Musk thistle, Carduus nutans L., is a biennial herbaceous species native to Europe, western Asia, and the Mediterranean (Hodgson and Rees 1976, Zwölfer and Harris 1984). It was first introduced into North America in the mid-1800s (Stuckey and Forsyth 1971) and is now recorded from 45 states in the United States and at least five Canadian provinces (McCarty 1978, Desrochers et al. 1988, USDA 2008). The introduction of exotic natural enemies was promoted and projected to at least five Canadian provinces (McCarty 1978, Desrochers et al. 1988). Upon establishment, R. conicus was widely redistributed as part of musk thistle biological control programs throughout the continental United States (Trumble and Kok 1982, Townsend et al. 1991, Lambdin and Grant 1992, Buntin et al. 1993, Gassmann and Kok 2002). As part of an integrated pest management (IPM) program against musk thistle, releases of R. conicus were made throughout Tennessee from 1989 through the 1990s (Grant and Lambdin 1993). The establishment of this weevil contributed to reductions (94%) in populations of musk thistle in original release sites between 1989 and 1995 (Lambdin and Grant 1996).

Early host specificity tests and host data from its native range suggested that R. conicus could feed and develop within the heads [the dense terminal cluster of sessile flowers exhibited by Carduus and other thistle species (Wofford 1989, Judd et al. 2002)] of several genera and species of thistles in the tribe Cynareae (Asteraceae) (Zwölfer and Harris 1984). However, the potential benefits of releasing R. conicus as a biological control agent were deemed to outweigh the potential limitations (Boldt 1997). Recent documentation of nontarget impacts of R. conicus on native
thistles in the north central and western United States, however, illustrates the importance of increasing efforts to assess the impact of these weevils on target and nontarget thistles in areas where they were released (Pemberton 2000, Sauer and Bradley 2008). The first record of R. conicus feeding on native North American thistles in the genus Cirsium was in Montana and Canada on C. undulatum (Nuttall) Sprengel and C. floridannii (Rydberg) Arthur (Rees 1977, 1978, Zwölfer and Harris 1984). Insect surveys of native thistles in California documented larvae of R. conicus, which is the developmental stage most damaging to plants, feeding on natural populations of 13 native Cirsium species (Goeden and Ricker 1987, Turner et al. 1987). One of these species [C. fontinale (Greene) Jepson variety obispoense J. T. Howell] is listed as Federally endangered [Turner and Herr 1996, United States Department of Agriculture (USDA) 2010], and another species, C. ciliolatum (Henderson) J. T. Howell, is listed as state endangered in California (Turner et al. 1987, USDA 2010).

Although the significance and impact of R. conicus on many of these native species are not yet known, studies of the feeding of R. conicus on Platte thistle, C. canescens Nuttall, have documented impacts because of larval feeding within the heads of this native thistle. Larvae of R. conicus were first documented feeding in the heads of C. canescens in Nebraska in 1993, and since that time R. conicus has been documented to infest >25% of observed C. canescens plants (Louda et al. 1997; Louda 1998, 2000). Additionally, feeding of R. conicus within thistle heads reduced the number of viable seeds in infested heads by 86%. These reductions in seed numbers can have direct negative impacts on populations of C. canescens, as this species is seed-limited and relies on newly produced seeds to sustain population levels over time (Louda 2000, Louda and Potvin 1995). Concern also exists that R. conicus will continue its host range expansion and use Pitcher’s thistle [C. pitcheri (Torrey) Torrey and Gray], a Federally listed threatened species that is closely related to C. canescens (Pavlovic et al. 1992, Louda 1998, 2000).

The impact of R. conicus on nontarget thistle species in the southern Appalachians is poorly known. Five native species of thistles in the genus Cirsium are found in Tennessee [tall thistle, C. altissimum (L.) Sprengel, soft thistle, C. carolinianum (Walter) Fernald & Schubert, field thistle, C. discolor (Muhlenberg ex Willklenow) Sprengel, yellow thistle, C. horridulum Michaux, and swamp thistle, C. muticum Michaux] [The University of Tennessee Herbarium (TENN) 2008, C. carolinianum and C. horridulum bloom from May through July (when Ca. nutans blooms and R. conicus is reproductively active). Consequently, because of their phenological concurrence with Ca. nutans and R. conicus in Tennessee, C. carolinianum and C. horridulum are the native species most at risk to nontarget feeding by R. conicus. Little is known, however, of the importance of seed production and current-seed germination and establishment to long-term sustainability of local populations of either thistle species. The remaining native thistles (C. altissimum, C. discolor, and C. muticum) bloom later in the year (approximately from August through October) and are not phenologically concurrent with the reproduction of R. conicus (Wofford 1989, Gleason and Cronquist 1991, Wiggins 2009). All five native species are widely distributed throughout the eastern United States, each occurring in at least 15 states in addition to Tennessee (USDA 2008). Nontarget activity of R. conicus on native thistles has not been reported in this region.

Two introduced Cirsium species [Canada thistle, C. arvense (L.) Scopoli, and bull thistle, C. vulgare (Savi Tenore)] also occur in Tennessee (TENN 2008), and both species are considered invasive in the state [Tennessee Exotic Pest Plant Council (TNEPPC) 2008]. Both C. arvense and C. vulgare have extensive flowering periods and can bloom from late June through October in the southern Appalachians (Wofford 1989). These two introduced species also can serve as hosts of R. conicus.

In field surveys conducted from 2004 through 2008, R. conicus was not observed in populations of naturally occurring nontarget native thistles (Wiggins 2009). Although nontarget feeding has not yet been detected in this region, controlled studies can help determine if nontarget feeding is possible, as well as quantify impacts (tissue damage, decreased seed numbers, and so forth) that these weevils may have on plants, should they begin using native thistles. Caged plant studies are an effective way to restrict herbivory to specific areas of plants, allowing quantification of the impacts of a known number of weevils on a known number of plant parts. Therefore, a study was initiated in 2008 using field-caged plants to evaluate the impact of larval feeding of R. conicus on seed production and assess host utilization of native (five species) and introduced (three species) thistles in eastern Tennessee.

Materials and Methods

Source of Weevils. Adult R. conicus were collected from field populations of Ca. nutans in Knox and Cumberland Counties, TN, for use in this study. From 22 April to 25 May 2008, adult R. conicus were shaken from bolting stems of Ca. nutans into sweep nets (38 × 70 centimeter canvas net bag). Adult weevils were then placed in a clear plastic container (31 × 31 × 41 centimeter with four 12-cm screened holes for ventilation) with bouquets of clipped Ca. nutans and taken to the laboratory, where they were held and observed for mating activity. Two pairs of copulating R. conicus were placed in one 29.6 ml cup (two male and two female weevils per cup) with a moistened cotton ball that was then sealed with a plastic lid and held in a growth chamber at 15°C until weevils were placed on caged plants in the field. Adult weevils were contained in cups no longer than 4 d before use in study.

Host Utilization Experiments. From 24 April to 27 May 2008, one to two populations (containing at least 40 plants each) of Ca. nutans and the seven Cirsium species that occur in Tennessee were selected, and plants from each population were caged to study the...
Table 1. Site information for Carduus nutans and seven Cirsium species at 14 field populations used to study the effects of Rhinocyllus conicus on caged plants, 2008 and 2009.

<table>
<thead>
<tr>
<th>Thistle species*</th>
<th>County</th>
<th>Duration 2008b</th>
<th>Duration 2009c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carduus nutans*</td>
<td>Knox</td>
<td>30 Apr–27 May</td>
<td>29 Apr–6 June</td>
</tr>
<tr>
<td>Cirsium altissimum</td>
<td>Roane</td>
<td>21 May–10 June</td>
<td>29 Apr–29 May</td>
</tr>
<tr>
<td>C. arvense*</td>
<td>Knox</td>
<td>6 May–13 June</td>
<td>19 May–30 June</td>
</tr>
<tr>
<td>C. carolinianum</td>
<td>Anderson</td>
<td>27 May–23 June</td>
<td>19 May–14 June</td>
</tr>
<tr>
<td>C. discolor</td>
<td>Bledsoe</td>
<td>2 May–30 May</td>
<td>8 May–7 June</td>
</tr>
<tr>
<td>C. horridulum</td>
<td>Cumberland</td>
<td>7 May–3 June</td>
<td>8 May–6 June</td>
</tr>
<tr>
<td>C. muticum</td>
<td>Morgan</td>
<td>13 May–20 Aug</td>
<td>N/A</td>
</tr>
<tr>
<td>C. vulgare*</td>
<td>Knox</td>
<td>8 May–17 July</td>
<td>25 May–22 June</td>
</tr>
<tr>
<td></td>
<td>Roane</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* One pop containing 60 plants each of C. altissimum, C. muticum, and C. vulgare was used during the study in 2008; one pop containing 30 plants of C. vulgare was used during the study in 2009. Two populations containing 30 plants each were used for all other species included in this study. Treatments (plants caged with adult R. conicus, plants caged without adult R. conicus, and uncaged plants) were divided equally among the plants within a pop (10–20 plants per treatment).

b Duration = the time period that treatments were applied to plants (Ca. nutans, C. arvense, C. carolinianum, C. discolor, C. horridulum, C. vulgare, 2008 and 2009; C. vulgare 2009) or that bags were left on the plants (C. altissimum, C. discolor, C. muticum, and C. vulgare 2008) before they were taken to the laboratory for processing.

N/A = not applicable; thistle species were not sampled at this site during this year.

a Introduced species.

impact of introduced weevils (Table 1). For plants that were caged with and without adult R. conicus, one nylon mesh bag (45 × 50 cm; Delnet pollination bags) was placed on the mainstem of each plant, and the opening of the bag was bunched together around the plant stem and tied with plastic flagging. In each study population, one of three treatments was applied to each plant (10–20 plants per treatment per population, 60 plants per thistle species) (Table 1): apical buds/flower heads of mainstems enclosed in mesh bags with four field-collected adult R. conicus (two male, two female), apical buds/flower heads of mainstems enclosed in mesh bags with no weevils (caged control), or plants flagged, but left uncaged as an open control. Treatments were left on the plants for 4 wk. After this time, plants of Ca. nutans, C. arvense, C. carolinianum, and C. horridulum were flowering and/or initiating seedling and, therefore, were clipped at ground level, placed in a plastic bag, and taken to the laboratory for processing. Weevils were removed from the bags of C. altissimum, C. discolor, C. muticum, and C. vulgare after 4 wk of exposure to weevils, and the cages were left on these species until collected from the field in mid-summer to fall to prevent other herbivorous insects from accessing the caged plant parts (Table 1). In the laboratory, heads were categorized as buds (flowers not yet present on head), flower heads (flowers present and seeds within head maturing or mature), and seed heads (majority of flowers dead, head senescing/seeds partially dispersed). The head width of buds, flower heads and seed heads per plant and numbers of seeds per flower head were measured and recorded. In addition, the number of eggs, larvae, pupae, and adults of R. conicus were recorded from all plant heads. Because no evidence of R. conicus reproduction was detected on the fall-blooming species, C. altissimum, C. discolor, or C. muticum, in 2008, only seed counts per treatment for these species were made in 2008, and these thistles were excluded from all studies in 2009.

This caged plant study was modified before it was repeated in 2009 (Table 1). Treatments of R. conicus were restricted to Ca. nutans, C. arvense, C. carolinianum, C. horridulum, and C. vulgare, as C. altissimum, C. discolor, and C. muticum do not bud or flower during the time the weevil larvae require those plant structures to be available. From 26 April to 25 May 2009, adult R. conicus were collected from field populations of Ca. nutans in Knox and Cumberland Counties, TN, and were processed for use in caged plant studies in the same manner implemented in 2008. As in 2008, weevils used in the field study were retained in cups for no longer than 4 d before use in the study. From 28 April to 30 June 2009, treatments were applied to plants from two populations (30 plants per population) of each thistle species, except for a single population of C. vulgare (no other suitable population was located) (Table 1). At each population, one of three treatments was applied to each of 30 plants (10 plants per treatment): apical buds/flower heads of mainstems enclosed in mesh bags with four field-collected adult R. conicus (two male, two female), apical buds/flower heads of mainstems enclosed in mesh bags with no weevils (caged control), or plants flagged, but left uncaged as an open control. Treatments remained in the field until a majority (ca. >50%) of the caged heads initiated flowering, after which plants were clipped at ground level, placed in a plastic bag, and taken to the laboratory for processing.

In the laboratory, plant height and head width diameter of buds, flower heads, and seed heads per plant were measured, and the number of eggs of R. conicus were recorded. Unlike 2008, where heads of plants were immediately dissected, heads of all plants were retained in a growth chamber (26°C) for 2 to 4 wk after removal from the field to allow maturation of immature R. conicus to adulthood. After this period, number of larvae, pupae and adults of R. conicus from all buds, flower heads, and seed heads, and numbers of seeds per flower head were recorded. Body length of adult R. conicus has been used as a measure of weevil fitness (i.e., smaller adults are believed to be less fit to mate and/or produce fewer offspring), as well as an indicator of host suitability (i.e., host plants that produce larger weevils are considered more suitable than those that produce smaller adults) (Rowe and Kok 1984, Turner et al. 1987). The lengths [distance (millimeter) between the anterior edge of the eyes to posterior tip of elytra] of all adult R. conicus recovered from plants of each thistle species caged with R. conicus in 2009 were measured using a Zeiss Stemi SVG microscope with an ocular micrometer calibrated with a Mini-scale measuring scale (0.1 mm increments) and recorded.
Data Analysis. For all thistle species in 2008 and five (Ca. nutans, C. arcense, C. carolinianum, C. horridulum, and C. vulgare) species in 2009, number of seeds per flower head was used as the response variable to assess influence of R. conicus on seed production. Cage treatment (i.e., caged with R. conicus, caged without R. conicus, no cage) was considered the independent variable. For Ca. nutans, C. arcense, C. carolinianum, C. horridulum, and C. vulgare in 2008 and 2009, number of eggs of R. conicus per head (buds, flower heads, or seed heads), number of R. conicus (total larvae, pupae, and adults) per head, and number of R. conicus per centimeter of plant head width diameter were considered as response variables that indicate utilization of thistle species by R. conicus. Additionally, in 2009 only, body length of adult R. conicus was considered the response variable that indicated suitability of each plant host for all adults reared from Ca. nutans, C. arcense, C. carolinianum, C. horridulum, and C. vulgare. Plant species was designated as the independent variable for these analyses of host utilization and host suitability. For Ca. nutans in 2009, length of recovered adult R. conicus was used as the response variable to determine the influence of caged plants on weevil development, and the caged status (i.e., caged or uncaged) of the plant was considered the independent variable.

Results and Discussion

This cage study documented several significant interactions among R. conicus and thistle species in Tennessee. Rhinocyllus conicus had a significant effect on seed production in both 2008 ($F_{1,86} = 5.23; P < 0.0001$) and 2009 ($F_{1,86} = 3.55; P = 0.0007$). Significantly fewer seeds per flower head were produced by plants caged with four adult R. conicus when compared with caged control plants of C. carolinianum and C. horridulum and uncaged control plants of C. carolinianum and C. vulgare in 2008 (Fig. 1A). Compared with caged control plants, both spring-blooming native thistle species were less attractive to R. conicus. For Ca. nutans in 2009, length of recovered adult R. conicus was used as the response variable to determine the influence of caged plants on weevil development, and the caged status (i.e., caged or uncaged) of the plant was considered the independent variable.

Table 2. Comparison of seed production (mean±SE) of flower heads of fall-blooming native thistle species caged with Rhinocyllus conicus and caged and uncaged control (no R. conicus applied) plants ($n = 20$ plants per treatment), 2008

<table>
<thead>
<tr>
<th>Thistle species</th>
<th>Treatment</th>
<th>Seeds (SE)</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirsium altissimum</td>
<td>Caged control</td>
<td>129.13 ± 11.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uncaged control</td>
<td>129.73 ± 6.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. conicus</td>
<td>136.00 ± 9.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. discolor</td>
<td>Caged control</td>
<td>147.54 ± 11.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uncaged control</td>
<td>152.54 ± 9.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. conicus</td>
<td>173.71 ± 8.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. muticum</td>
<td>Caged control</td>
<td>95.02 ± 3.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uncaged control</td>
<td>100.71 ± 6.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. conicus</td>
<td>94.51 ± 4.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tukey-Kramer tests detected no significant ($P = 0.05$) differences among treatments within individual species.

All data were analyzed by one-way analysis of variance (ANOVA) (ANOVA: PROC MIXED; SAS Institute 2005). Significance for all ANOVA tests was set at $P < 0.05$. Before analysis, all data were transformed using either rank [number of seeds per flower head (both years), R. conicus per head (2008), R. conicus per centimeter of head width (2008), body lengths of R. conicus, and body lengths of R. conicus from caged and uncaged Ca. nutans] or the natural log ($x + 0.5$) [number of eggs of R. conicus (both years), R. conicus per head (2009), and R. conicus per centimeter of head width (2009)]. When significant ANOVA results were obtained, post hoc analysis was performed using the Tukey-Kramer test ($\alpha = 0.05$) to detect differences among means. Original mean and standard error values are reported in Fig. 1 and Tables 2 through 4.

Table 3. Comparison of oviposition (avg no. of eggs per head (mean ± SE)) of Rhinocyllus conicus among thistle species caged with R. conicus ($n = 20$ plants per species), 2008 and 2009

<table>
<thead>
<tr>
<th>Thistle species</th>
<th>Eggs of R. conicus/head</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carduus nutans*</td>
<td>12.15 ± 3.03a</td>
<td>13.73 ± 1.98a</td>
<td></td>
</tr>
<tr>
<td>C. arcense*</td>
<td>2.22 ± 0.44b</td>
<td>0.10 ± 0.03c</td>
<td></td>
</tr>
<tr>
<td>C. carolinianum</td>
<td>7.10 ± 0.83a</td>
<td>1.99 ± 0.52b</td>
<td></td>
</tr>
<tr>
<td>C. horridulum</td>
<td>9.13 ± 1.48a</td>
<td>13.24 ± 1.73a</td>
<td></td>
</tr>
<tr>
<td>C. vulgare*</td>
<td>3.19 ± 0.92b</td>
<td>0.16 ± 0.09c</td>
<td></td>
</tr>
</tbody>
</table>

* Different letters within the same column denote significant ($P = 0.05$) differences among means as detected by Tukey-Kramer tests.
* Introduced species.
tive thistle species showed significant reductions (by ~85% in *C. carolinianum* and ~35% in *C. horridulum*) in seed numbers because of larval feeding of *R. conicus* (Fig. 1A). No significant differences were observed in seeds per flower head among treatments in fall-blooming thistles (*C. altissimum, C. discolor,* and *C. muticum*; Table 2). Seed production of the four *Cirsium* thistles caged with *R. conicus* in 2009 was not significantly reduced when compared with controls (Fig. 1B). Although there were no significant differences in seeds per flower head among treatments on plants of *Ca. nutans* in 2008, significantly fewer seeds were produced by plants of *Ca. nutans* caged with *R. conicus* than by caged control plants in 2009 (~46% reduction) (Fig. 1).

The feeding of *R. conicus* on *Ca. nutans* has been well documented and was expected during this study. Likewise, no feeding by *R. conicus* was expected on the fall-blooming species in 2008, as these species do not develop buds necessary for larval development during the time *R. conicus* is reproductively active in this region. Despite larval feeding and development in heads of spring- and summer-blooming *Cirsium* species enclosed in cages with *R. conicus*, nontarget feeding has not been documented on *C. carolinianum* or *C. horridulum* in naturally occurring populations (Wiggins 2009). Although the possibility of feeding by *R. conicus* on the nontarget native species in this study exists, the extent to which it may occur in natural populations, if ever, remains unclear.

Although the impact of *R. conicus* on seed numbers of *Ca. nutans* was as expected in 2009, the lack of differences in seed production among control plants and plants exposed to *R. conicus* in 2008 is not indicative of these plant/insect interactions in Tennessee. Only flower heads of each species were included in the analyses, because it is difficult to accurately determine potential seed numbers in buds or general seed numbers in seed heads that have begun to senesce and lose seeds. However, many buds and seed heads were present on plants of *Ca. nutans* when the plants were collected from the field in 2008, and statistical estimates were made on a small number of flower heads of *Ca. nutans* (*n* = 12; only one head present for caged control treatment). When seed heads also were included in the analyses (total heads analyzed = 48), significant (*F* = 7.62; *P* = 0.0014) treatment effects were documented. Fewer seeds were observed in open control plants (mean  = 199.97) than heads caged with *R. conicus* (mean  = 199.41) or caged control plants (mean  = 279.20), and significant differences were observed between heads caged with *R. conicus* and caged control plants. The provision of allowing more than ~50% of heads to begin flowering in 2009 instead of leaving plants in the field for a set amount of time allowed for more uniform seed counts from greater numbers (*n* = 120) of flower heads of *Ca. nutans* in 2009.

On thistle heads caged with *R. conicus*, significant differences were observed among egg numbers of *R. conicus* on thistle species in 2008 (*F* ~161~ = 8.64; *P* < 0.0001) and 2009 (*F* ~144~ = 105.21; *P* < 0.0001). Significantly more eggs per head were recorded on *Ca. nutans* (an introduced species) and the native species *C. carolinianum* and *C. horridulum* than on the introduced species *C. arvense* and *C. vulgar* (Table 3). In 2009, significantly more eggs were recorded on *Ca. nutans* and *C. horridulum* than *C. carolinianum*, and all three of these thistle species had significantly more eggs per head than *C. arvense* and *C. vulgar* (Table 3). Both *C. arvense* and *C. vulgar* bud and bloom later in the year than *Ca. nutans*, and are not used as frequently as host plants by *R. conicus* (G.J.W., unpublished data). Therefore, less oviposition is expected on these species when compared with *Ca. nutans*. Oviposition on plants of both *C. carolinianum* and *C. horridulum* was comparable to that observed on *Ca. nutans* in 2008, and in 2009, similar numbers of eggs of *R. conicus* were observed on *Ca. nutans* and *C. horridulum*. Consistently high egg numbers of *R. conicus* on *C. horridulum* could be because of the head widths of this thistle species: of the eight study species, the head width of *C. horridulum* is second in size only to *Ca. nutans*. However, *R. conicus* oviposited on *C. carolinianum* much less in 2009 than the previous year. The reason for differences in egg numbers on *C. carolinianum* between years is unclear, but oviposition of *R. conicus* on this species in both years indicates acceptability as a potential host species.

Further examination of heads caged with *R. conicus* showed that thistle species significantly affected the numbers of weevils per head in 2008 (*F* ~67~ = 13.03; *P* < 0.0001) and 2009 (*F* ~32~ = 17.89; *P* < 0.0001). Significantly more *R. conicus* were recorded per head of *Ca. nutans* than from *C. arvense, C. horridulum,* and

<table>
<thead>
<tr>
<th>Thistle species</th>
<th><em>R. conicus</em>/head 2008</th>
<th><em>R. conicus</em>/head 2009</th>
<th><em>R. conicus</em>/cm head width 2008</th>
<th><em>R. conicus</em>/cm head width 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carduus nutans</em></td>
<td>8.61 ± 1.68b</td>
<td>7.04 ± 0.90a</td>
<td>3.08 ± 0.50a</td>
<td>3.07 ± 0.39a</td>
</tr>
<tr>
<td><em>Cirsium arvense</em></td>
<td>1.29 ± 0.05d</td>
<td>1.18 ± 0.08b</td>
<td>1.70 ± 0.07ab</td>
<td>1.55 ± 0.12ab</td>
</tr>
<tr>
<td><em>C. carolinianum</em></td>
<td>4.06 ± 0.45ab</td>
<td>2.38 ± 0.54b</td>
<td>3.69 ± 0.40a</td>
<td>2.48 ± 0.54ab</td>
</tr>
<tr>
<td><em>C. horridulum</em></td>
<td>3.15 ± 0.30c</td>
<td>2.36 ± 0.28b</td>
<td>1.51 ± 0.13b</td>
<td>1.24 ± 0.13b</td>
</tr>
<tr>
<td><em>C. vulgar</em></td>
<td>1.54 ± 0.19cd</td>
<td>2.00 ± 0.98ab</td>
<td>1.22 ± 0.11b</td>
<td>1.75 ± 1.52ab</td>
</tr>
</tbody>
</table>

* Different letters within the same column denote significant (*P* = 0.05) differences among means as detected by Tukey-Kramer tests.

* Introduced species.
C. vulgare in 2008 and C. arvense, C. carolinianum, and C. horridulum in 2009 (Table 4). Additionally, C. carolinianum had significantly more R. conicus per head than C. vulgare, and both native thistles had significantly more R. conicus per head than C. arvense in 2008 (Table 4). Thistle species also significantly influenced the numbers of weevils per centimeter head width in 2008 ($F_{1, 67} = 7.55; P < 0.001$) and 2009 ($F_{1, 82} = 3.86; P = 0.0063$). Significantly more R. conicus per centimeter of plant head width were observed in Ca. nutans and C. carolinianum than C. horridulum and C. vulgare in 2008, with Ca. nutans only having significantly more R. conicus per centimeter head width than C. horridulum in 2009. All Cirsium species had similar numbers of R. conicus per head and R. conicus per centimeter of head width in 2009 (Table 4). The levels of infestation of both native thistles (C. carolinianum and C. horridulum) in 2008 by R. conicus compared with C. arvense and C. vulgare (both introduced thistles) indicate that these native species may be as suitable a host species, although nontarget activity in naturally occurring populations has not been observed in either native species (Wiggins 2009).

Although the mean number of R. conicus per head can be influenced by the head width of the plant (i.e., larger heads provide greater area for more larvae), the mean R. conicus per centimeter of plant head width diameter is standardized to a uniform unit (centimeter). The relatively smaller average head size of C. carolinianum (~10 mm diameter) may render it less preferable or attractive to R. conicus as a host plant compared with Ca. nutans (~35 mm diameter), but also may enable greater damage by fewer weevils per head than Ca. nutans should nontarget feeding occur in natural populations.

The number of R. conicus per head and per centimeter plant head width followed similar trends as egg loads of R. conicus for most thistle species during both years (Tables 3 and 4). However, mean egg numbers per head on C. horridulum were not significantly different from Ca. nutans either year, yet the number of R. conicus per head and per head width on C. horridulum were significantly less than Ca. nutans in both years. These differences may indicate that R. conicus is capable of laying high numbers of eggs on C. horridulum, but few of those eggs hatch or result in larval development within the head. Unlike the other thistle species in this study, C. horridulum has leaf-like outer bracts that enclose the head. These bracts may provide an obstacle through which it is more difficult for newly hatched R. conicus to maneuver into the head and receptacle within and account for lower levels of R. conicus per centimeter of plant head width despite high numbers of eggs per head.

Mean body lengths of adult R. conicus reared on different thistle species ranged from 5.38 mm (C. carolinianum) to 5.74 mm (C. vulgare), but no significant ($F_{1, 370} = 0.60; F = 0.6617$) differences in body lengths were documented. Additionally, no significant ($F_{1, 107} = 1.28; P = 0.2589$) differences were documented in body lengths of R. conicus from caged ($\bar{x} = 5.5$ mm) versus uncaged ($\bar{x} = 5.63$ mm) flower heads of Ca. nutans. Because R. conicus were collected in large numbers from uncaged plants of only Ca. nutans, this test was performed only for plants of Ca. nutans. The similarity between R. conicus reared from caged and uncaged Ca. nutans demonstrates the lack of impact of caging on the development of the weevils inside of flower heads. The body lengths of R. conicus reported here are similar to those documented in other studies (Rowe and Kok 1984, Turner et al. 1987). The similarity in body lengths of R. conicus reared from different thistle species indicates that each of these species is a potentially acceptable host.

Other studies have confirmed the host preference of R. conicus for Ca. nutans (Arnett and Louda 2002) and have demonstrated its ability to reduce seed production long after initial releases and subsequent establishment in an area (Kok 2001). Nonetheless, R. conicus continues to expand its host range to include native Cirsium species (Pemberton 2000, Sauer and Bradley 2008). Future studies could be conducted on chemical or other cues that may be used by R. conicus to search for and/or determine suitable host plants. These studies may provide a better understanding of the factors that enable R. conicus to expand its host range to thistle species native to North America.

This study is the first documentation of the ability of R. conicus to develop, and accordingly reduce viable seed numbers, in heads of either C. carolinianum or C. horridulum. Results from this study demonstrate this weevil has the ability to use these native thistles as host species, with significant reductions in seed numbers of both native species observed in cage studies the first year of the study. R. conicus readily oviposited on both native thistles at similar levels to those observed on Ca. nutans in 2008, and both native thistles exhibited significantly greater egg loads than C. arvense or C. vulgare both years. Furthermore, infested heads of C. carolinianum contained numbers of R. conicus per centimeter of plant head width similar to Ca. nutans in 2008 and 2009, and both native species contained numbers of R. conicus per centimeter of plant head width similar to C. arvense and C. vulgare during at least 1 yr of the study. This information further signifies the suitability of C. carolinianum and C. horridulum as host species for R. conicus.

The ramifications of nontarget feeding to these native thistle species at the population level, however, are unclear. No studies are known to demonstrate whether C. carolinianum or C. horridulum are seed-limited, or the importance and reliance on current seed to the sustainability of populations. Additionally, no studies were conducted to demonstrate population reductions for these native thistles during the course of this research. However, several general plant characteristics that may predispose a species to susceptibility to head-feeding insects (discussed in Louda and Potvin 1995) are present in C. carolinianum and infer potential population impacts should nontarget feeding occur. C. carolinianum is a biennial plant (Gleason and Cronquist 1991, Keil 2006), and biennials often rely more on current seed for regeneration (Louda and...
Potvin 1995). Furthermore, as with many other Cirsium species, C. carolinianum produces large inflorescences relative to plant size, which simultaneously enhances the likelihood of pollination while attracting inflorescence and seed feeders (Louda and Potvin 1995). Finally, unlike some Cirsium species that may persist perennially via rhizomes or root sprouts (e.g., C. arvense and C. horridulum, respectively), C. carolinianum is known to reproduce only via seeds with no known vegetative mechanism for reproduction (Gleason and Cronquist 1991, Keil 2006). Thus, C. carolinianum relies solely on seed production to sustain local populations. Because C. horridulum may reproduce through both seed production and using existing root sprouts, this species may not be at as great a risk as C. carolinianum for population impacts because of non-target seed feeding by R. conicus. Future studies of both C. carolinianum and C. horridulum that limit herbivores, seed production, and seed dispersal could address the potential impacts that R. conicus may have on plant populations over time.

The evidence of the potential for R. conicus to use these species is tempered by the fact that no nontarget activity has been observed in naturally occurring populations of either species. It is important to note, however, that R. conicus was present in Nebraska but not observed using C. canescens in annual monitoring efforts for 16 yr before its initial documentation on this native species (Louda 1998). Monitoring of C. carolinianum and C. horridulum should be considered in land-management areas where conservation of native species is a priority. These monitoring efforts could provide early detection of nontarget feeding by R. conicus if it should occur on these native species and improve information on which to base appropriate management decisions.

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