $F_{(1,18)} = 17.1$; $P = 0.0006$). The absence of a grass-density effect at the release site (where *A. zoegana* was abundant; $F_{(1,18)} = 1.48$; $P = 0.24$) is consistent with the hypothesis that the moth causes a reduction in the density of bolted plants (from about 58.1/0.25 m^2, the overall average for low grass density plots at the check site) similar to the reduction caused by high grass density alone (at the check site).

The *A. zoegana* larval population increased substantially at the check site in June 1994 to levels similar to that at the release site (Fig. 3). Because the June 1994 population represents insects that started attacking the plants in August 1993, we excluded both the October 1993 and the June 1994 observations from the analysis to determine the possible effect of high versus low *A. zoegana* populations on spotted knapweed densities. Density of bolted knapweed plants was 39% lower at the release site (37.8 ± 4.5 plants/0.25 m^2) than at the check site (62.2 ± 9.4) in the low grass density plots ($F_{(1,13)} = 6.82$; $P = 0.02$). However, there was no effect at the high grass density plots at the release site and at the check site. Although bolted knapweed densities continued to decrease in 1993 and 1994 at the low grass

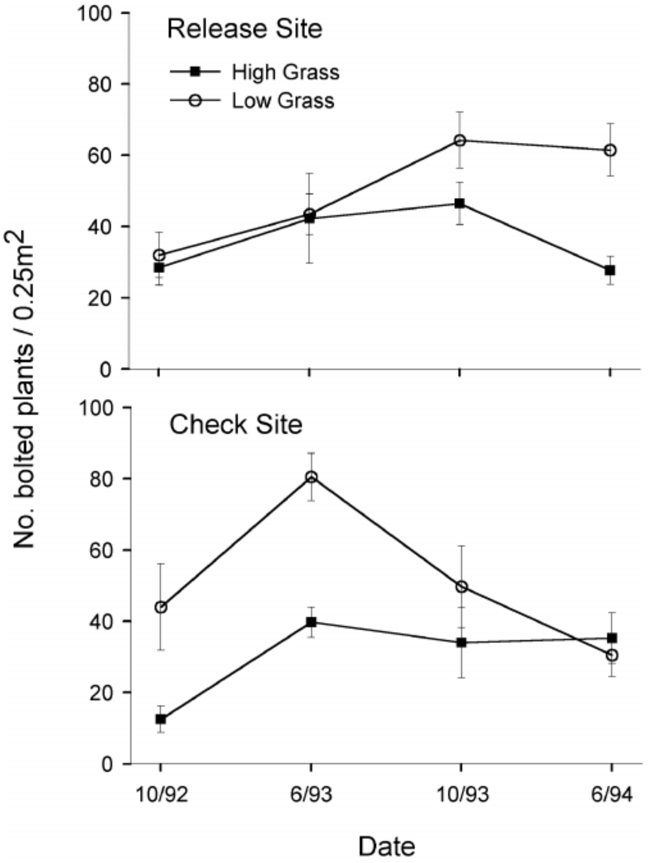

FIG. 7. Density of bolted spotted knapweed plants at the release and check sites in plots with high and low grass density (mean ± SE).

density plots at the check site, there was not a similar decrease at the release site, where the *A. zoegana* infestation continued to remain high. While there is some indication that *A. zoegana* may be reducing the density of bolted knapweed plants in this habitat, especially at low grass densities, the results are not definite and more studies are needed to resolve this question. The fact that bolted knapweed plant densities at the high *A. zoegana*–high grass density plots were not lower than at the low *A. zoegana*–high grass density plots suggests that the detrimental effects of the moth and grass at this site are substitutive rather than additive.

There were fewer rosettes (38%) in the high grass density plots than in low grass density plots (46.9 ± 6.2 [SE] vs 75.8 ± 10.5 plants/0.25 m^2; $F_{(1,28)} = 5.71$; $P = 0.02$; Fig. 8). *A. zoegana*, as a main effect, did not significantly affect rosette density ($F_{(1,28)} = 1.95$; $P = 0.17$); the October 1993 and June 1994 data were excluded from analysis as discussed above. The grass species were presumably competing with knapweed rosettes, but the presence of *A. zoegana* did not further reduce knapweed rosette populations at plots with either high or low grass density.

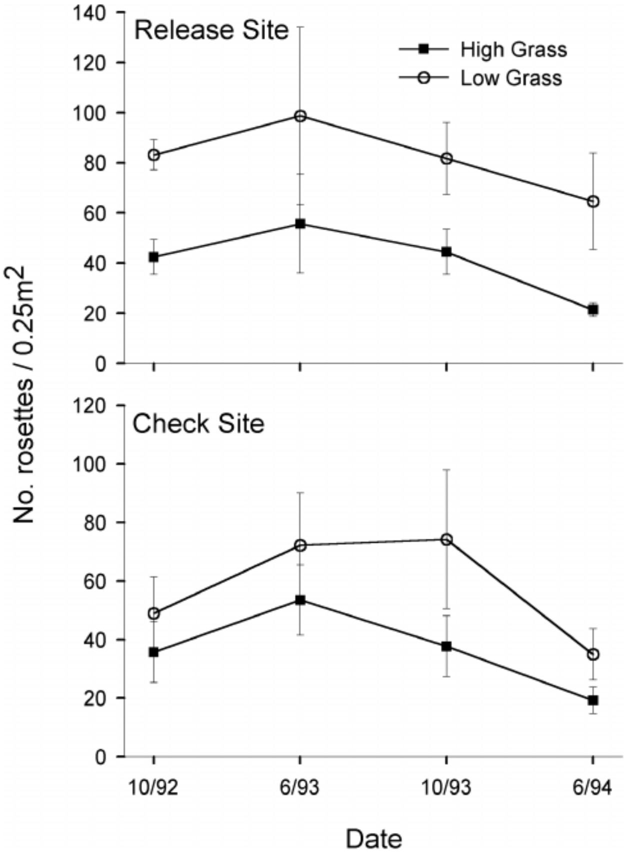

FIG. 8. Density of spotted knapweed rosettes at the release and check sites in plots with high and low grass density (mean ± SE).

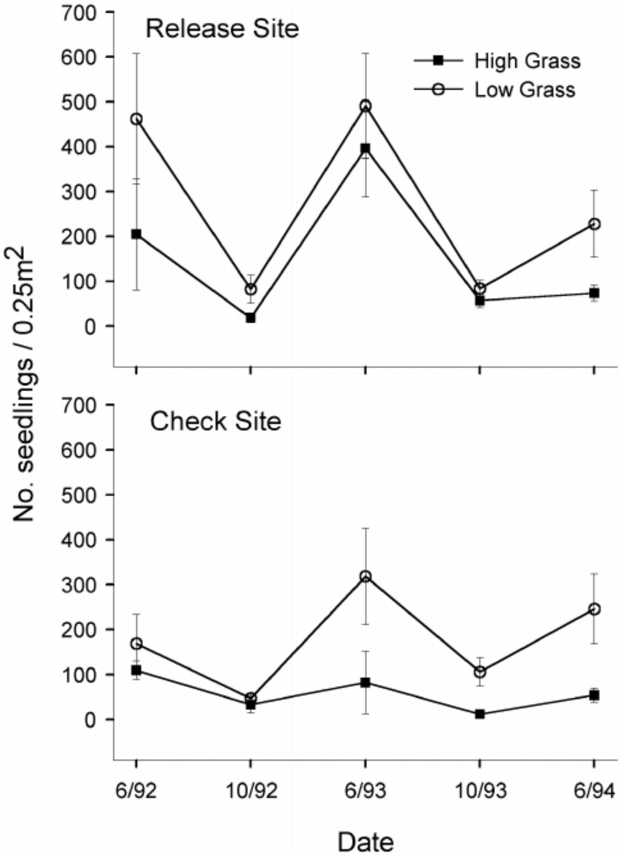

FIG. 9. Density of spotted knapweed seedlings at the release and check sites in plots with high and low grass density (mean ± SE).

Overall, the density of knapweed seedlings was higher (65%) at the release site (210.3 ± 35.7 [SE] plants/0.25 m^2) than at the check site (126.7 ± 23.1 plants/0.25 m^2; $F_{(1,54)} = 7.9$; $P = 0.007$) and higher (100%) at the low grass density plots (225.2 ± 33.3 plants/0.25 m^2) than at the high grass density plots (112.4 ± 26.5 plants/ 0.25 m^2; $F_{(1,54)} = 22.7$; $P = 0.0001$) (Fig. 9). Seedling density was significantly higher in spring (244.5 ± 32.2) than in autumn (59.2 ± 8.7) ($F_{(1,63)} = 46.1$; $P = 0.0001$). The density of seedlings during spring was higher at the release site (309.8 ± 49.7 plants/0.25 m^2) than at the check site (169.8 ± 33.4 plants/0.25 m^2; $F_{(1,43)} = 4.65$; $P = 0.04$), but there was no difference in seedling density between sites in the autumn when seedling densities were low (60.9 ± 11.3 vs 57.0 ± 14.1). There was no difference in seedling density at the release and check sites in spring 1994, when the moth infestation levels at the two sites were similar. The higher seedling density in spring of 1992 and 1993 at the release site was possibly due to *A. zoegana*-caused stress to mature knapweed plants, which may prevent these older plants from monopolizing moisture and soil nutrients, thus permitting greater survival of seedlings. Grass competition presumably explains the lower density of seedlings at the high grass density plots compared to the low grass density plots, which occurred regardless of the presence of *A. zoegana*.

Root Damage

Feeding by the larvae caused noticeable destruction to knapweed roots. Heavily attacked roots were often truncated due to necrosis of the root below the area of larval feeding. These truncated roots often had an abnormal proliferation of small lateral roots. In June 1994, 14% of the plants sampled in the release site had tap roots less than 5 cm in length; average root length of healthy spotted knapweed plants exceeds 30 cm (unpublished data). Live larvae were occasionally found in the roots of dead plants, suggesting that the larvae contributed to the demise of the plants.

CONCLUSION

A. zoegana was well established at the release site and spread 140 m to the check site during the course of this study. The moth appeared to reduce the size or number of many morphological features of established knapweed plants, and it may have reduced bolted plant density in plots with low grass density, but it may have caused an increase in knapweed seedlings in the spring. Grass competition also appeared to reduce many growth features and may have reduced the number of knapweed rosettes, bolted plants, and seedlings.

Due to experimental limitations, these results are clearly preliminary, but the reductions in knapweed biomass, height, number of stems, and number of capitula, etc. in the release sites suggest that *A. zoegana* has potential as a biocontrol agent in the intermountain region of Montana. Portions of this study should be further explored in future studies, particularly under different environmental conditions and with more site replication and/or control of *A. zoegana* numbers. Understanding the interaction of biological control agents such as *A. zoegana* and interspecific plant competition will be important in the attempt to reduce spotted knapweed's competitive edge over native and preferred vegetation.

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REFERENCES

Boggs, K. W., and Story, J. M. 1987. The population age structure of spotted knapweed (*Centaurea maculosa*) in Montana. *Weed Sci.* **35,** 194–198.

Callaway, R. M., DeLuca, T. H., and Belliveau, W. M. 1999. Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa. Ecology* **80,** 1196–1201.

Conover, W. J. 1980. "Practical Nonparametric Statistics," 2nd ed. Wiley, New York.

Davis, E. S., Fay, P. K., Chicoine, T. K., and Lacey, C. A. 1993. Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. *Weed Sci.* **41,** 57–61.

Fitzpatrick, S. M. 1989. A potential collection method for *Agapeta zoegana* (Lepidoptera: Cochylidae), a knapweed-root-feeding moth. *J. Entomol. Soc. Br. Columbia* **86,** 55–62.

Groh, H. 1944. "Canadian Weed Survey: 2nd Annual Report." Canada Department of Agriculture.

Harris, P., and Clapperton, M. J. 1997. An exploratory study on the influence of vesicular–arbuscular mycorrhizal fungi on the success of weed biological control with insects. *Biocontrol Sci. Technol.* **7,** 793–201.

Jacobs, J. S., and Sheley, R. L. 1997. Relationships among Idaho fescue defoliation, soil water, and spotted knapweed emergence and growth. *J. Range Manage.* **50,** 258–262.

Lacey, C. 1989. Knapweed management: A decade of change. *In:* "Proceedings of the 1989 Knapweed Symposium" (P. Fay and J. Lacey, Eds.), pp. 1–6. Montana State Univ., Bozeman.

Levy, E. B., and Madden, E. A. 1933. The point method of pasture analysis. *New Zealand J. Agric.* **46,** 267–275.

Müller, H. 1989a. Structural analysis of the phytophagous insect guilds associated with the roots of *Centaurea maculosa* Lam., *C. diffusa* Lam., and *C. vallesiaca* Jordan in Europe: Field observations. *Oecologia* **78,** 41–52.

Müller, H. 1989b. Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.: Cochylidae). *Weed Res.* **29,** 103–111.

Müller, H., Schroeder, D., and Gassmann, A. 1988. *Agapeta zoegana* (L.) (Lepidoptera: Cochylidae), a suitable prospect for biological control of spotted and diffuse knapweed, *Centaurea maculosa* Monnet de la Marck and *Centaurea diffusa* Monnet de la Marck (Compositae) in North America. *Can. Entomol.* **120,** 109–124.

Müller-Schärer, H. 1991. The impact of root herbivory as a function of plant density and competition: Survival, growth, and fecundity of *Centaurea maculosa* in field plots. *J. Appl. Ecol.* **28,** 759–776.

Sheley, R. L., Jacobs, J. S., and Carpinelli, M. F. 1998. Distribution, biology, and management of diffuse (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). *Weed Technol.* **12,** 353–362.

Steinger, T., and Müller, H. 1992. Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of intraspecific plant competition and soil nitrogen availability. *Oecologia* **91,** 141–149.

Story, J. M., Boggs, K. W., and Good, W. R. 1991. First report of the establishment of *Agapeta zoegana* L. (Lepidoptera: Cochylidae) on spotted knapweed, *Centaurea maculosa* Lamarck, in the United States. *Can. Entomol.* **123,** 411–412.

Watson, A. K., and Renney, A. J. 1974. The biology of Canadian weeds: *Centaurea diffusa* and *C. maculosa. Can. J. Plant Sci.* **54,** 687–701.