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Occurrence of *Trichosiocalus horridus* (Coleoptera: Curculionidae) on Native *Cirsium altissimum* Versus Exotic *C. vulgare* in North American Tallgrass Prairie

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ABSTRACT Postrelease studies can provide data with which to evaluate expectations based on prerelease tests of biological control insects. In 2004, we observed *Trichosiocalus horridus* Panzer (Coleoptera: Curculionidae), the rosette weevil introduced into North America against Eurasian thistles, feeding on native tall thistle, *Cirsium altissimum* L. Spreng., in tallgrass prairie. In this study, we examined the rosette weevil's use of tall thistle, compared with its use of the co-occurring exotic bull thistle, *C. vulgare* (Savi) Tenore. For both thistle species, we quantified weevil frequency, abundance, and seasonal variation in incidence, using both timed observations at two sites over two growing seasons (2004, 2005) and dissections of thistle flowering shoots from 13 sites (2005). Based on prerelease information, we expected the Eurasian thistle to be the quantitatively preferred host plant for this Eurasian weevil. Instead, we found that both the frequency of infestation and the mean number of adult rosette weevils per plant were at least as high, and sometimes higher, on the native thistle as on the exotic thistle. Furthermore, adult weevil phenology coincided on the two host species. This study provides new quantitative evidence of nontarget feeding by another weevil released for thistle biological control; and it raises important questions for further research. We conclude that continued new releases, as well as augmentation of existing populations, of *T. horridus* should wait until more research is done on the impact of the nontarget occurrence now reported for this biological control insect.

KEY WORDS biological control of weeds, *Cirsium*, ecological risk assessment, *Trichosiocalus horridus*, weed control

Nonindigenous organisms can cause extensive damage in natural ecosystems and inflict significant economic losses in agricultural systems (National Research Council 2002, Pimentel et al. 2005). One control strategy for exotic plants has been the deliberate introduction of exotic insect species as weed biological control agents (Julien and Griffiths 1998). Debate over the adequacy of ecological risk assessments and the prediction of nontarget host use for such deliberate introductions continues (Louda et al. 2003a, b, 2005b; Hoddle 2004a, b; Louda and Stiling 2004; Messing and Wright 2006). Although a few reports of serious unintended ecological damage, such as population level nontarget effects, have been published (Louda et al. 1997, 2003b; Pemberton 2000; van Lenteren et al. 2006), the comprehensiveness of the re-

search on nontarget effects has been challenged (Howarth 1991; Simberloff and Stiling 1996, 2000). Furthermore, Thomas and Reid (2007) also reported recently that postrelease monitoring studies generally lack a quantitative assessment of biological control agent efficacy. For example, they found that, in Australia, 75% of postrelease studies to assess the impact of biological control agents recorded only effects on individual plant characteristics (i.e., growth and seed production), rather than effects on weed population densities. Thus, further information on postrelease performance and feeding by introduced biological control agents, such as this study of nontarget incidence of a thistle biological control agent, are warranted.

Biological control of thistles (*Carduus* spp., *Cirsium* spp.) has a long history in the United States (Goeden 1978, Julien and Griffiths 1998, USDA-ARS 2005). Recently, Pemberton (2000) found 23 known cases of nontarget feeding on species in the genus *Cirsium* in the continental United States, the Caribbean, and Hawaii. Population effects of such nontarget feeding on native thistles by two exotic insects used as thistle biological control agents have been reported. In the first case, Louda et al. (1997) reported extensive non-

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target impacts of *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae), the Eurasian flower-head weevil, on multiple species of native thistles in nature reserves and two National Parks in the central United States.

A rapid population increase by *R. conicus* occurred on both Platte thistle (*Cirsium canescens* Nutt.) and wavyleaf thistle [*C. undulatum* (Nutt.) Spreng.] after the weevil invaded Sand Hills prairie sites that had no exotic thistles (Louda 1998). Studies of this invasion have shown that *R. conicus* has significantly reduced seed production of both native thistles (Louda 2000; Louda and Arnett 2000; Louda et al. 2003a, 2005b) and that *R. conicus* is contributing to the observed population decline of Platte thistle (Rose et al. 2005). Furthermore, *R. conicus* now represents a potential quantitative threat to a close relative of Platte thistle, the federally threatened Pitcher's thistle, *C. pitcheri* (Torr.) Torrey and Gray (Louda et al. 2005a), a rare species in the United States and Canada around the Great Lakes of North America.

In the second case, Louda and O'Brien (2002) reported significant nontarget feeding by another exotic weevil, *Larinus planus* Fabricius (Coleoptera: Curculionidae). This adventitious Eurasian weevil was deliberately released in Gunnison, CO, against Canada thistle (*Cirsium arvense* L. Scop.). Release occurred after contemporary host specificity tests that showed that *L. planus* accepted but did not prefer large-headed North American thistles; based on this study, *L. planus* was not expected to affect native thistles (McClay 1990). However, *L. planus* is now more abundant on the native Tracy's thistle [*Cirsium undulatum* variety *tracyi* (Rydb.) Welsh] than on the targeted Canada thistle around Gunnison, and feeding on Tracy's thistle by *L. planus* caused large (58.1%) reductions in viable seed (Louda and O'Brien 2002). Such losses have significant, negative effects on key demographic parameters for populations of this already sparse native thistle (Dodge 2005).

Thus, quantitative evidence from these two case histories, emerging from research stimulated by serendipitous ecological observations rather than by planned postrelease monitoring studies, shows that the potential for nontarget effects can be underestimated in the standard prerelease assessments of insect host specificity (Gassmann and Louda 2001; Louda et al. 2003a, b, 2005a, b). Retrospective, post-release studies of such cases provide data with which to examine the relationship between predictions based on prerelease tests and subsequent field dynamics. Such information can be used to enhance design of prerelease studies for subsequent potential biological control agents to improve prediction of nontarget host use and impact under field conditions (Louda et al. 2003a, b).

The rosette weevil, *Trichosirocalus horridus* Panzer, was originally released in North America as a biological control agent against two Eurasian thistles, *Carduus nutans* L. and *C. macrocephalus* Desf. (Julien and Griffiths 1998). In 2004, we observed *T. horridus* feeding on native tall thistle (*Cirsium altissimum* L.

Spreng.) in the tallgrass prairie region of eastern Nebraska in the central Great Plains, United States. In this study, our first objective was to quantify the frequency of infestation and the abundance of *T. horridus* on tall thistle. Our second objective was to compare the weevil's use of the native tall thistle to its use of the co-occurring Eurasian bull thistle, *Cirsium vulgare* (Savi) Tenore.

We hypothesized that bull thistle would be the predominant host plant for the rosette weevil, based on prerelease tests and on their known interaction in Europe (Zwölfer 1988, Gassmann and Kok 2002). We expected the new native host plant to receive primarily "spillover" feeding from high populations of *T. horridus* on individuals near exotic host plants, as reported in other cases of nontarget feeding (Blossey et al. 2001, Rand and Louda 2004, Rand et al. 2004, Russell et al. 2007). We addressed three specific questions. First, what is the frequency of *T. horridus* occurrence on the native versus the exotic thistle species? Second, how does abundance of *T. horridus* per plant vary between the two thistles? Third, what are seasonal patterns of *T. horridus* abundance on the native versus the exotic thistle host species?

Materials and Methods

Natural History. *Trichosirocalus horridus*, the rosette weevil, was previously classified as *Ceuthorrhynchidius horridus* (Panzer); recently, it was divided into two sibling species: *T. briesei* sp. n. and *T. mortadelo* sp. n. (Alonso-Zarazaga and Sánchez-Ruiz 2002). For subsequent identification, we deposited voucher specimens from this study at the University of Nebraska State Museum, Division of Entomology. The rosette weevil was introduced into the United States from Italy in 1974 against weedy Eurasian species of *Carduus* and *Cirsium*, after prerelease host specificity testing (Ward et al. 1974, Kok 1975). Official releases are reported for 17 states (USDA 1996), but no records are available for Nebraska (M. Coffin, personal communication). Establishment of *T. horridus* at the initial release sites in Virginia was confirmed by 1977 (Kok and Trumble 1979), and feeding on the co-occurring native field thistle, *Cirsium discolor* (Muhl. ex Willd.) Spreng., was reported (McAvoy et al. 1987). We found no other published reports of nontarget feeding by *T. horridus* on any other native North American thistle.

The biology of *T. horridus* has been summarized recently (Gassmann and Kok 2002, Piper and Coombs 2004). Adults are active in spring and fall, and oviposition occurs early, most likely in May in Nebraska when plants are emerging. Eggs are deposited into the midrib or main veins on the underside of thistle leaves, especially on young rosettes. Time to egg hatch is on the order of 2 wk; larvae develop and mature in 6–8 wk and pupate in the soil. The larval stage is the most destructive, because larvae feed on the meristematic tissues in the center of rosettes, causing tissue necrosis and often delaying flowering. We observed *T. horridus* adults feeding on developing young leaves and vegetative shoots of both tall and bull thistles. Adults es-

tivate during the summer heat and become active again in fall. There is one generation per year.

Bull thistle (*C. vulgare*) is a Eurasian species that has invaded many regions of North America since its introduction during colonial times (Great Plains Flora Association 1986, Randall and Rejmanek 1993). Although bull thistle is listed as a noxious weed in two states adjacent to Nebraska—Colorado and Iowa (USDA 2005), it is not a listed weed in Nebraska (McCarty et al. 1967, Stubbendieck et al. 1994). Tall thistle (*C. altissimum*) is a native North American species. It is the most common native thistle in eastern Nebraska (Great Plains Flora Association 1986), but plants occur only sparsely in small patches (Andersen and Louda 2008).

Both thistles are taprooted, monocarpic, short-lived perennials usually found in roadsides, ditches, and disturbed areas (Great Plains Flora Association 1986, Silvertown and Smith 1989). Once juvenile rosettes reach flowering size, they bolt (form an inflorescence-bearing stem), flower, and die. The two thistles are similar in flowering phenology in Nebraska, bolting May–late June and flowering late July–early October (Great Plains Flora Association 1986). Both species depend strictly on seed production for reproduction (Silvertown and Smith 1989, Jackson 1998).

Study Sites. We used two open grassland sites in Lancaster County, NE, for weekly timed observations of insect abundance through the growing seasons of 2004 and 2005. These were Sutton Farm (40°52'33.13" N; 96°31'10.95" W) and Pioneers Park Nature Center (40°46'34.44" N; 96°47'10.03" W). Both sites were surrounded by fields and stands of tallgrass prairie. At these sites, tall thistle and bull thistle co-occurred in small "mixed patches," allowing us to compare insect abundances on the two thistles in the same local environment. Although both thistles were relatively abundant at both sites in 2004, the numbers of bolting bull thistles unexpectedly decreased at Sutton Farm in 2005. Although more mixed patch sites would have been useful, their availability in eastern Nebraska is limited (Andersen and Louda 2008).

Weevil use of both thistles also was studied at 11 additional sites in Lancaster County, NE, in 2005. These sites had single-species patches of thistles, which we call "isolated patches"; each isolated patch was comprised solely of only one of the two thistle species ($n = 6$ for tall thistle; $n = 5$ for bull thistle; exact site locations in Takahashi 2006). Initially, we categorized each isolated patch as dense (≥ 0.2 thistles/m²) or sparse (< 0.2 thistles/m²). Mean density of the dense patches was 0.7 ± 0.08 for tall thistle ($n = 3$) and 9.1 ± 8.65 for bull thistle ($n = 2$). Mean density of the sparse patches was 0.2 for tall thistle ($n = 2$) and 0.1 for bull thistle ($n = 2$), including estimated density at two sites that were mowed in midsummer ($n = 1$ for each thistle species).

Sampling Design

Timed Observations. The occurrence of adult rosette weevils on the two host plant species was quan-

tified using weekly timed observations on plants at the two mixed patch sites. At each site early in the flowering season (26 June 2004, 16 May 2005), 10 bolting plants of each thistle species were randomly selected and marked for observation. Each week through both seasons (to 28 August 2004 and 15 October 2005), each marked plant was observed for 5 min between 1700 and 1900 hours. Additional times of observation (0900–1100 and 1300–1500 hours) in 2004 showed similar patterns (Takahashi 2006); however, because these data were not independent estimates of daily insect occurrence, they are not presented here.

Thistle Shoot Dissections. Because hidden adults and internally feeding larvae cannot be detected by observation in situ, in 2005, we also collected thistle flowering shoots for dissection. Each shoot was a flower head plus its subtending stem. We counted both *T. horridus* adults on, and curculionid larvae in, per shoot separately. We were not able to rear any weevil larvae to eclosion in this study, so curculionid larval identity could not be determined unambiguously. However, only one other adult weevil was observed feeding on these two thistles: the native weevil *Baris* sp. nr. *subsimplis* Casey. Consequently, as a first report, we present the total curculionid larval counts, which represent the larvae of both weevils. If the larvae occur in the same proportion as the adults, 28.1% of the larvae would be expected to be *T. horridus* ($n = 114$ total adult weevils). Because no observational bias by plant host species or by stand type should occur in these counts, comparison between native and exotic thistle hosts provides baseline data for future research. We also examined internal feeding damage; however, it could not be used as evidence of *T. horridus* occurrence because the internal damage caused by this weevil was not consistently distinctive (Takahashi 2006). Thus, adult counts are unambiguous, but larval counts and feeding evidence must be interpreted with caution until further research is done.

Shoot samples of two types (random, damaged) were collected for dissection in all 13 sites—2 mixed-patch sites and the added 11 isolated-patch sites—at 3-wk intervals in 2005 (16 May–30 September). First, at each site, we collected one flowering shoot, generally the main branch, from each of five randomly selected plants from one (isolated patch) or both (mixed patch) thistle species ($n = 5$ shoots per species per site per date; total $n = 75$ for bull thistle and total $n = 79$ for tall thistle). Second, we collected a sample of shoots with external evidence of damage (added shoots: $n = 5$ damaged shoots per species per site per date; total $n = 70$ for bull thistle and total $n = 70$ for tall thistle). The aim in collecting damaged shoots for dissection was to correlate specific types of external damage with the presence of particular internal-feeding herbivores.

Statistical Analyses. The weekly observational count data were used to compare adult rosette weevil infestation frequencies on the native thistle to those on the exotic thistle. We first scored each plant for presence/absence of adult weevils and then we compared the

frequencies on the two host plant species. Infestation frequency was defined as the number of plants per host species with *T. horridus* adults present on each date. We tested this measure of adult weevil frequency between the two thistle species using the Wilcoxon two-sample test. We also compared differences in adult weevil abundances, defined as the mean number of adult weevils per plant, between the two thistle species, using Student's *t*-test for two samples with unequal variances. Finally, to compare seasonal patterns in adult weevil abundance between the two thistle host species, we plotted and evaluated mean number (SE) of *T. horridus* weevils observed per plant each week on each thistle species at each site.

Additional information on frequency and abundance of cryptic adult weevils, as well as evidence on the occurrence of curculionid larvae, by host plant species was provided by dissection of the shoot samples collected in 2005. The dissection data were from the first two sampling periods in 2005 (16–20 May and 5–7 June) because no *T. horridus* were observed in the third survey (27–30 June) or later in the season. For the shoot samples, we first tested the effect of host plant species (tall, bull), patch density (dense, sparse), and their interaction on the abundance of cryptic adult weevils and separately on the abundance of internally feeding curculionid larvae, using a two-way factorial analysis of variance (ANOVA); we found no interaction effects (Takahashi 2006). Also, we tested whether there was an interaction among patch density, patch type (single-species, mixed), and shoot sample type (random, damaged) in explaining the frequency or abundance of *T. horridus* adults, or separately of curculionid larvae, using the log likelihood *G* test. Again, we found no interactions (both $G < 0.02$, $P \gg 0.20$; Takahashi 2006). Thus, we pooled the shoot dissection data for weevil adults, and separately for weevil larvae, by host plant species across all patches and sites in the analyses of overall patterns of weevil adult or of weevil larval, frequency, and abundance by thistle host species.

To compare frequencies of cryptic adult weevils on native versus on exotic thistle species, we calculated the proportion of all sampled shoots per host species that were infested in each site on each sampling date. We tested the mean difference in proportion infested (arcsine-transformed) between the two thistle host species using a two-sample *t*-test. To compare the relatively low, non-normal mean abundances of cryptic adult weevils on shoots of the two thistle hosts, we used the Wilcoxon test. In this case, we first examined shoot samples from the two sampling methods (random, damaged) separately; then we pooled sample types and evaluated the overall patterns between the two thistle host species.

To compare curculionid larval infestation frequencies in shoots of the two host plants, we used Student's *t*-test on the observed proportion of shoots infested (arcsine-transformed). To compare mean number of curculionid larvae in the pooled shoot samples for the native tall thistle versus the exotic bull thistle, we used the nonparametric Wilcoxon test (as above). Finally,

when marginal statistical differences occurred, we also calculated statistical power ($1 - \beta$), which is the probability of detecting a difference between the two means, using the estimated SD ($\hat{\sigma}$) from a nonparametric one-way ANOVA. All statistical tests were done using SAS v.8.2 (SAS Institute 2005).

Results

Occurrence and Phenology of Adult *T. horridus* in Timed Observations. At the two main study sites, *T. horridus* adults occurred on both thistle species in mixed patches. In 2004, the first adult was observed in the last week of June, and no *T. horridus* adults were observed after the third week in July (Fig. 1). At Sutton Farm, the average number of *T. horridus* per plant declined after the first observation on the native tall thistle and on the exotic bull thistle (Fig. 1A). At Pioneers Park, *T. horridus* was observed only in late June and only on the native thistle (Fig. 1B). Overall, the frequency of weevil occurrence on native tall thistle was not distinguishable from that on the exotic bull thistle (Table 1).

In 2005, when sampling began earlier (16 May 2005 versus 26 June 2004), the first adult was observed a week earlier, in the third week of June; no *T. horridus* adults were observed after the last week in June (Fig. 1C and D). The numbers of adult *T. horridus* observed on plants peaked again in mid- to late June (Fig. 1). However, peak abundance of *T. horridus* adults per plant was lower and a week earlier in 2005 than in 2004, with the pattern clearest at Sutton Farm (Fig. 1). At Sutton Farm, there were no statistical differences in the frequency (Table 1) or abundance (Fig. 1C) of *T. horridus* adults per plant between native and exotic thistle hosts. At Pioneers Park, however, *T. horridus* adults in 2005 occurred earlier than in 2004 (Fig. 1), and they were marginally more frequent on bull thistle than on native tall thistle in the second week of June (Table 1; $P = 0.093$; power $[1 - \beta] = 0.460$). Overall, adult *T. horridus* frequency and abundance in the mixed patches generally were similar on the native thistle as on the exotic thistle in both years.

Frequency and Abundance of *T. horridus* Adults in Shoot Samples From 13 Sites. In the randomly sampled shoots from all 13 patch sites in 2005, cryptic (hidden) *T. horridus* adults occurred on native tall thistle as frequently and abundantly as on exotic bull thistle (Fig. 2). Few *T. horridus* adults were found in May, early in the growing season, and all were on bull thistle (Fig. 2A). In June, however, the frequency of *T. horridus* adults on sampled shoots trended toward being higher on native tall thistle than on bull thistle (Fig. 2A), although the difference was not statistically significant given the low power of the test with the sample sizes available ($t_{12} = -1.45$; $P = 0.17$; power $[1 - \beta] = 0.268$). Similarly, adult weevil abundance per random shoot was low on both thistle species, but also trended toward being higher on the native tall thistle than on bull thistle; again, these means were not statistically different, likely reflecting the low power of the test (Fig. 3A; $z = -1.23$, $P = 0.22$; power $[1 -$

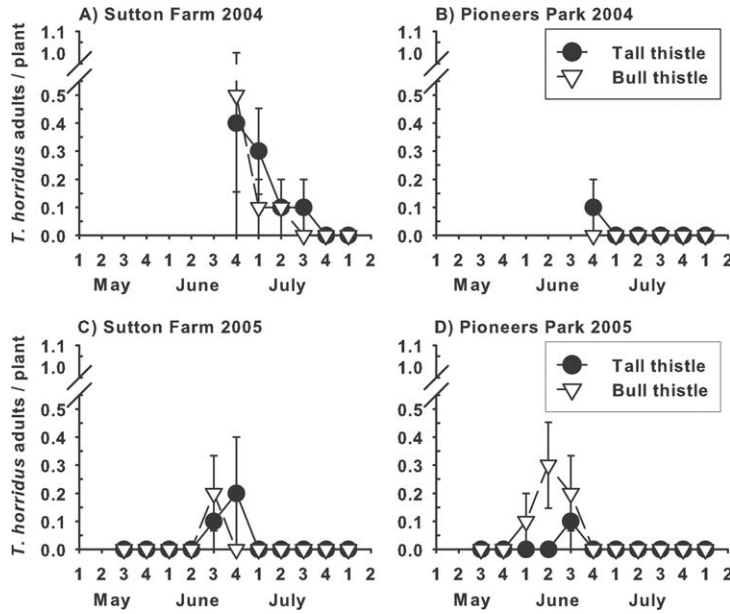


Fig. 1. Seasonal pattern of occurrence of *T. horridus* adults on both native tall thistle (*C. altissimum*) and exotic bull thistle (*C. vulgare*) in mixed species patches at the two main sites in eastern Nebraska tallgrass prairie. Mean number of *T. horridus* adults per plant was sampled using 5-min observation periods ($n = 10$ plants per species per date) in 2004 at (A) Sutton Farm and (B) Pioneers Park Nature Center and in 2005 at (C) Sutton Farm and (D) Pioneers Park Nature Center. No *T. horridus* were observed later than mid-July in either season.

$\beta] = 0.277)$. Overall, the results showed that *T. horridus* adult abundance was at least equal on the native tall thistle to that on the targeted bull thistle; in fact, both frequency and abundance actually trended toward being greater on the native, nontarget thistle than on the exotic, targeted species.

On the damaged shoots, those with external evidence of insect feeding, cryptic *T. horridus* adult numbers in general were at least equal on the native tall thistle as on bull thistle and trended toward being higher on the native species overall (Fig. 2B; $t_{12} = -1.79$; $P = 0.09$; power $[1 - \beta] = 0.383$). In early season (May), *T. horridus* adults in these samples were

found only on tall thistle and not on bull thistle (Fig. 2B), which is opposite of the pattern for the random shoot samples. In fact, per damaged shoot, the abundance of cryptic adult weevils was significantly greater on native tall thistle than on bull thistle (Fig. 3B; $z = -2.18$; $P = 0.03$), and this difference was greater in June than in May (Fig. 2B). Finally, when the shoot samples from randomly sampled and damaged sampled shoots were analyzed together, we again found equal, or greater, *T. horridus* adult infestation frequency (Fig. 2C; $t_{43,2} = -1.96$; $P = 0.06$) and abundance (Fig. 3C; $z = -2.33$; $P = 0.02$) on the native tall thistle than on bull thistle.

Table 1. Probability ($P < Z$) that the frequency of *T. horridus* adults on native tall thistle (*Cirsium altissimum*) plants was equal to that on the co-occurring Eurasian bull thistle (*C. vulgare*) plants each week at the two main study sites (Wilcoxon two-sample test)

	Week								
	May		June				July		
	3	4	1	2	3	4	1	2	
2004									
Sutton Farm	—	—	—	—	—	1.000	0.314	1.000	0.379
Pioneers Park	—	—	—	—	—	0.379	—	—	—
2005									
Sutton Farm	—	—	—	—	0.589	0.379	—	—	—
Pioneers Park	—	—	0.379	0.093	0.589	—	—	—	—

Infestations of *T. horridus* adults were assessed using 5-min observations per plant for 10 plants of each species per week at Sutton Farm and Pioneers Park. Dash indicates data not available. The results show that, over the season in both years, the frequency of *T. horridus* generally was equal on the native and exotic thistles.

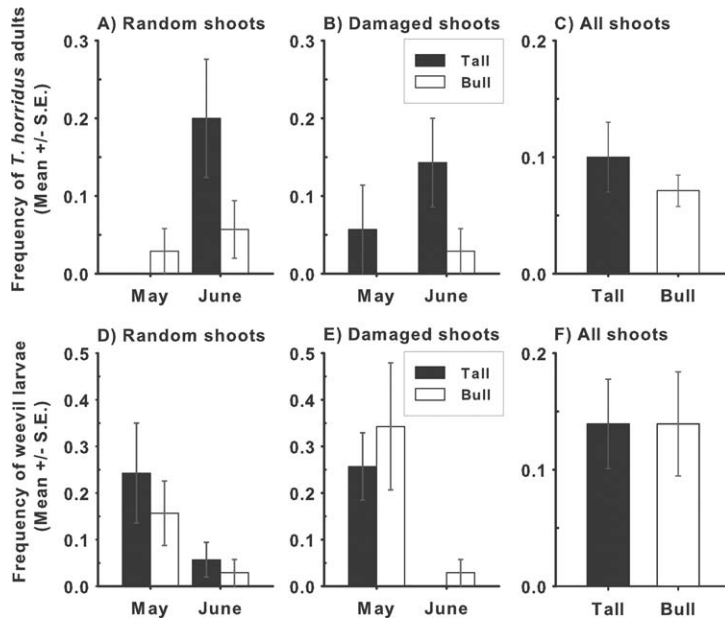


Fig. 2. Mean frequency of infestation by *T. horridus* adults and by curculionid larvae for dissected flowering shoots of native tall thistle, *C. altissimum*, or exotic bull thistle, *C. vulgare*, by shoot sample type and overall in 2005. Shoots were sampled from two main sites with mixed species patches plus 11 single species ("isolated") patches ($n = 6$ for tall thistle; $n = 5$ for bull thistle) in Lancaster County, NE. Mean frequency of *T. horridus* adults on (A) randomly sampled shoots, (B) shoots with evidence of insect feeding, and (C) randomly sampled and damaged shoots both combined. Mean frequency of curculionid larvae inside (D) randomly sampled shoots; (E) damaged shoots, showing evidence of insect feeding; and (F) randomly sampled and damaged shoots combined. Data were arcsine-square root transformed for the statistical analyses. Means (SE) presented are back-transformed values.

Frequency and Abundance of Curculionid Larvae.

Both frequency of shoot infestation and abundance of curculionid larvae were similar on the two thistle hosts in 2005. First, in randomly collected shoots, mean frequency of curculionid larvae per shoot was equal for tall and bull thistles, both in May ($t_{12} = -0.61$; $P = 0.51$) and in June ($t_{12} = -0.61$; $P = 0.55$), with higher frequencies of infestation in May than in June (Fig. 2D). Although curculionid larvae tended to be more abundant on tall thistle than on bull thistle in these samples (Fig. 3D), the observed difference was not statistically significant ($z = -0.9$; $P = 0.36$; power $[1 - \beta] = 0.232$).

Second, in the damaged shoots, the mean infestation frequency of curculionid larvae per shoot also was equal for tall and bull thistles in May 2005 ($t_{12} = 0.56$; $P = 0.58$) when the majority of the larvae were found (Fig. 2E). Additionally, we found no evidence of any difference in the abundances of weevil larvae (number per shoot) between the two host species in damaged shoots (Fig. 3E; $z = 0.81$; $P = 0.42$). Similarly, we did not find any difference in the mean infestation frequencies (Fig. 2F; $t_{54} = -0.09$; $P = 0.93$) and abundance of weevil larvae (Fig. 3F; $z = -0.72$; $P = 0.47$) on the two host plants when the shoot samples from the two sampling methods were combined. The relative proportion of *T. horridus* larvae to native weevil larvae remains unknown, because no curculionid larvae were reared successfully. However, the striking

result in these data are that the frequency and abundance of curculionid larvae were equal between shoots of the native and exotic thistle hosts, consistent with the frequencies and abundances of adult *T. horridus*.

Discussion

Host Plant Occurrence of *T. horridus*. The field data showed that *T. horridus* is now using a newly acquired native host plant, tall thistle (*C. altissimum*), as frequently and intensively as it uses the targeted, exotic host plant, bull thistle (*C. vulgare*) in tallgrass prairie. Contrary to our initial expectation for a Eurasian weevil, we found no difference in the frequency, abundance, or phenology of *T. horridus* use of the native tall thistle compared with that of the Eurasian bull thistle. Both frequency and abundance of *T. horridus* adults per plant were similar for both thistles in all field observations and dissection data, and often might have been higher on the native thistle had the power of the inconclusive tests been higher. Our ability to detect differences, given the variances and sample sizes, was low for some of the statistical tests. However, the consistent trends toward greater abundances on the native thistle suggest that our conclusion of "no difference" is conservative. Also, although the temporal occurrence of *T. horridus* adults varied between years and sites, the patterns were consistent by thistle spe-

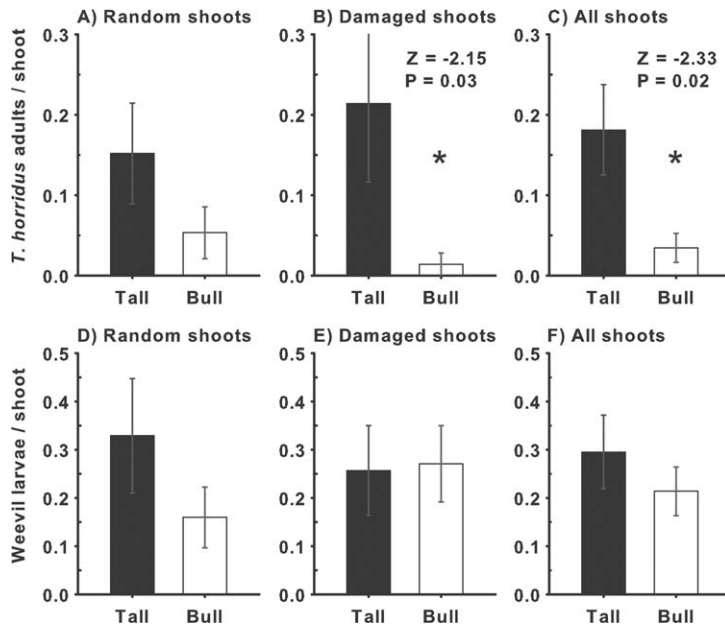


Fig. 3. Abundance (SE) of *T. horridus* adults and curculionid larvae per shoot for collected shoots of the native tall thistle, *C. altissimum*, and its exotic host, bull thistle (*C. vulgare*). Samples were collected from the 2 mixed species patches and 11 isolated single thistle species patches ($n = 6$ for tall thistle; $n = 5$ for bull thistle) in eastern Nebraska in 2005. Mean number of *T. horridus* adults per shoot from: (A) randomly selected shoots; (B) shoots with evidence of insect feeding; (C) randomly sampled and damaged shoots both combined; mean number of curculionid larvae per shoot from (D) randomly selected shoots; (E) shoots with evidence of insect feeding; and (F) randomly sampled and damaged shoots both combined. Probabilities of the observed differences between the host plant species were computed by using Wilcoxon two-sample rank tests.

cies between growing seasons. Furthermore, no evidence emerged of higher *T. horridus* occurrence on the targeted exotic thistle host under field conditions. Thus, this study contributes new quantitative evidence showing equivalent use by *T. horridus* of a second, nontargeted, newly acquired, native, host plant compared with its use of a co-existing, targeted, Eurasian, host plant.

Our findings for tall thistle parallel the early evidence for *T. horridus* feeding on native field thistle (*C. discolor*) in Virginia in the single previous report of nontarget feeding by this weevil (McAvoy et al. 1987). In that case, native field thistle experienced variable levels of infestation 1981–1985. Frequency of *T. horridus* averaged 6% of the field thistles examined, compared with 20% of bull thistle, 54% of musk thistles (*C. nutans*), and 20% of plumeless thistles (*Carduus acanthoides* L.) (McAvoy et al. 1987). However, in 1982, the native thistle was more heavily infested by *T. horridus* (44%) than any of the targeted thistle species. In our study, 20 yr later on a different native thistle, the proportions were similar: an average of 5.1% of the tall thistles observed in the field were infested (8.4% in 2004; 2.5% in 2005) and 9.4% of the tall thistle shoots dissected in 2005 had *T. horridus* adults. These results extend the earlier findings of nontarget feeding by *T. horridus*. Both studies showed that native *Cirsium* species can be as or more susceptible to *T. horridus* as the targeted Eurasian species, and both document the

variability in the level of *T. horridus* infestation between years. Together, the data available suggest *T. horridus* has the potential of imposing an equivalent effect on native species as on the related targeted weed. Consequently, we conclude that further research, involving monitoring and quantification of the interaction strength and conditions determining the variation in the interaction of *T. horridus* with native *Cirsium* species, is merited before further spread or augmentation of this biological control agent.

Questions Raised by the Added Evidence of Nontarget Feeding by *T. horridus*. The data here raise at least three important questions and issues. First, do the data here represent the beginning stage or the stable stage of *T. horridus* population growth and use of nontarget native hosts in this region? Because no records of releases of *T. horridus* in Nebraska are available (M. Coffin, personal communication), it is unknown how long *T. horridus* has been established in Nebraska. Given the high dispersal capability reported for *T. horridus* adults (McAvoy et al. 1987, Piper and Coombs 2004), and our evidence that *T. horridus* occurred on tall thistle in isolated patches, our findings may represent a new geographic expansion or invasion. Alternatively, if present conditions represent an older set of colonizations, the weevil population either is still in a lag phase of population growth or is limited by environmental conditions. Thus, the first issue raised by this study is the need for more information

to differentiate these alternatives and to predict population growth of *T. horridus* on native tall thistle populations in tallgrass prairie.

Second, does *T. horridus* feeding on tall thistle, especially in combination with that quantified for native herbivores (Guretzky and Louda 1997, Jackson 1998, Louda and Rand 2002), contribute to a reduction in its flowering, seed production, and population density? Damage caused by insect herbivores on developing tall thistle rosettes and flowering shoots is extensive (Guretzky and Louda 1997), and it can cause mortality, abortion of flowering shoots, and reduction of reproductive success (Young 2003; T. Suwa and S. Louda, unpublished data). Because floral damage and seed losses by native insect herbivores limit seed reproduction of both tall and bull thistles in this region (Jackson 1998, Louda and Rand 2002, Tenhumberg et al. 2008) and because recruitment of these thistle species is limited by seed availability (Young 2003; J. Eckberg et al., unpublished data), we hypothesize that increasing foliage and reproductive losses could negatively affect tall thistle population size. Further research on the demographic consequences of adding *T. horridus* to the herbivore guild of tall thistle is required to evaluate this hypothesis and the impact of the nontarget use reported here.

Finally, what are the implications of these data for ecological risk assessments, both contemporary reassessment of risk from *T. horridus* and future assessments for other potential biological control agents? The results here clearly support the suggestions (Louda et al. 2003a, b, 2005a, b) that additional ecological data, such as evidence on the parameters determining host use and choice under varied environmental conditions, could improve prediction of such nontarget interactions. Tests of host specificity, along with records of host incidence in the indigenous region, have been shown to be necessary but not sufficient to predict some patterns of host use by biological control agents in the field (Louda et al. 1997, 2005a; Arnett and Louda 2002; Louda and O'Brien 2002). Nor can individual preference and performance of insects under quarantine conditions predict interaction strengths and population consequences under field conditions in a new environment (Louda et al. 2003a, b, 2005b; Sheppard et al. 2005). Furthermore, community-level interactions, including indirect effects or lagged cumulative negative impacts, cannot be predicted from laboratory results (Crooks and Soulé 1999, Pearson et al. 2000, Louda et al. 2005b, Simberloff 2005). These observations and the results presented here argue for further research on potential nontarget effects to improve prediction of outcomes postrelease. The findings here, consistent with the earlier data, strongly suggest that, under field conditions, native thistles can be as vulnerable to feeding and nontarget impact by this biological control agent as are the targeted Eurasian thistles. Thus, we conclude that further distribution of *T. horridus*, or augmentation of existing ineffective populations, should stop until more research on the potential quantitative impacts of *T. hor-*

ridus on native thistles, as well as exotic thistles, are conducted.

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