

# Indirect effect of early-season infestations of *Trichosiocalus horridus* on *Rhinocyllus conicus* (Coleoptera: Curculionidae)

Lindsey R. Milbrath<sup>1</sup> and James R. Nechols\*

Department of Entomology, Kansas State University, Manhattan, KS 66506-4004, USA

Received 23 April 2003; accepted 29 September 2003

## Abstract

Field experiments were conducted to determine whether early vegetative feeding by *Trichosiocalus horridus* alters musk thistle (*Carduus nutans*) as a resource for the later-arriving *Rhinocyllus conicus*. Results showed that thistles with high larval densities of *T. horridus* (66 per plant) produced more and shorter flower stems, significantly fewer flower heads, and delayed flowering by 1 week when compared to uninfested thistles. Also, colonization and oviposition on flower heads by adult *R. conicus* were as much as three and five times lower, respectively, on thistles heavily infested with *T. horridus* than on uninfested thistles. As a result, 63% fewer *R. conicus* adults developed from *T. horridus*-infested thistles. Musk thistles that were infested with lower densities of *T. horridus* larvae (<20 per plant) also produced multiple stems that were usually shorter than uninfested thistles. However, no differences were observed in flower head production or in the behavioral responses of *R. conicus* to *T. horridus*-infested and uninfested plants. In a greenhouse experiment, the mortality rate of *R. conicus* was higher on musk thistles that were heavily infested by *T. horridus*, implying a change in musk thistle quality. However, this effect was apparent only at low *R. conicus* larval densities. *T. horridus* can indirectly and negatively affect *R. conicus* through a variety of mechanisms, but only when densities of *T. horridus* are high. This finding may have important implications for the continued redistribution of both weevils, which were imported for the classical biological control of musk thistle, a noxious weed in pastures and rangeland.

© 2003 Elsevier Inc. All rights reserved.

**Keywords:** Indirect competition; Classical biological control; Weeds; *Trichosiocalus horridus*; *Rhinocyllus conicus*; Musk thistle; *Carduus nutans*

## 1. Introduction

A widely accepted hypothesis in biological control of weeds is that stress from multiple sources is necessary to exert sufficient control of the target plant (Harris, 1981, 1991). This may involve a broad range of stresses, such as herbivory and plant competition (e.g., McEvoy et al., 1993), or consist of multiple biological control agents, provided that the herbivores act in a complementary and compatible manner (e.g., James et al., 1992). Insects that feed on different plant tissues and/or occur at different times of the year impose different kinds of stress on weeds. Importantly, they also avoid direct competition (Harris, 1991). However, indirect interactions be-

tween spatially and temporally isolated herbivores can still occur if one or both of the herbivores alter the host plant that serves as a common resource, i.e., the host plant mediates the interaction (see review by Wootton, 1994). These indirect interactions may result in outcomes that are positive (facilitation—Damman, 1989; Gange and Brown, 1989; Masters et al., 2001; Pilon, 1992; Strauss, 1991; Tschardtke, 1989; Williams and Myers, 1984), negative (competition—English-Loeb et al., 1993; Faeth, 1986; Harrison and Karban, 1986; Hoffman and Moran, 1992; Moran and Whitman, 1990; Petersen and Sandström, 2001; Strauss, 1991; Tindall and Stout, 2001; Williams and Myers, 1984), or neutral (Bounfour and Tanigoshi, 2001). To date, very few published studies have focused on spatially and temporally separated herbivores (but see Hoffman and Moran, 1992; Pilon, 1992; Strauss, 1991). Indirect interactions between herbivores may have important consequences for the host plant and, ultimately, for the level of

\* Corresponding author. Fax: 1-785-532-6232.

E-mail address: [jnechols@oznet.ksu.edu](mailto:jnechols@oznet.ksu.edu) (J.R. Nechols).

<sup>1</sup> Present address: USDA-ARS, Grassland, Soil and Water Research Laboratory, Temple, TX 76502, USA.

biological control attained in the case of weeds. Therefore, investigations of this kind are germane to the overall evaluation of biological control programs for weeds.

Musk, or nodding, thistle (*Carduus nutans* L. species group) was introduced from its native Eurasian/North African range into North America over 150 years ago (Stuckey and Forsyth, 1971). It became a noxious weed in pasture and rangeland habitats, especially in overgrazed situations (Dunn, 1976; Fick and Peterson, 1995). Musk thistle grows primarily as a biennial or winter annual and is propagated only through seed (McCarty and Scifres, 1969). Individual plants can produce 10,000 or more seeds, although only 35% may be viable (McCarty, 1982). Dormant seed remains viable for up to 15 years (Burnside et al., 1981).

A classical biological control program was initiated for musk thistle in the United States and Canada in the 1960s. These efforts resulted in the establishment of two weevils: *Trichosiromus horridus* (Panzer) and *Rhinocyllus conicus* Froelich (Kok, 2001). *T. horridus* adults have an extended oviposition period that lasts from late fall through spring. Under warm conditions during this period, eggs are laid and larvae feed within the vegetative crowns. Destruction of the apical meristem results in the growth of secondary buds, such that infested thistles often produce multiple flower stems instead of a single stem. These stems are generally shorter, bear fewer flower heads, and may flower later compared to uninfested thistles (Cartwright and Kok, 1985; Woodburn, 1997). In contrast to *T. horridus*, *R. conicus* larvae infest flower heads (capitula) that are produced in the spring and early summer (Surlles and Kok, 1976). Feeding on the receptacle, and later callus, tissue results in the abortion of developing ovules, and young achenes also may be directly fed upon, limiting the production of viable seed (Kok, 2001; McCarty and Lamp, 1982; Shorthouse and Lalonde, 1984). Both weevils have been redistributed within the United States and natural dispersal has occurred, with *R. conicus* occupying a larger range (Andres and Rees, 1995). Northeast Kansas is one region where both weevils are established and have an overlap in their distributions (Hilbert and Brooks, 2000).

Current activities to continue the redistribution of these weevils is predicated on the assumption that the two species act in a complementary manner because they have somewhat different phenologies, and because they feed on different parts of the plant (Kok, 2001; Nechols, 2000). However, no experiments have been done to determine the relative role of each weevil in reducing viable seed production, or to assess whether early vegetative feeding by *T. horridus* causes indirect adverse effects on *R. conicus* by altering plant quality and/or quantity. In addition, concerns regarding the ecological safety of these weevils due to feeding by *R. conicus* on nontarget, native thistle hosts (Louda et al., 1997; Turner and Herr,

1996; Turner et al., 1987), and the potential for *T. horridus* to do the same (McAvoy et al., 1987), underscores the need to examine the interaction between the two weevils. Therefore, our objectives were to determine if the seasonal synchrony between ovipositing *R. conicus* and musk thistle flower head availability is altered (phenological effect); the acceptability of musk thistle flower heads for oviposition by *R. conicus* is altered (behavioral effect); the quantity of plant tissue available to developing *R. conicus* larvae is altered (resource limitation effect); and the quality of musk thistle heads for developing *R. conicus* larvae is altered (physiological effect).

## 2. Materials and methods

### 2.1. Insect colonies

Adult *T. horridus* were field-collected annually between 1998 and 2001 in the fall from musk thistle, and overwintered at 5 or 10 °C and a photoperiod of 10:14 h (L:D) in an environmental growth chamber. Adults were held in ventilated, plexiglass boxes containing bouquets of musk thistle leaves in cotton-plugged, water-filled shell vials. To obtain *T. horridus* eggs, 15–30 weevils (1:2 male:female) were placed in ventilated, 2-liter paper containers with thistle bouquets and held at a thermoperiod of 20:10 °C (10:14 h) and a photoperiod of 10:14 h (L:D). Leaves were dissected every 2–7 days for eggs that were then placed in petri dishes lined with moist filter paper layered over wet cotton. Eggs were stored at 2–3 °C in darkness for 1–4 months (Kok and McAvoy, 1983). Subsequently, the eggs were transferred to 25 °C and allowed to develop until the embryonic head capsule had darkened (24–48 h pre-hatch). Batches of 10–13 eggs were aspirated with water into individual Pasteur pipettes, the pipette tip was wrapped with Parafilm (American National Can, Chicago, IL), and the pipettes were refrigerated for approximately 1 week until eggs were needed to artificially infest musk thistle plants in field and greenhouse experiments. Preliminary tests showed that neither refrigeration nor storage in water affected the hatching rate.

The adult *R. conicus* used in the greenhouse experiment were reared from mature flower heads of musk thistle that were field-collected in July. Adults were held in an environmental growth chamber in ventilated, plexiglass boxes that contained moist paper towels. From July to September, the photophase and temperature were gradually shortened/decreased from 14:10 to 10:14 h (L:D) and from 25 to 5 °C, respectively. Adults were held under these latter conditions for an additional 100 days to terminate dormancy.

Voucher specimens of *T. horridus* and *R. conicus* were deposited in the Kansas State University Museum of

Entomological and Prairie Arthropod Research under Lot No. 140.

## 2.2. Field experiments

Between 1998 and 2001, field experiments were conducted to determine whether *T. horridus* altered the availability, acceptability, and/or quantity of musk thistle flowers for *R. conicus*.

### 2.2.1. 1998–1999, Keats, KS

In fall 1998, a field site was selected near Keats, Riley, KS (39° 14.92'N, 96° 43.50'W, 380 m), that had a history of musk thistle infestation and populations of *R. conicus* and *T. horridus*. In October, a 0.25-ha plot was fenced off to exclude cattle, and frame cages 0.8 × 0.8 × 0.2 m (length × width × height) were placed over naturally occurring musk thistle rosettes (one plant per cage) to isolate them from field populations of *T. horridus*. Cages had a window screen top and were large enough to allow for the expansion of rosettes. The experimental plant density of the natural stand was 0.04 musk thistle/m<sup>2</sup>. The initial diameter of thistle rosettes ranged from 15 to 30 cm. The phenological development of caged thistles through bolting was similar to nonexperimental thistles growing near the field plot. To minimize interspecific plant competition on musk thistles, grasses and other vegetation within the cages and in the surrounding areas were clipped or mowed to maintain an average height of 10 cm.

The experiment had a 3 × 3 factorial treatment structure in a Latin square design. Because of the varying terrain at the field site, treatments were grouped by location within the site (first blocking factor). Rosette diameters measured in December (when fall growth had ceased and prior to freezing of the ground) served as a second blocking factor because the growth response of thistles to *T. horridus* damage had previously been shown to interact with thistle size (Sieburth et al., 1983). There were nine replications (blocks), each having three levels of *T. horridus* infestation (0, 25, or 50 eggs per plant) and three levels of *R. conicus* infestation (excluded with a closed bag, natural infestation with an open or sham bag, and natural infestation with no bag) for a total of 81 experimental units. Exclusion of *R. conicus* was included in this and the subsequent field experiment because these experiments also were designed to examine a separate objective involving the weevils' impact on musk thistle flower head and seed production.

Musk thistle plants designated for *T. horridus* treatments were infested in late March 1999 by inserting the tips of two or four Pasteur pipettes (depending on infestation level) that contained 12–13 mature (within 1 day of hatching) eggs into the main vein of thistle leaves. Eggs were collected as described above. This procedure

was used because naturally deposited eggs and larvae cannot be counted in the field without destroying the plant. To account for possible effects on thistle plants from mechanical injury, three empty pipette tips were inserted into each control thistle. Thistle plants were not available to establish an unwounded control. Six additional caged thistles (nonexperimental) also were infested with 25 or 50 eggs. These thistles were dissected for larvae after 1 month to estimate the actual infestation levels of the experimental thistles. In addition, to obtain estimates of natural infestations of *T. horridus*, 10 musk thistle plants were dug from the ground in late April/early May at each of three locations in Northeast Kansas. The plants were dissected and the number of *T. horridus* larvae was counted.

When bolting began in late April 1999, frame cages were opened and a 0.25% solution of permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, Eliminator, Gro Tec, Madison, GA] was applied to the grass immediately outside the cages to exclude late-ovipositing *T. horridus*. Frame cages were removed by mid-May when *R. conicus* began ovipositing. All of the flower buds (capitula) that developed on each plant were individually covered by (1) closed or (2) open (sham) nylon mesh bags (23 × 23 mesh), or (3) left unbagged. To exclude *R. conicus*, bags were tied off at the top and bottom (on the rachis just below the bud) with twist ties. To test for unintended effects resulting from closed bags, sham bags were tied to the rachis only, leaving the top and most of the bottom open. To provide additional access for *R. conicus* adults, a 3 × 13-cm square was cut in the side of the bag. Flowers in all treatments were hand-pollinated with a small paintbrush to ensure uniform pollination. Bags remained in place until 1 week before seed heads were harvested. All seed heads were then covered with a closed bag to prevent seed loss due to birds and storms.

To determine seasonal activity of *R. conicus*, the total number of adults (males and females) on each plant, and the number of eggs per flower head were counted at least once a week. Adults were counted during the mid-morning hours when they were active. To assess *R. conicus* survival within heads, seed heads were clipped and placed in 0.5-liter containers 10–14 days after full bloom (just prior to seed dispersal) or when they had aborted. Seed heads were held in the laboratory at ~25°C and a photoperiod of 16:8 h (L:D). The number of adult *R. conicus* that emerged or were dissected out of the head was counted.

To quantify the impact of *T. horridus* on thistle development, each plant was examined about once a week beginning in the spring and continuing until plant death. On each sampling date, the number of flower stems, stem heights, and the number of heads per stem and per plant were recorded. To estimate the amount of food available to developing *R. conicus* larvae, head

receptacle diameters were measured to the nearest 0.1 mm at full bloom or abortion of the head. A relationship between receptacle diameter and biomass was then developed using flower heads from greenhouse-grown thistles. Heads were measured at full bloom, the florets and bracts were removed and the remaining receptacle tissue was dried at 50 °C for 2 days and then weighed to the nearest mg. Simple linear regression was performed using the  $\ln(\text{dry mass})$  on diameter (SAS Institute, 1999). The linear relationship between receptacle diameter and receptacle dry mass for flower heads at full bloom was described by the equation:  $\ln(\text{dry mass}) = 2.93 + 0.10 \times \text{diameter}$  ( $R^2 = 0.93$ ,  $F_{1,34} = 460.17$ ,  $P < 0.0001$ ).

#### 2.2.2. 1999–2000, Westmoreland, KS

The experiment was established at a 0.18-ha field site near Westmoreland, Pottawatomie, KS (39° 26.09'N, 96° 20.43'W, 409 m). The experimental design was a  $4 \times 2$  factorial treatment structure in a randomized complete block design. There were 10 blocks (replications), each of which had four levels of *T. horridus* infestation (artificial infestation in the fall, spring, both fall and spring, or excluded) and two *R. conicus* infestation levels (natural infestation or excluded) for a total of 80 experimental units.

Musk thistle rosettes were transplanted for increased uniformity of plot conditions and plant size. In late September, 2 weeks prior to transplanting, a 0.64-m<sup>2</sup> area was cleared of vegetation for each experimental plant by applying glyphosate (*N*-(phosphonomethyl)glycine, Roundup, Monsanto, St. Louis, MO) at 4.7 liter/ha. This zone was established to reduce effects of competing grasses on thistles without disrupting the soil profile. In addition, surrounding grass and other vegetation were mowed at regular intervals to maintain a minimum height of 10 cm. Frame cages were placed in the ground 1 week before transplanting and a 0.25% permethrin solution was applied inside the cages to eliminate any *T. horridus* adults. Greenhouse-germinated musk thistle seedlings, 10–15 cm in diameter, were transplanted on 8 October. Plants were spaced 2 m apart, resulting in a density of 0.25 musk thistle/m<sup>2</sup>.

Musk thistle plants designated for *T. horridus* infestation were inoculated with five mature eggs from a laboratory colony on 7 December or 15 mature eggs on 13 March, or both. The number of *T. horridus* eggs used for each treatment was based on the quantity of eggs that could be harvested from the laboratory colony. However, the majority of eggs are oviposited in the spring. Eggs were placed directly in the crown of the thistles and covered by moist cotton to prevent desiccation before hatching. Six additional caged thistles were infested with *T. horridus* at experimental rates in the fall or spring and then dissected in April to provide estimates of actual densities of *T. horridus* in the ex-

perimental plants. Four uninfested thistles were left in frame cages until April and then dissected. Results confirmed that the cages were effective in preventing the invasion of natural populations of *T. horridus*.

*Rhinocyllus conicus* was excluded from plants by applying sprays of 0.25% permethrin to the developing flower heads at 2-week intervals. Sprays were discontinued on heads that were about to flower to prevent interfering with pollinators, and no spraying was done after *R. conicus* adults were no longer present in the field. Applications of *Bacillus thuringiensis* Berliner subsp. *kurstaki* (DiPel ES, Valent BioSciences, Walnut Creek, CA) at 1.8 liter/ha were applied weekly to flower heads in all treatments to minimize infestations of the sunflower moth, *Homoeosoma electellum* (Hulst). Applications began on an individual head just prior to bloom and continued until head senescence.

Data collection and analyses were similar to the 1998–1999 experiment, except that head receptacle diameters were measured 4 days after full bloom (when the maximum head size was achieved). The linear relationship between receptacle diameter and receptacle dry mass for flower heads at 4 days post-bloom on greenhouse-grown thistles was described by the equation:  $\ln(\text{dry mass}) = 3.03 + 0.11 \times \text{diameter}$  ( $R^2 = 0.94$ ,  $F_{1,46} = 759.32$ ,  $P < 0.0001$ ).

#### 2.2.3. 2000–2001, Keats, KS

A field experiment, using natural infestations of both *T. horridus* and *R. conicus*, was conducted on a fenced, 0.02-ha site located at 39° 14.69'N, 96° 43.66'W (366 m) and approximately 0.5 km from the 1998–1999 experimental site to determine how high densities of *T. horridus* alter musk thistle for *R. conicus*. The experimental design was a one-way treatment structure in a randomized complete block design. There were 20 blocks (replications), each of which had two levels of *T. horridus* infestation (natural infestation or excluded) for a total of 40 experimental units. The field plots were established as described previously except that no frame cages were used. Naturally germinated thistles surrounding the field plots were destroyed in the fall and additional *T. horridus* adults were released into the plot area in March to ensure high infestation levels. Greenhouse-germinated musk thistle seedlings, 10–15 cm in diameter, were transplanted to experimental plots on 28 September, 2000. *T. horridus* was excluded by applying sprays of 0.25% permethrin, and sunflower moth was excluded with applications of DiPel ES at 1.8 liter/ha at 2- or 1-week intervals, respectively. Five additional transplanted thistles (nonexperimental) were dug from the ground in late April and dissected to quantify natural infestation levels by *T. horridus*. Data were collected using procedures described previously.

#### 2.2.4. Statistical analyses

Plants were omitted from analysis if they had died prematurely (e.g., in the rosette stage) from unknown causes, were contaminated by naturally occurring (nonexperimental) infestations of *T. horridus*, or displayed no signs of damage and developed a central, primary flower stem after having been artificially infested with *T. horridus* eggs.

Correlations were obtained between numbers of adult *R. conicus* per plant, plant height, and number of flower heads per plant for different sampling dates using PROC CORR (SAS Institute, 1999). Mortality rates for *R. conicus* were calculated as  $(1 - \text{number of adults produced} / \text{number of eggs oviposited})$ . Data on mortality rates and the percentage of flower heads receiving *R. conicus* eggs were subjected to arcsine square root transformation. Data were analyzed with analysis of variance using PROC MIXED. The 1999 data on adult *R. conicus* production and mortality rates were subjected to analysis of covariance to adjust for the effect of sunflower moth, using the proportion of moth-infested heads as the covariate (PROC MIXED, SAS Institute, 1999). Means were separated, as necessary, using Fisher's protected least significant difference test (LSMEANS, SAS Institute, 1999). For most analyses, data from individual sampling dates were combined to represent three periods of *R. conicus* seasonal activity: (Early) the first 3–4 weeks of activity in which 50% of *R. conicus*' seasonal egg production were oviposited and adult *R. conicus* densities were high; (Mid) the next 1–2 weeks in which an additional 40–45% of the season's eggs were oviposited and *R. conicus* numbers were decreasing; and (Late) the last 1–3 weeks in which the remaining 5–10% of the eggs were oviposited and *R. conicus* adults disappeared.

#### 2.3. Greenhouse experiment

A greenhouse experiment was conducted in 2002 to test whether the quality of musk thistle tissue for developing *R. conicus* larvae was altered by prior infestation by *T. horridus*. Intraspecific competition may affect development and survival among *R. conicus* larvae. Therefore, the experiment incorporated this factor. The experiment had a  $2 \times 2$  factorial treatment structure and was set up in a completely randomized design. There were 10 replications, each of which had two levels of *T. horridus* infestation (artificially infested or not) and two levels of *R. conicus* infestation (low or high density) for a total of 40 experimental units.

Musk thistles were grown from seed in 7.6-liter pots in a greenhouse at 21:15°C and approximate natural daylengths supplemented with artificial light for 2 months. The thistles were then vernalized by transferring them to 8°C and a photoperiod of 9:15 h (L:D) for 54 days (Medd and Lovett, 1978). Following vernali-

zation, thistles were placed in a greenhouse at 15:10°C and a photoperiod (L:D) of 14:10 h. They were fertilized every other week using one-half strength Peters Professional 20-20-20 fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH). Initial rosette diameters of experimental thistles were 32–36 cm. Thistles designated for *T. horridus* damage were infested by placing 30 mature eggs into the crown of each plant (19 eggs followed by 11 eggs 1 week later). Eggs were obtained from a laboratory colony of *T. horridus*. Five nonexperimental thistles also were infested. Dissections 3 weeks later yielded a mean of  $27.4 \pm 1.9$  larvae per plant, which is high for potted thistles (see Sieburth et al., 1983). The temperature was raised to 25:20°C 6 weeks after infesting to allow for severe feeding damage by *T. horridus* larvae to occur without stimulating rapid thistle growth.

Following cold storage, *R. conicus* were transferred to screened cages ( $60 \times 55 \times 90$  cm [length  $\times$  width  $\times$  height]) in the greenhouse, each containing potted musk thistle plants, for feeding, mating, and oogenesis. When the first flower head of an experimental plant was fully exposed, i.e., the bottom of the receptacle was visible, its receptacle diameter was measured. Five to 10 adult *R. conicus* females were enclosed on the head with a mesh bag and left until the desired density of eggs was oviposited (2–3 days). The maximum number of eggs per head for the low-density treatment was adjusted according to the relationship  $y = 3.0\pi r^2$ , where  $y$  = maximum number of eggs and  $r$  = (receptacle diameter + 1.0 cm)/2. Previous field data showed that fully exposed heads gained an additional 1.0 cm in diameter by full bloom. Also, the maximum number of adults produced per cm<sup>2</sup> of receptacle area (measured at full bloom) was around 3.0. This value was chosen to ensure that development or mortality were likely not affected by intraspecific larval competition (e.g., Dowd and Kok, 1981) but that food was not overabundant and that each head received a comparable egg load. The maximum number of eggs was doubled for the high-density treatment.

Heads were bagged 30 days after oviposition to catch early-emerging *R. conicus* adults and harvested 14 days later. Heads were held in 0.5-liter containers at 25:20°C and a photoperiod (L:D) of 14:10 h for 14 days to ensure complete weevil development. Larval entrance holes were counted to confirm that *R. conicus* treatment densities were achieved using the number of entrance holes/ $\pi r^2$ , where  $r$  = receptacle radius measured at full bloom. Adult weevils that emerged or were dissected out of the heads were frozen immediately, then dried at 50°C for 2 days and individually weighed to the nearest 0.1 mg. Differences in body length were extrapolated by measuring the length of the left elytrum to the nearest 0.05 mm using an ocular micrometer at 10 $\times$ . Previous measurements had shown a highly significant linear relationship between elytral length and total body length ( $\text{Body} = 1.426 \times \text{Elytrum}$ ,  $R^2 = 0.99$ ,  $F_{1,24} = 24,603.2$ ,

$P < 0.0001$ ). Each adult was sexed by dissecting it and inspecting the genitalia.

Mortality rates were calculated as  $(1 - \text{number of adults produced} / \text{number of larval entrance holes})$  and arcsine square root-transformed. Data on calculated larval *R. conicus* densities, percent mortality, and adult male and female dry mass and elytral length were analyzed with analysis of variance using PROC MIXED (SAS Institute, 1999). The sex ratio of adults produced was analyzed with the *G* test (Sokal and Rohlf, 1995).

### 3. Results

#### 3.1. Effect of *T. horridus* on musk thistle growth and development

##### 3.1.1. 1998–1999

Dissections of musk thistle plants in three pastures in Northeast Kansas revealed a wide range of larval densities for naturally occurring populations of *T. horridus* as follows (mean  $\pm$  SD):  $17.0 \pm 10.5$ ,  $19.2 \pm 15.5$ , and  $33.8 \pm 27.2$  larvae per plant (range: 0–77;  $n = 10$ ).

Inoculation of experimental musk thistle rosettes at rates of 25 or 50 *T. horridus* eggs per plant resulted in mean spring densities of  $13.3 \pm 8.0$  and  $18.3 \pm 1.5$  larvae per plant, respectively ( $n = 3$ ). The corresponding ranges of larval infestations were 5–21 and 17–20 larvae per plant, respectively.

Experimental thistles that had these rates of *T. horridus* infestation produced more flower stems than uninfested thistles ( $2.0 \pm 0.2$  and  $1.0 \pm 0.2$ , respectively;  $P < 0.001$ ). In addition, flower stems of uninfested thistles were taller than infested thistles from May through June ( $P < 0.001$ ; Fig. 1, Top), the period during which *R. conicus* was active.

##### 3.1.2. 1999–2000

Manipulating the rate and seasonal infestation schedule of *T. horridus* eggs on musk thistle rosettes resulted in spring larval densities of about 2 [ $n = 1$ ] (fall inoculation),  $9.8 \pm 3.3$  [ $n = 5$ ] (spring inoculation), and approximately 12 (fall and spring inoculation data combined) larvae per plant. Thistles infested at these densities produced more flower stems than uninfested thistles ( $2.6 \pm 0.1$  and  $1.0 \pm 0.2$ , respectively;  $P < 0.001$ ). Flower stem heights of *T. horridus*-infested and uninfested thistles were similar during most of the season when *R. conicus* activity was greatest (Fig. 1, Middle).

##### 3.1.3. 2000–2001

Musk thistles exposed to natural populations of *T. horridus* had spring infestation rates of  $66.2 \pm 22.9$  larvae per plant ( $n = 5$ ). Plants infested by *T. horridus* produced  $6.7 \pm 0.5$  flower stems, more than uninfested

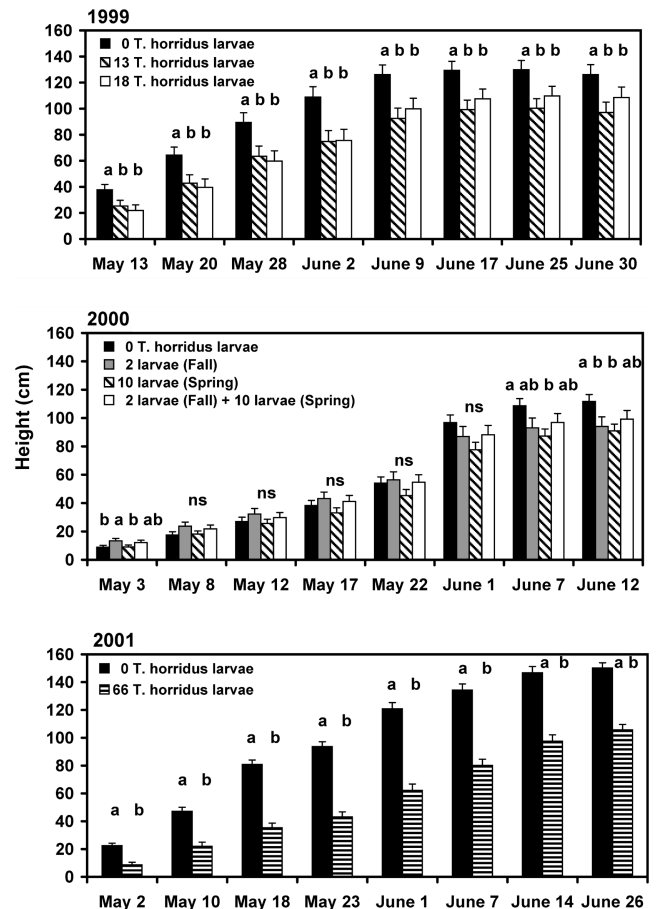


Fig. 1. Heights (Mean  $\pm$  SE) of bolted musk thistle stems in response to different infestations of *Trichosiromus horridus*. (top) 1999, (middle) 2000, and (bottom) 2001. Dates shown reflect the period of seasonal reproductive activity for *Rhinocyllus conicus*. For each date, means denoted by a different letter are significantly different (Fisher's protected LSD,  $P \leq 0.05$ ) (ns—nonsignificant differences).

plants ( $3.9 \pm 0.5$  stems;  $P < 0.001$ ). Uninfested plants typically had a large central stem and additional secondary stems. Uninfested thistles were taller than infested plants throughout the season ( $P < 0.001$ ; Fig. 1, Bottom).

#### 3.2. Synchrony between *R. conicus* adults and seasonal flower production

In all years, adult *R. conicus* appeared on thistle plants during the last week in April, shortly after bolting of musk thistle had commenced and several days prior to the appearance of flower heads. The number of adults increased rapidly and remained at high densities for about 4 weeks before declining over the subsequent 3- to 4-week period. Low levels of adults were observed until the middle or end of June. Oviposition occurred as soon as flower buds were accessible to adult *R. conicus*, usually by the first week of May. Ninety percent of all eggs were oviposited by the first week of June.

### 3.2.1. 1998–1999

There were no significant ( $P > 0.05$ ) differences between *T. horridus*-infested and uninfested thistles in the appearance of the first musk thistle flower heads or the schedule of flower head production from early May to mid-June, the period during which most of the oviposition by *R. conicus* occurs (Fig. 2).

### 3.2.2. 1999–2000

The first appearance of flower heads was similar for all *T. horridus*-infested and uninfested thistles. The rate of flower head production was low for uninfested thistles and spring-infested thistles. Thistles inoculated in both the fall and spring showed a trend for intermediate levels of flower head production. The most flower heads—almost twice the numbers observed on uninfested and spring-inoculated thistles—were produced by plants that were inoculated with *T. horridus* in the fall only. Differences in flower head production among *T. horridus* treatments occurred during the period of peak oviposition by *R. conicus* (Fig. 3).

### 3.2.3. 2000–2001

Thistles that were naturally infested by *T. horridus* produced flower heads approximately 1 week later than uninfested thistles (10 May and 2 May, respectively). Infested thistles also produced fewer flower heads than uninfested thistles throughout the entire period when *R. conicus* was reproductively active ( $P < 0.009$ , Fig. 4).

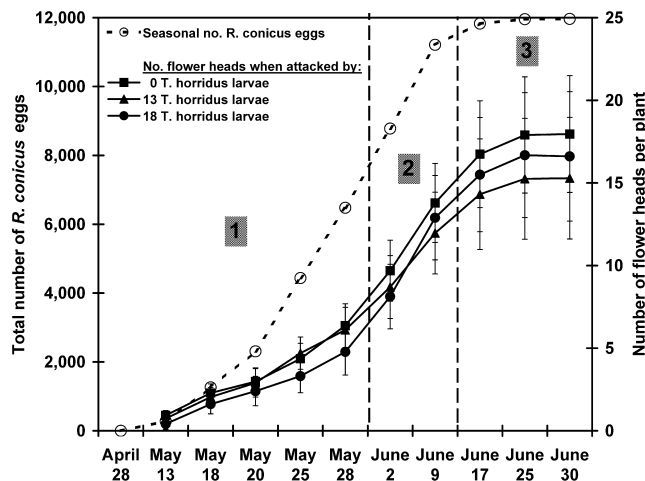


Fig. 2. Relationship between seasonal egg production of *Rhinocyllus conicus* and musk thistle flower head production at different levels of *Trichosirocalus horridus* infestation, 1999. Vertical lines denote when ~50 and 90% of *R. conicus*'s seasonal egg production were oviposited—(1) Early-season heads received an average of 29 *R. conicus* eggs per head, (2) mid-season heads received 7 eggs per head, and (3) late-season heads received 1 egg per head. Means for flower heads within each date were not significantly different ( $P > 0.05$ ).

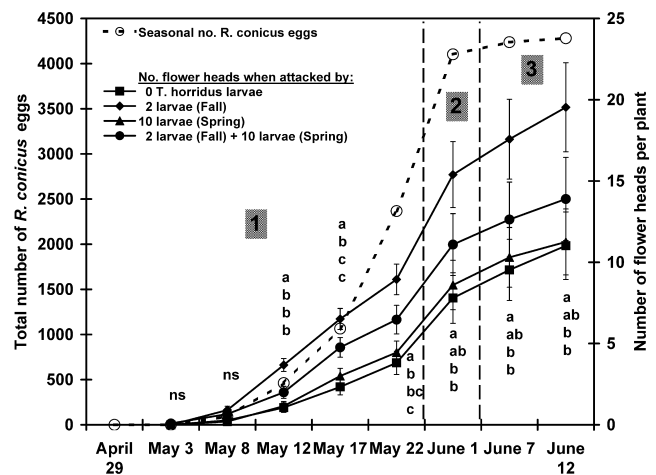


Fig. 3. Relationship between seasonal egg production of *Rhinocyllus conicus* and musk thistle flower head production at different levels of *Trichosirocalus horridus* infestation, 2000. Vertical lines denote when ~50 and 90% of *R. conicus*'s seasonal egg production were oviposited—(1) Early-season heads received an average of 15 *R. conicus* eggs per head, (2) mid-season heads received 2 eggs per head, and (3) late-season heads received 0 eggs per head. Means for flower heads within each date denoted by different letters were significantly different (Fisher's Protected LSD,  $P < 0.05$ ) (ns—nonsignificant differences).

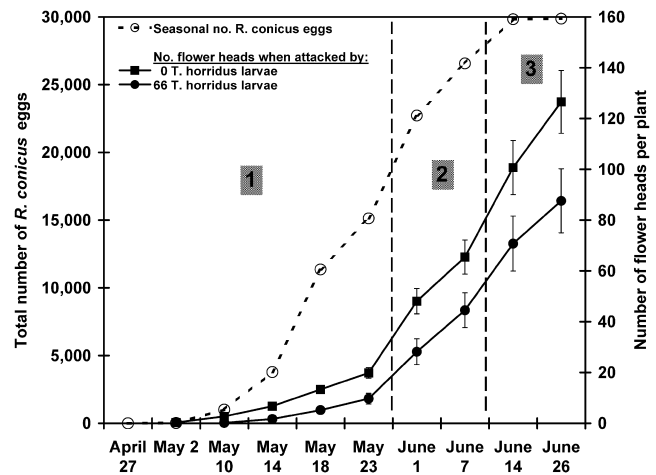


Fig. 4. Relationship between seasonal egg production of *Rhinocyllus conicus* and musk thistle flower head production at different levels of *Trichosirocalus horridus* infestation, 2001. Vertical lines denote when ~50 and 90% of *R. conicus*'s seasonal egg production were oviposited—(1) Early-season heads received an average of 32 *R. conicus* eggs per head, (2) mid-season heads received 6 eggs per head, and (3) late-season heads received 0.3 eggs per head. Means for flower heads within each date were significantly different except for May 2 ( $P < 0.05$ ).

## 3.3. Recruitment and oviposition by *R. conicus* (Table 1)

### 3.3.1. 1998–1999

There were no seasonal differences in adult *R. conicus* densities, or in the number of eggs oviposited per plant, among *T. horridus* treatments. Similarly, the number of *R. conicus* eggs per flower head was not affected by *T.*

*horridus* infestation. The percentage of flower heads receiving eggs, and the number of eggs per head, both declined over the course of the season (Table 1).

### 3.3.2. 1999–2000

The per-plant densities of *R. conicus* adults and eggs were not statistically different among *T. horridus* treatments. However, among thistles infested by *T. horridus* in the fall, there was a trend for more *R. conicus* adults in May, and more eggs through the beginning of June. The number of *R. conicus* eggs per flower head did not differ significantly among treatments.

### 3.3.3. 2000–2001

More *R. conicus* adults colonized uninfested thistles than infested thistles ( $P < 0.008$ ). This pattern persisted until late in the season. In addition, more eggs were deposited on uninfested thistles than on infested thistles. The difference was greatest from the end of April to early June, when a high proportion of eggs

were laid. During the first week of oviposition, when no flower heads were available on *T. horridus*-infested plants, approximately 1000 *R. conicus* eggs (50 eggs per plant) were laid on thistles not infested by *T. horridus*. Mean egg densities on individual flower heads also were greater on uninfested thistles for most of the season.

In all years, there was a strong, positive correlation ( $P < 0.05$ ) between numbers of adult *R. conicus* and flower heads per plant, and between *R. conicus* adults and plant height. Correlations were significant in May but generally were not significant later in the season.

### 3.4. Abundance of larval food (Table 2)

#### 3.4.1. 1998–1999

The number and maximum receptacle diameter of flower heads that formed during the season were similar between *T. horridus*-infested and uninfested thistles. As a result, the quantity of receptacle tissue available to *R.*

Table 1

Seasonal densities (mean  $\pm$  SE) of *Rhinocyllus conicus* adults and eggs and mean percentages of heads with eggs on *Trichosirocalus horridus*-infested and uninfested musk thistles<sup>a</sup>

Dates	Number of <i>T. horridus</i> larvae per plant	Adult <i>R. conicus</i> per plant	New eggs per plant	Eggs per infested head	% Heads with eggs <sup>b</sup>
<i>1999</i>					
28 April–28 May	0	2.6 $\pm$ 0.6 a	156.1 $\pm$ 32.6 a	31.4 $\pm$ 2.7 a	100.0 (99.9–100) a
	13	2.2 $\pm$ 0.6 a	123.6 $\pm$ 34.7 a	23.9 $\pm$ 2.9 a	100.0 (100) a
	18	2.6 $\pm$ 0.6 a	130.1 $\pm$ 35.8 a	30.5 $\pm$ 3.0 a	100.0 (100) a
29 May–9 June	0	0.9 $\pm$ 0.3 a	95.5 $\pm$ 22.4 a	7.9 $\pm$ 1.2 a	99.1 (93.8–99.6) a
	13	0.9 $\pm$ 0.3 a	94.1 $\pm$ 24.1 a	8.1 $\pm$ 1.3 a	98.0 (87.1–99.9) a
	18	1.4 $\pm$ 0.3 a	111.7 $\pm$ 25.0 a	8.3 $\pm$ 1.3 a	92.7 (81.1–99.0) a
10–30 June	0	0.1 $\pm$ 0.1 a	14.3 $\pm$ 5.5 a	1.9 $\pm$ 0.3 a	40.9 (9.3–77.5) a
	13	0.2 $\pm$ 0.1 a	11.0 $\pm$ 6.0 a	1.8 $\pm$ 0.3 a	57.1 (15.9–92.9) a
	18	0.2 $\pm$ 0.1 a	18.6 $\pm$ 6.2 a	2.2 $\pm$ 0.3 a	35.9 (5.0–76.1) a
<i>2000</i>					
29 April–22 May	0	0.9 $\pm$ 0.6 a	33.4 $\pm$ 22.2 a	22.2 $\pm$ 4.1 a	84.3 (54.7–99.4) a
	~2 (F) <sup>c</sup>	3.1 $\pm$ 0.9 a	109.5 $\pm$ 33.3 a	15.0 $\pm$ 5.1 a	99.1 (70.7–100) a
	10 (S) <sup>c</sup>	1.2 $\pm$ 0.6 a	33.0 $\pm$ 21.1 a	16.7 $\pm$ 3.7 a	97.5 (77.9–100) a
	2 + 10 (F + S)	2.2 $\pm$ 0.7 a	82.9 $\pm$ 27.2 a	11.9 $\pm$ 4.3 a	93.4 (62.7–98.1) a
23 May–1 June	0	0.5 $\pm$ 0.2 a	30.8 $\pm$ 10.8 a	6.4 $\pm$ 1.3 a	57.9 (24.4–87.7) a
	~2 (F)	0.5 $\pm$ 0.3 a	82.0 $\pm$ 16.2 a	3.8 $\pm$ 1.7 a	61.8 (17.2–96.5) a
	10 (S)	0.6 $\pm$ 0.2 a	35.5 $\pm$ 10.2 a	6.3 $\pm$ 1.1 a	31.3 (5.4–66.5) a
	2 + 10 (F + S)	0.4 $\pm$ 0.2 a	30.2 $\pm$ 13.2 a	1.8 $\pm$ 1.7 a	39.3 (6.9–78.6) a
2–12 June	0	0.07 $\pm$ 0.02 a	3.8 $\pm$ 1.9 a	—	0.3 (–0.8–0.8) a
	~2 (F)	0 $\pm$ 0.04 a	4.9 $\pm$ 2.8 a	—	0.5 (0.2–3.3) a
	10 (S)	0 $\pm$ 0.02 a	4.8 $\pm$ 1.8 a	—	0 (–0.1–1.6) a
	2 + 10 (F + S)	0 $\pm$ 0.03 a	0.4 $\pm$ 2.3 a	—	0 (–0.6–0.6) a
<i>2001</i>					
27 April–23 May	0	7.0 $\pm$ 0.6 a	635.1 $\pm$ 53.7 a	41.8 $\pm$ 1.7 a	100.0 (99.8–100) a
	66	2.5 $\pm$ 0.6 b	131.8 $\pm$ 55.0 b	22.2 $\pm$ 1.7 b	99.9 (99.6–100) a
24 May–7 June	0	4.6 $\pm$ 0.5 a	378.8 $\pm$ 36.7 a	7.7 $\pm$ 0.4 a	89.5 (83.8–94.0) a
	66	2.4 $\pm$ 0.5 b	205.5 $\pm$ 37.6 b	6.4 $\pm$ 0.4 b	84.4 (77.8–90.0) b
8–26 June	0	0.2 $\pm$ 0.1 a	99.7 $\pm$ 11.9 a	2.6 $\pm$ 0.4 a	11.6 (7.7–16.2) a
	66	0.2 $\pm$ 0.1 a	69.0 $\pm$ 12.2 b	2.0 $\pm$ 0.4 a	12.8 (8.6–17.6) a

<sup>a</sup> Means within columns and for each set of dates followed by different letters were significantly different (Fisher's Protected LSD,  $P \leq 0.05$ ).

<sup>b</sup> Means shown were back-transformed from arcsine square root values. Numbers in parentheses are 95% confidence intervals.

<sup>c</sup> F = fall inoculation, S = spring inoculation.



Table 2

Seasonal production of flower heads and quantity (mean  $\pm$  SE) of musk thistle receptacle tissue available to *Rhinocyllus conicus* larvae as influenced by *Trichosirolcalus horridus* infestation of musk thistles<sup>a</sup>

Dates	Number of <i>T. horridus</i> larvae per plant	New flower heads per plant	Maximum receptacle diameter (mm)	Receptacle mass per plant (mg)
<i>1999</i>				
28 April–28 May	0	6.4 $\pm$ 1.3 a	26.7 $\pm$ 0.9 a	1828 $\pm$ 374 a
	13	6.1 $\pm$ 1.4 a	23.2 $\pm$ 0.9 b	1401 $\pm$ 387 a
	18	4.8 $\pm$ 1.4 a	27.0 $\pm$ 1.0 a	1583 $\pm$ 399 a
29 May–9 June	0	7.4 $\pm$ 1.4 a	17.8 $\pm$ 0.8 a	955 $\pm$ 214 a
	13	5.9 $\pm$ 1.5 a	17.6 $\pm$ 0.8 a	750 $\pm$ 223 a
	18	8.1 $\pm$ 1.5 a	19.4 $\pm$ 0.8 a	1238 $\pm$ 232 a
10–30 June	0	4.2 $\pm$ 1.3 a	15.2 $\pm$ 0.7 a	372 $\pm$ 137 a
	13	3.4 $\pm$ 1.3 a	14.5 $\pm$ 0.9 a	357 $\pm$ 144 a
	18	4.0 $\pm$ 1.4 a	15.8 $\pm$ 0.8 a	399 $\pm$ 150 a
<i>2000</i>				
29 April–22 May	0	3.8 $\pm$ 0.7 c	30.9 $\pm$ 1.2 a	3390 $\pm$ 539 a
	~2 (F) <sup>b</sup>	8.9 $\pm$ 0.9 a	26.8 $\pm$ 1.6 a	4150 $\pm$ 691 a
	10 (S) <sup>b</sup>	4.4 $\pm$ 0.7 bc	28.4 $\pm$ 1.2 a	2414 $\pm$ 528 a
	2 + 10 (F + S)	6.5 $\pm$ 0.9 b	28.3 $\pm$ 1.5 a	3625 $\pm$ 651 a
23 May–1 June	0	4.0 $\pm$ 1.0 a	23.2 $\pm$ 1.2 a	1291 $\pm$ 305 a
	~2 (F)	6.5 $\pm$ 1.3 a	17.4 $\pm$ 1.7 b	1202 $\pm$ 398 a
	10 (S)	4.1 $\pm$ 1.0 a	20.1 $\pm$ 1.2 ab	1121 $\pm$ 298 a
	2 + 10 (F + S)	4.7 $\pm$ 1.2 a	17.4 $\pm$ 1.5 b	1017 $\pm$ 373 a
2–12 June	0	3.2 $\pm$ 0.7 a	12.5 $\pm$ 1.1 a	397 $\pm$ 102 a
	~2 (F)	4.2 $\pm$ 1.0 a	12.5 $\pm$ 1.7 a	366 $\pm$ 139 a
	10 (S)	2.7 $\pm$ 0.7 a	12.4 $\pm$ 1.3 a	325 $\pm$ 99 a
	2 + 10 (F + S)	2.9 $\pm$ 0.9 a	11.8 $\pm$ 1.5 a	266 $\pm$ 128 a
<i>2001</i>				
27 April–23 May	0	19.9 $\pm$ 2.1 a	31.2 $\pm$ 0.8 a	12,968 $\pm$ 1362 a
	66	9.7 $\pm$ 2.1 b	29.2 $\pm$ 0.8 b	5704 $\pm$ 1362 b
24 May–7 June	0	45.6 $\pm$ 4.9 a	25.1 $\pm$ 0.6 a	17,195 $\pm$ 1765 a
	66	34.8 $\pm$ 5.0 b	25.6 $\pm$ 0.6 a	12,907 $\pm$ 1765 b
8–26 June	0	61.1 $\pm$ 6.5 a	20.0 $\pm$ 0.7 a	12,627 $\pm$ 1421 a
	66	42.8 $\pm$ 6.6 b	20.3 $\pm$ 0.7 a	9582 $\pm$ 1421 a

<sup>a</sup> Means within columns and for each set of dates followed by different letters were significantly different (Fisher's Protected LSD,  $P \leq 0.05$ ).

<sup>b</sup> F = fall inoculation, S = spring inoculation.

*conicus* larvae was not different ( $P > 0.05$ ) between the two groups of experimental plants (Table 2).

### 3.4.2. 1999–2000

Thistles that were infested by very low densities of *T. horridus* in the fall produced more flower heads in early spring than uninfested thistles ( $P < 0.001$ ). Fall-infested plants also produced significantly more flower heads than plants that were infested either in the fall and spring or in spring only. In contrast, uninfested thistles produced larger flower heads during mid-season (late May to early June) than thistles that were infested in the fall only or both fall and spring. There were no differences in head size among *T. horridus*-infested thistles. The estimated quantity of receptacle tissue available to *R. conicus* larvae did not differ throughout the season among *T. horridus* treatments.

### 3.4.3. 2000–2001

In contrast to 1999 and 2000, thistles infested at higher *T. horridus* densities produced fewer flower heads

over the entire season than uninfested thistles ( $P < 0.04$ ). Early-season flower heads of infested plants were slightly smaller than uninfested plants. Thistles infested by *T. horridus* produced less receptacle biomass during the first two-thirds of the season than uninfested thistles ( $P < 0.03$ ).

## 3.5. Production of *R. conicus* adults (Table 3)

### 3.5.1. 1998–1999

Neither the number of *R. conicus* adults produced nor the rate of mortality (egg-adult) differed between *T. horridus*-infested and uninfested thistles (Table 3).

### 3.5.2. 1999–2000

There were no differences in the number of *R. conicus* adults produced or mortality rates among *T. horridus* treatments.

### 3.5.3. 2000–2001

Approximately 2.5 times fewer adult *R. conicus* developed from *T. horridus*-infested thistles than from

Table 3

Mean  $\pm$  SE numbers of *Rhinocyllus conicus* adults developing, and mean percentages of preadult mortality, from *Trichosirocalus horridus*-infested and uninfested musk thistles<sup>a</sup>

Year	Dates	Number of <i>T. horridus</i> larvae per plant	New <i>R. conicus</i> adults per plant	% Mortality (egg to adult) <sup>b</sup>
1999	28 April–28 May	0	68.9 $\pm$ 14.5 a	65.8 (59.1–72.2) a
		13	43.8 $\pm$ 15.8 a	77.2 (70.4–83.3) a
		18	62.7 $\pm$ 16.9 a	68.2 (60.3–75.6) a
	29 May–9 June	0	11.2 $\pm$ 3.3 a	85.5 (78.0–91.7) a
		13	7.8 $\pm$ 3.5 a	82.8 (73.9–90.1) a
		18	13.1 $\pm$ 3.5 a	86.9 (78.5–93.5) a
	10–30 June	0	0.8 $\pm$ 0.6 a	85.0 (60.4–98.7) a
		13	2.1 $\pm$ 0.8 a	90.1 (63.0–100.0) a
		18	1.2 $\pm$ 0.8 a	79.5 (48.0–98.2) a
2000	29 April–22 May	0	24.9 $\pm$ 11.2 a	62.7 (41.9–81.3) a
		~2 (F) <sup>c</sup>	76.0 $\pm$ 16.9 a	58.2 (36.1–82.1) a
		10 (S) <sup>c</sup>	35.9 $\pm$ 11.2 a	34.7 (18.2–53.2) a
		2 + 10 (F + S)	41.0 $\pm$ 13.8 a	61.0 (38.8–81.0) a
	23 May–1 June	0	4.4 $\pm$ 2.1 a	59.9 (23.1–91.1) a
		~2 (F)	3.2 $\pm$ 3.1 a	66.4 (18.6–98.7) a
		10 (S)	6.9 $\pm$ 2.0 a	71.3 (37.8–94.7) a
		2 + 10 (F + S)	0.5 $\pm$ 2.6 a	76.5 (28.1–100.0) a
	2–12 June	0	0 $\pm$ 0.2 a	—
		~2 (F)	0 $\pm$ 0.3 a	—
		10 (S)	0.4 $\pm$ 0.2 a	—
		2 + 10 (F + S)	0.2 $\pm$ 0.3 a	—
	27 April–23 May	0	135.6 $\pm$ 13.7 a	83.0 (80.1–85.7) a
		66	40.1 $\pm$ 13.7 b	80.9 (77.9–83.8) a
		0	46.2 $\pm$ 5.1 a	84.5 (81.7–87.2) a
	24 May–7 June	66	25.6 $\pm$ 5.1 b	84.6 (81.7–87.3) a
		0	3.4 $\pm$ 0.9 a	83.7 (63.5–96.7) a
	8–26 June	0	3.1 $\pm$ 0.9 a	71.0 (48.3–89.2) a
		66		

<sup>a</sup> Means within columns and for each set of dates followed by different letters were significantly different (Fisher's Protected LSD,  $P \leq 0.05$ ).

<sup>b</sup> Means shown were back-transformed from arcsine square root values. Numbers in parentheses are 95% confidence intervals.

<sup>c</sup> F = fall inoculation, S = spring inoculation.

uninfested thistles. However, rates of *R. conicus* mortality did not differ significantly between *T. horridus*-infested and uninfested plants.

### 3.6. *Trichosirocalus horridus*-induced changes in thistle quality and effect of *R. conicus* larval densities (Table 4)

The percentage mortality of *R. conicus* (larvae-adults) was higher from thistles that were infested by *T. horridus* in the greenhouse than from uninfested thistles ( $P < 0.002$ ), but only in thistle heads that had lower larval densities of *R. conicus* (Table 4). Mean adult body size and weight for *R. conicus* were lower at the higher intra-specific larval density than at the lower one ( $P < 0.001$ ). The sex ratio of *R. conicus* did not differ from a 1:1 ratio in any of the treatments ( $G$  test,  $P > 0.05$ ).

## 4. Discussion

Experimental evidence indicates that *T. horridus* modifies musk thistle in a variety of ways that indirectly affect *R. conicus*. Furthermore, the data suggest that the

effect on *R. conicus* may range from negative to neutral, depending on the level and timing of infestation by *T. horridus*. Specific negative effects included asynchrony between *R. conicus* and musk thistle, lower colonization of thistles by *R. conicus* adults and fewer eggs laid, and reduced quantity and quality of floral resources available for developing *R. conicus* larvae. To our knowledge, only Strauss (1991) has similarly demonstrated indirect effects (negative to positive) among the chrysomelid beetle *Blepharida rhois* (Forster), the cerambycid beetle *Oberea ocellata* Haldeman, and whitetail deer (*Odocoileus virginianus* [Boddaert]), whose feeding on the shared host, smooth sumac (*Rhus glabra* L.), are separated both in space and time.

### 4.1. Synchrony between *R. conicus* adults and seasonal flower production

In all years, the appearance of adult *R. conicus* coincided with musk thistle bolting (stem elongation). Colonization occurred several days before flower heads were available, and oviposition began as soon as flower buds were accessible to adults. The short preoviposition

Table 4

Effect of *Trichosiocalus horridus* infestation and *Rhinocyllus conicus* larval density on *R. conicus* mortality and adult body size<sup>a</sup>

<i>T. horridus</i>	<i>R. conicus</i> larvae/cm <sup>2</sup> <sup>b</sup>	% Mortality (larvae to adult)	Dry mass <sup>c</sup> (mg)	Left elytral length <sup>c</sup> (mm)
Absent	3.1 ± 0.3	3.7 (0.4–10.0)	9.2 ± 0.2	4.39 ± 0.04
Absent	6.3 ± 0.3	19.8 (11.1–30.4)	7.6 ± 0.2	4.13 ± 0.04
Present	3.4 ± 0.3	23.1 (13.7–34.1)	9.3 ± 0.2	4.46 ± 0.04
Present	6.9 ± 0.3	14.9 (7.3–24.5)	7.3 ± 0.2	4.07 ± 0.04
Factor	<i>P</i> values			
<i>T. horridus</i>	0.143	0.055	0.894	0.891
<i>R. conicus</i>	<0.001	0.190	<0.001	<0.001
<i>T. horridus</i> × <i>R. conicus</i>	0.650	0.004	0.345	0.099

<sup>a</sup> Percentages of mortality are back-transformed from arcsine square root values. Numbers in parentheses are 95% confidence intervals. For all other data, means are followed by standard errors.

<sup>b</sup> Based on the area of the flower head at full bloom.

<sup>c</sup> Neither sex nor any other interaction terms were significant ( $P > 0.05$ ).

period that we observed is consistent with the one reported by Smith and Kok (1987) in Virginia.

In 2001, when *T. horridus* larval densities were high, development of the first musk thistle heads was delayed considerably compared to plants that were kept free of *T. horridus* or that experienced low *T. horridus* infestations (1999 and 2000). This delay prevented *R. conicus* from ovipositing on *T. horridus*-infested thistles for about 1 week. In Australia, Woodburn (1997) reported a delay in musk thistle flower head production of 2 weeks due to *T. horridus* infestation. This more extended delay was associated with larval densities of *T. horridus* that were nearly twice as high as those we observed in Kansas. Based on our field ovipositional data, we estimated that 3–4% of the seasonal eggs laid by *R. conicus* are deposited during the first week that flowers are available. Because *R. conicus* appears to have a fixed oviposition period, this delay represents a direct loss in reproduction that cannot be replaced. The shift in plant phenology in 2001 also resulted in considerably fewer (50%) available flower heads for *R. conicus* females during the period of peak oviposition. Continuous, heavy oviposition by *T. horridus*, with subsequent waves of larval feeding on developing meristems, prevented secondary and tertiary flower stems that had initiated growth from elongating further. Eventually some flower stems were able to develop normally. Nevertheless, the repeated destruction of stems would be expected to delay the production of flower heads. In contrast, low densities of *T. horridus* larvae did not interfere with the bolting of secondary flower stems.

In addition to *T. horridus*, other environmental factors may influence secondary stem development, and, ultimately, flower head production of musk thistles. For example, Woodburn (1997) attributed secondary stem development in musk thistle, in part, to increased fertility. In our studies, larger, more vigorous, plants, and an early hard frost may have contributed to early secondary stem development in thistles in 2001 because both *T. horridus*-infested and uninfested plants had

more stems than in 1999 and 2000. However, because the interaction between *T. horridus* and other factors is unknown, it is unclear whether the magnitude of the reduction of flower heads from *T. horridus* that was observed in 2001 would have been different if comparably heavy *T. horridus* infestations had occurred on the smaller, less productive, experimental thistles in 1999 and 2000.

#### 4.2. Recruitment and oviposition by *R. conicus*

The multiple-stemmed plants associated with *T. horridus*' feeding damage typically were shorter than uninfested plants. This observation is consistent with the findings of other workers (Cartwright and Kok, 1985; Woodburn, 1997). However, differences in heights varied from year to year. In 2001, when flower stems were the tallest, and differences in heights between *T. horridus*-infested and uninfested thistles were the greatest (twofold or more), we observed significantly lower rates of colonization and oviposition on *T. horridus*-infested thistles. These results were not observed in other years when plants were shorter and differences in heights were smaller. Therefore, it is possible that *R. conicus* has a threshold response with respect to plant height. Alternatively, *R. conicus* adults may respond to differences in the abundance of flower heads, which, in 2001, were more numerous on thistles that were not infested by *T. horridus* and associated with the most *R. conicus* adults.

In contrast to the reduced flowering observed under high *T. horridus* densities, plants that were artificially inoculated in the fall of 1999 with very low infestations of *T. horridus* (around 2 larvae per plant) produced more flower heads in spring 2000 than uninfested thistles. Thus, it appears that the plants may have been stimulated, a phenomenon similar to that reported by Cartwright and Kok (1985). The differences in colonization and oviposition behavior observed in our experiments occurred only when adult female *R. conicus* could move freely among thistles with contrasting growth

forms and sizes. Therefore, it is unclear how they would respond to a situation in which all available musk thistles were shorter.

#### 4.3. Abundance of larval *R. conicus* food

Delayed flower head production in musk thistles that were heavily damaged by *T. horridus* had the long-term consequence of reducing the total amount of resource available to *R. conicus* larvae. About 50% less dry mass of receptacle tissue was available to larvae early in the season as compared to control plants. Reduction in tissue was related to a decrease in the number of flower heads produced, but not to large changes in the average size of flower heads. Woodburn (1997) noted a similar pattern between musk thistle head numbers and size. This large change in food quantity undoubtedly played a significant role, in addition to decreased oviposition, in the greatly depressed production of adult *R. conicus* relative to undamaged thistles.

Although both low and high densities of *T. horridus* did not occur in the same year, comparisons of plant and *R. conicus* responses were made in all years between musk thistles that had or had not been infested by *T. horridus*. Therefore, it is reasonable to conclude that the effects on *R. conicus* were attributable to *T. horridus*-induced changes in thistles. Furthermore, in 2001, when *T. horridus* densities were highest, and effects on thistles were greatest, plants were generally the largest and most vigorous of the 3 years. Because larger thistles have been shown to exhibit relatively greater tolerance to *T. horridus* damage than smaller ones (Sieburth et al., 1983), the dramatic effects on plant growth and reproduction that we observed in 2001 are impressive and suggest that an even greater impact may have occurred if higher *T. horridus* densities had coincided with the smaller thistles that were prevalent in 1999 and 2000.

#### 4.4. *Trichosiocalus horridus*-induced changes in thistle quality and *R. conicus* larval densities

Survival of *R. conicus* to adulthood was lower on musk thistles exposed to high densities of *T. horridus* than on plants free of *T. horridus*, but this difference was evident only at lower *R. conicus* densities. At high larval densities of *R. conicus*, similar mortality rates were observed, regardless of *T. horridus* damage. Direct, intraspecific competition among *R. conicus* larvae due to overcrowding of flower heads appears to have had a stronger negative effect on preadult survival and adult body size than *T. horridus*-induced changes in plant quality (indirect interspecific competition). Goeden and Ricker (1985) also noted that *R. conicus* larvae experience intense scramble competition on Italian thistle, *Carduus pycnocephalus* L.

*Trichosiocalus horridus* appears to trigger changes in musk thistle that reduce the quality of the flower head

for developing *R. conicus*. Other types of plant stress also have been shown to negatively affect the development of *R. conicus*. For example, results of a greenhouse study by Dowd and Kok (1983) showed that *R. conicus* that were reared on water-stressed musk thistles experienced a higher rate of mortality and had smaller body sizes than adults that developed on well-watered plants. Differences in fertilization also influenced survival.

It is unknown whether the differences in musk thistle quality observed in our greenhouse experiment would be expressed in the field. For example, Sieburth et al. (1983) showed that potted thistles are more sensitive to stress than field-grown plants. Furthermore, our observations were made only on the first flower head produced; whereas, under field conditions, *R. conicus* larvae develop in an array of heads. Whether or not a change in plant quality affects larvae developing in heads produced later in the season when intraspecific competition is minimal is unknown. However, observed rates of mortality in the field were very high throughout the season (~80%), so other factors may obscure the effects of decreased plant quality on late-season *R. conicus* larvae. These factors may be density-independent, such as the physical dislodgment of eggs from wind and rain (Surles et al., 1975).

#### 4.5. Impact of *T. horridus*-induced plant changes on populations of *R. conicus*

The combined effects of high densities of *T. horridus* on musk thistle resulted in an estimated 63% reduction in the number of *R. conicus* adults produced. Although other environmental factors may influence the seasonal survival of *R. conicus*, including parasitism, adult mortality in flower heads, and overwintering mortality (Goeden and Ricker, 1980; Kok, 1976), indirect interspecific competition with *T. horridus* represents an additional source of mortality that significantly influences the population dynamics of *R. conicus*. This negative impact can be attributed to three sources—altered synchrony between *R. conicus* and musk thistle (4% of the total reduction), reduced resource availability (40%), and lower egg recruitment (56%) (Table 5). The majority of the impact occurred early in the season.

#### 4.6. Recommendations for musk thistle biological control

Based on our results, it appears that *T. horridus* has the potential for reducing populations of *R. conicus* when infestations of the former on musk thistle are high. The musk thistle-mediated effects on *R. conicus* occurred under conditions of low plant competition, a situation that would likely exist when habitats have been recently disturbed. It is unclear whether higher densities of surrounding vegetation would influence the indirect interaction between the two weevils. However, it is likely that

Table 5

Calculations and estimates of indirect effects of *Trichosirocalus horridus*-induced changes in musk thistle on reductions in *Rhinocyllus conicus* populations, Keats, KS, 2001

Indirect effect	Reduction in <i>R. conicus</i> adult production per <i>T. horridus</i> -infested plant	
	Number	% <sup>a</sup>
<i>Altered synchrony between R. conicus and musk thistle</i> ( <i>no oviposition on T. horridus</i> -infested plants from 2 to 10 May)		
~50 eggs/non- <i>T. horridus</i> infested plant × (1 – 0.90 preadult mortality <sup>b</sup> ) =	5	4
<i>Reduced resource availability</i>		
Early season (10 May to 23 May)		
Assumed equivalent egg densities (22.2 per head) to factor out lower recruitment (see below)		
9.7 heads per <i>T. horridus</i> -infested plant – (19.9 heads [total] – 1.4 heads [produced 2–10 May]) per uninfested plant = 8.8 fewer heads/plant		
8.8 heads/plant × (22.2 eggs/head × [1 – 0.82 mortality]) =	35.2	30
Mid-season (24 May to 7 June)		
(34.8 heads per <i>T. horridus</i> -infested plant × 84.4% receiving eggs) – (45.6 heads per uninfested plant × 89.5% receiving eggs) = 11.4 fewer heads/plant receiving eggs		
11.4 heads/plant × (~7 eggs/head × [1 – 0.85 mortality]) =	12.0	10
<i>Lower egg recruitment (Early season, through 23 May)</i>		
22.2 eggs/head [ <i>T. horridus</i> -infested plants] – 41.8 eggs/head [uninfested plants] = 19.6 fewer eggs/head		
These eggs were distributed over 18.6 heads/plant: (19.9 heads [total] – 1.4 heads [produced 2–10 May]) per non- <i>T. horridus</i> infested plant		
18.6 heads/plant × (19.6 eggs/head × [1 – 0.82 mortality]) =	65.6	56 100%

<sup>a</sup>% = number lost/116.4; 68.8 *R. conicus* adults produced per *T. horridus*-infested plant – 185.5 adults per uninfested plant = 116.4 fewer total adults/plant.

<sup>b</sup>The first flower heads for each plant received around 110 total eggs per head, resulting in a preadult mortality rate of ~90%.

infestations of *T. horridus* will vary among different locations and years. Unfortunately, little data on *T. horridus* densities in North America have been published (but see Andres and Rees, 1995; Kok and Mays, 1989). Infestations of *T. horridus* that are consistently low, as appears to be the case for some locations in Kansas, will likely not affect *R. conicus* populations. In contrast, sites that harbor high densities may depress populations of *R. conicus*. In either case, our data suggest that interactions between musk thistle weevils are complex and warrant careful experimental evaluation of the combined and individual impacts of *R. conicus* and *T. horridus* on musk thistle under a variety of environmental conditions—including relative densities of the two weevils—before decisions about redistributing one or both weevils are made.

## Acknowledgments

We thank Darrel Westervelt and Karen Krouse for the use of their land; Lucas Robison, Jason Sweet, Nicole Sweet, Mary Milbrath, Shauna Dendy, Xiaoli Wu, Neil Miller, Megan Murphy, Justin Schmitz, Soledad

Villamil, Erika Jensen, and Allan Fritz for technical assistance; Tom Loughin for statistical advice; and David Margolies, Walter Fick, Randall Higgins, Sonny Ramaswamy, Walter Dodds (all from Kansas State University), and two anonymous reviewers for providing helpful suggestions on earlier drafts of the manuscript. We also acknowledge the following colleagues who contributed information and ideas during discussions: Ernst Horber (Kansas State University), Loke Kok (Virginia Polytechnic Institute and State University), Svata Louda (University of Nebraska), and Ben Puttler (University of Missouri). This research was funded in part by a grant to L.R.M. from the Sigma Xi Grants-in-Aid of Research program, and by Regional Research Project W-185. This is contribution no. 03-285-J from the Kansas Agricultural Experiment Station.

## References

- Andres, L.A., Rees, N.E., 1995. Musk thistle. In: Nechols, J.R., Andres, L.A., Beardsley, J.W., Goeden, R.D., Jackson, C.G. (Eds.), *Biological Control in the Western United States: Accomplishments and Benefits of Regional Research Project W-84, 1964–1989*, No. 3361, Univ. California, Div. Agric. Nat. Resources, Oakland, CA, pp. 248–251.

- Bounfour, M., Tanigoshi, L.K., 2001. Host plant-mediated interactions between *Tetranychus urticae* and *Eotetranychus carpinii borealis* (Acari: Tetranychidae). *Exp. Appl. Acarol.* 25, 13–24.
- Burnside, O.C., Fenster, C.R., Evetts, L.L., Mumm, R.F., 1981. Germination of exhumed weed seed in Nebraska. *Weed Sci.* 29, 577–586.
- Cartwright, B., Kok, L.T., 1985. Growth responses of musk and plumeless thistles (*Carduus nutans* and *C. acanthoides*) to damage by *Trichosiocalus horridus* (Coleoptera: Curculionidae). *Weed Sci.* 33, 57–62.
- Damman, H., 1989. Facilitative interactions between two lepidopteran herbivores of *Asimina*. *Oecologia* 78, 214–219.
- Dowd, P.F., Kok, L.T., 1981. Impact of larval crowding on survival of *Rhinocyllus conicus* (Coleoptera: Curculionidae). *Environ. Entomol.* 10, 472–473.
- Dowd, P.F., Kok, L.T., 1983. Influence of soil conditions on *Carduus* thistles and development of the thistle head weevil, *Rhinocyllus conicus* (Coleoptera: Curculionidae). *Environ. Entomol.* 12, 439–441.
- Dunn, P.H., 1976. Distribution of *Carduus nutans*, *C. acanthoides*, *C. pycnocephalus*, and *C. crispus*, in the United States. *Weed Sci.* 24, 518–524.
- English-Loeb, G.M., Karban, R., Hougén-Eitzman, D., 1993. Direct and indirect competition between spider mites feeding on grapes. *Ecol. Appl.* 3, 699–707.
- Faeth, S.H., 1986. Plant mediated interactions between seasonal herbivores: enough for evolution or coevolution? In: Spencer, K.C. (Ed.), *Chemical Mediation of Coevolution*. Academic Press, New York, pp. 391–414.
- Fick, W.H., Peterson, D.E., 1995. Musk Thistle: Identification and Control. Kansas State Univ. Coop. Ext. Pub. L231 (revised), Manhattan, KS.
- Gange, A.C., Brown, V.K., 1989. Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia* 81, 38–42.
- Goeden, R.D., Ricker, D.W., 1980. Mortality of *Rhinocyllus conicus* (Col.: Curculionidae) in milk thistle flowerheads in southern California. *Prot. Ecol.* 2, 47–56.
- Goeden, R.D., Ricker, D.W., 1985. Seasonal asynchrony of Italian thistle, *Carduus pycnocephalus*, and the weevil, *Rhinocyllus conicus* (Coleoptera: Curculionidae), introduced for biological control in southern California. *Environ. Entomol.* 14, 433–436.
- Harris, P., 1981. Stress as a strategy in the biological control of weeds. In: Papavizas, G.C. (Ed.), *Biological Control in Crop Protection*, Beltsville Symp. Agric. Res. 5, Allanheld, Osmun & Co., Totowa, NJ, pp. 333–340.
- Harris, P., 1991. Classical biocontrol of weeds: its definition, selection of effective agents, and administrative-political problems. *Can. Entomol.* 123, 827–849.
- Harrison, S., Karban, R., 1986. Effects of an early-season folivorous moth on the success of a later-season species, mediated by a change in the quality of the shared host, *Lupinus arboreus* Sims. *Oecologia* 69, 354–359.
- Hilbert, B., Brooks, H.L., 2000. Biological Control of Musk Thistle in Kansas. Kansas State Univ. Coop. Ext. Pub. L873, Manhattan, KS.
- Hoffman, J.H., Moran, V.V., 1992. Oviposition patterns and the supplementary role of a seed-feeding weevil, *Rhyssomatus marginatus* (Coleoptera: Curculionidae), in the biological control of a perennial leguminous weed, *Sesbania punicea*. *Bull. Entomol. Res.* 82, 343–347.
- James, R.R., McEvoy, P.B., Cox, C.S., 1992. Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaeae*): an experimental analysis. *J. Appl. Ecol.* 29, 589–596.
- Kok, L.T., 1976. Overwintering mortality of caged thistle-head weevils, *Rhinocyllus conicus*, in Virginia. *Environ. Entomol.* 5, 1105–1108.
- Kok, L.T., 2001. Classical biological control of nodding and plumeless thistles. *Biol. Control* 21, 206–213.
- Kok, L.T., Mays, W.T., 1989. Comparison of the seasonal occurrence of *Trichosiocalus horridus* (Panzer) (Coleoptera: Curculionidae) in Virginia between 1981–83 and 1979. *J. Entomol. Sci.* 24, 465–471.
- Kok, L.T., McAvoy, T.J., 1983. Refrigeration, a practical technique for storage of eggs of *Trichosiocalus horridus* (Coleoptera: Curculionidae). *Can. Entomol.* 115, 1537–1538.
- Louda, S.M., Kendall, D., Connor, J., Simberloff, D., 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* 277, 1088–1090.
- Masters, G.J., Jones, T.H., Rogers, M., 2001. Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia* 127, 246–250.
- McAvoy, T.J., Kok, L.T., Mays, W.T., 1987. Dispersal of *Trichosiocalus horridus* (Panzer) (Coleoptera: Curculionidae) in southwest Virginia. *J. Entomol. Sci.* 22, 324–329.
- McCarty, M.K., 1982. Musk thistle (*Carduus thoermeri*) seed production. *Weed Sci.* 30, 441–445.
- McCarty, M.K., Lamp, W.O., 1982. Effect of a weevil, *Rhinocyllus conicus*, on musk thistle (*Carduus thoermeri*) seed production. *Weed Sci.* 30, 136–140.
- McCarty, M.K., Scifres, C.J., 1969. Life Cycle Studies with Musk Thistle. *Res. Bull. Univ. Nebraska, Lincoln Agric. Exp. Stn.* 230, Lincoln, NE.
- McEvoy, P.B., Rudd, N.T., Cox, C.S., Huso, M., 1993. Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaeae* populations. *Ecol. Monogr.* 63, 55–75.
- Medd, R.W., Lovett, J.V., 1978. Biological studies of *Carduus nutans* (L.) ssp. *nutans*. II. Vernalization and phenological development. *Weed Res.* 18, 369–372.
- Moran, N.A., Whitman, T.G., 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology* 71, 1050–1058.
- Nechols, J.R., 2000. Biological control of musk thistle: a reassessment. In: Follet, P.A., Duan, J.J. (Eds.), *Nontarget Effects of Biological Control*. Kluwer, Norwell, MA, pp. 245–259.
- Petersen, M.K., Sandström, J.P., 2001. Outcome of indirect competition between two aphid species mediated by responses in their common host plant. *Funct. Ecol.* 15, 525–534.
- Pilson, D., 1992. Aphid distribution and the evolution of goldenrod resistance. *Evolution* 46, 1358–1372.
- SAS Institute, 1999. SAS/STAT User's Guide, Version 8th ed., SAS Institute, Cary, NC.
- Shorthouse, J.D., Lalonde, R.G., 1984. Structural damage by *Rhinocyllus conicus* (Coleoptera: Curculionidae) within the flowerheads of nodding thistle. *Can. Entomol.* 116, 1335–1343.
- Sieburth, P.J., Kok, L.T., Lentner, M., 1983. Factors influencing the effectiveness of *Trichosiocalus horridus* (Panzer) in the control of *Carduus* thistles. *Crop Prot.* 2, 143–151.
- Smith II, L.M., Kok, L.T., 1987. Influence of temperature on oviposition, quiescence, and mortality of *Rhinocyllus conicus* (Coleoptera: Curculionidae). *Environ. Entomol.* 16, 971–974.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*, third ed. W.H. Freeman and Co, New York.
- Strauss, S.Y., 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* 72, 543–558.
- Stuckey, R.L., Forsyth, J.L., 1971. Distribution of naturalized *Carduus nutans* (Compositae) mapped in relation to geology in northwestern Ohio. *Ohio J. Sci.* 71, 1–15.
- Surles, W.W., Kok, L.T., 1976. Pilot studies on augmentation of *Rhinocyllus conicus* (Col.: Curculionidae) for *Carduus* thistle control. *Environ. Entomol.* 5, 901–904.

- Surles, W.W., Pienkowski, R.L., Kok, L.T., 1975. Mortality of the immature stages of *Rhinocyllus conicus*, a thistle head weevil, in Virginia. *Environ. Entomol.* 4, 371–372.
- Tindall, K.V., Stout, M.J., 2001. Plant-mediated interactions between the rice water weevil and fall armyworm in rice. *Entomol. Exp. Appl.* 101, 9–17.
- Tscharntke, T., 1989. Attack by a stem-boring moth increases susceptibility of *Phragmites australis* to gall-making by a midge: mechanisms and effects on midge population dynamics. *Oikos* 55, 93–100.
- Turner, C.E., Herr, J.C., 1996. Impact of *Rhinocyllus conicus* on a non-target, rare, native thistle (*Cirsium fontinale*) in California. In: Moran, V.C., Hoffmann, J.H. (Eds.), *Proc. IX Int. Symp. Biol. Contr. Weeds*, 19–26 January, 1996, Stellenbosch, South Africa, University of Cape Town, Cape Town, South Africa, p. 103.
- Turner, C.E., Pemberton, R.W., Rosenthal, S.S., 1987. Host utilization of native *Cirsium* thistles (Asteraceae) by the introduced weevil *Rhinocyllus conicus* (Coleoptera: Curculionidae) in California. *Environ. Entomol.* 16, 111–115.
- Williams, K.S., Myers, J.H., 1984. Previous herbivore attack of red alder may improve food quality for fall webworm larvae. *Oecologia* 63, 166–170.
- Woodburn, T.L., 1997. Establishment in Australia of *Trichosirocalus horridus*, a biological control agent for *Carduus nutans*, and preliminary assessment of its impact on plant growth and reproductive potential. *Biocontrol Sci. Technol.* 7, 645–656.
- Wootton, J.T., 1994. The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.* 25, 443–466.