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Proceedings of the X International Symposium on Biological Control of Weeds
4-14 July 1999, Montana State University, Bozeman, Montana, USA
Neal R. Spencer [ed.]. pp. 551-567 (2000)

Predicting Non-Target Ecological Effects of Biological Control Agents: Evidence from *Rhinocyllus conicus*

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Abstract

The discovery and quantification of significant non-target ecological effects of *Rhinocyllus conicus* on native species in the northcentral USA provides the opportunity to experimentally evaluate factors that might help predict non-target host plant use, magnitude of direct impact with transference, and indirect effects mediated by trophic interactions. The relevance for biocontrol risk assessment of at least four important ecological relationships has emerged from these studies so far. These relationships include the role played in interaction outcome and ecological risk by: 1) ecological similarity of potential host plants, as well as phylogenetic distance; 2) synchrony of critical stages between insect and potential host plant(s), as well as acceptability; 3) population limiting processes of potential host plants; and, 4) overlap of feeding niche within the native guild of species dependent upon the host plants. In addition, this case makes it clear that host specificity and preference/performance criteria are necessary but insufficient to quantify potential ecological risk. In the selection of biocontrol agents, knowledge of the ecological relationships should help to quantify the risks inherent in deliberate introductions of new species.

Keywords: insect-plant, biological control of weeds, indirect effects, Curculionidae, ecological interactions, plant population dynamics

One of the greatest challenges for science is to understand nature, and specifically the interactions and interconnections of the natural world, well enough to predict the outcome of altering environmental conditions or community composition. Thus, the fundamental challenge of predicting the outcome of deliberate introductions to suppress the population growth of an exotic species, which underlies both success and risk in classical biological control, is basically an ecological one. Such prediction is clearly a difficult assignment, since it requires a conceptual understanding of the population consequences of interactions within communities as well as knowledge of the evolutionary and phylogenetic background influencing species interactions. We are only now beginning to develop such understanding.

Various strategies have been proposed to improve the predictive understanding required for successful, safe biocontrol. We suggest that experimental assessment of ongoing biological control projects, specifically including the study of related, potential non-target species and their dependent consumer guilds, is an under-utilized approach to enhancing our predictive ability. Further examination of population and community dynamics within projects that are already established represents an efficient strategy to improve both the success rate and the safety of future biocontrol efforts. Systematic observations of the patterns, plus experimental tests of the factors hypothesized to determine

the outcome of interactions, are needed both in cases where biological control has succeeded and in cases where it has directly or indirectly fallen short. Such studies should provide a basis for increased success and decreased risk in future biocontrol efforts.

The quantification of significant non-target ecological effects of the flowerhead weevil (*Rhinocyllus conicus* Froel.), released for the biological control of musk thistle (*Carduus nutans* L.), in combination with extensive data on the weevil's biology, provides a special opportunity to apply this approach. Here, we summarize evidence from our studies of thistles and *R. conicus* and use it to illustrate the utility of intensive, ecological studies in biological control.

At least four ecological relationships have emerged as relevant to understanding and predicting the host range expansion of *Rhinocyllus conicus* onto native *Cirsium* species, Platte thistle (*C. canescens* Nutt.) and wavyleaf thistle (*C. undulatum* Spreng.) in sand prairie. These relationships, and the ecological principles underlying them, should provide further insight into risk assessment and prediction of nontarget effects with other biocontrol agents. The principles revolve around the discovery of the role played in species interactions and ecological risk by: 1) ecological similarity of potential host plants, as well as phylogenetic distance; 2) phenological synchrony of critical life history stages between insect and potential host plant(s), as well as acceptability; 3) population limiting processes of potential host plants; and, 4) overlap of feeding niche within the native guild of species dependent upon the host plants.

The "rules of thumb" that can be derived from these relationships and our results in the study of this specific case, thus, revolve around the clear need to gather ecological data on potential nontarget species identified through host specificity testing. First, we need to believe the data from our standard feeding, oviposition, and preference-performance tests; if potential nontarget feeding is found, the possible consequences need to be studied further. Both "choice" and "no choice" tests provide valuable information. Although alternative potential host species were evaluated in the early studies of *Rhinocyllus conicus*, no further intensive study was undertaken to quantify the conditions under which such interactions could have a significant effect on native populations (Gassmann and Louda 2000). Environmental safety considerations suggest we now assume that host species accepted in feeding trials, and especially those on which early larval development can occur, need further assessment of potential population impacts to quantify this dimension of environmental safety.

Second, it is possible to identify the more vulnerable nontarget species within the array of phylogenetically-related ones, using ecological criteria. These are the nontarget species that should be used for "choice"/"no choice" feeding, oviposition, and preference-performance tests if the goal is to quantify ecological risks. Ecological traits, such as the phenology, morphology, life history and structure of dependent feeding guilds, help explain the patterns we have found and the quantitative impact we have documented. As far as we can tell, the nontarget species evaluated in the case of *R. conicus* were chosen from among the phylogenetically-related species by economic importance and by accessibility rather than by ecological criteria. Ecological criteria would have identified the higher vulnerability of Platte thistle, *Cirsium canescens*, over that of a related, co-occurring wavyleaf thistle, *C. undulatum*, in our system.

And, third, our results suggest that we need to measure the population and demographic consequences of augmenting the feeding guild on vulnerable nontarget native species. This will include a determination of limiting factors for the plant population and

better quantification of the dependent species with life histories that are phenologically synchronized on the required resources. Our data to date suggest that if populations of potential nontarget species are limited by their consumers under normal conditions, then the addition of another consumer has a high probability of having a significant ecological effect on an acceptable nontarget plant species and its dependent species.

In order to build the case for these recommendations, we briefly review some of the natural history of the species involved. Then, we summarize the data available on the population dynamics of the native species prior to the establishment of *R. conicus,* and the evidence that is now available as a baseline from which to assess the population growth and impact of *R. conicus* on the native species in our system. These data provide the foundation for the generalizations suggested on critical ecological relationships involved in the development of nontarget effects, and on ways to quantify those potential effects prior to the decision to release.

Natural History Background

Study Sites and Plants. Our research on thistles summarized here was performed 1984-1999 at two preserves that are 330 km apart in Nebraska, Arapaho Prairie (Arthur Co.) and Niobrara Valley Preserve (Brown Co.), and at the University of Nebraska's Cedar Point Biological Station in Ogallala, NE. Both preserves contain characteristic sandhills prairie vegetation (Keeler *et al.* 1980, Kaul 1989). Arapaho has not been grazed since 1978, whereas our sites at the Niobrara have been continuously grazed by cattle at a moderate stocking rate.

Two native thistles commonly co-occur in sand prairie in this region. Wavyleaf thistle (*Cirsium undulatum* (Nutt.) Spreng.) occurs in various grassland types from Texas to southern Canada. The range of the second species, Platte thistle (*C. canescens* Nutt.), is more restricted. Platte thistle occurs only in prairie on sands and gravels in the upper Missouri River drainage of the upper Great Plains in central and western Nebraska, eastern Wyoming and northeast Colorado (Great Plains Flora Asso. 1986). Since distributions of both are local and patchy (Great Plains Flora Assoc. 1986) neither is a major weed (McCarty *et al.* 1967, McCarty and Scifres 1967). In Nebraska, musk thistle, *Carduus nutans* L. subsp. *leiophyllous* (Petr.) Stoj. & Stef. (Kartesz 1998), is concentrated on moister, loamy soils, especially in the eastern and south-central parts of the state.

All three thistles are tap-rooted, short-lived perennials that grow as rosettes for some years before they bolt, flower, and set seed. Wavyleaf thistle is iterocarpic in our region, flowering several times before dying (McCarty *et al.* 1967). Platte thistle is monocarpic, growing as a rosette for 1 - 5 yr., flowering, and then dying after its sole reproductive episode (Lamp and McCarty 1981, Louda and Potvin 1995). Musk thistle is described as biennial (Great Plains Flora Asso. 1986). The phenological sequence of flowering among these thistles in our area is: Platte and musk and, then later, wavyleaf (Louda 1998b, A.E. Arnett and S.M. Louda, unpublished data).

Native Insects. A characteristic set of insects feeds on the inflorescences of thistles (Zwoelfer 1965, 1988; Redfern 1983). At least five native insects commonly feed on or in developing flowerheads of the two native thistles at our sites. These are: (i) two picture-winged flies (*Paracantha culta* Wiedeman, *Orellia occidentale* [Snow]: Tephritidae); (ii) two pyralid moths, *Pyrausta subsequalis plagialis* Haim. (Subfamily Pyraustinae) and *Homeosoma [stypticellum] impressale* Hulst (Subfamily Phycitinae); plus (iii) adults of a native weevil, *Baris subsimilis* Casey (Curculionidae), whose larvae feed internally on

stems and roots (Lamp 1980, Louda and Potvin 1995, S.M. Louda, unpub. data). There are no published records of these native insects developing on flowerheads of the exotic *Carduus* spp. in Nebraska (Lamp 1980, Lamp and McCarty 1982a,b,c).

On Platte thistle, three insects feed sequentially within the main flower heads (Lamp and McCarty 1979, 1982a,c). The first is the tephritid *P. culta,* which oviposits on unopened 10 - 16 mm heads (Lamp and McCarty 1982a,c), heads that are also used by *R. conicus* (S.M. Louda and A.E. Arnett, unpublished data). Next, the less common fly (*O. occidentalis*) oviposits on larger flowerheads (18 - 22 mm) that are just opening (Lamp and McCarty 1979, 1982a,c). Finally, the larger pyralid moth, *H. impressale*, oviposits among the florets of larger, open heads (Lamp and McCarty 1979, 1982a,c).

On wavyleaf thistle, the native weevil (*B. subsimilis*) occurs first and commonly causes abortion of the initial, terminal flowerhead (S. M. Louda, T. Tesar, and J. Burger, unpublished data). The most common tephritid on wavyleaf thistle is *O. occidentalis*, since it is typically more temporally synchronous with the development of wavyleaf flowerheads than is *P. culta*. However, the pyralid moth, *H. impressale*, does the most damage to wavyleaf thistle flowerheads among the native insects. In some years, many of the flowerheads that develop, most of which look undamaged externally, contain only seed fragments and frass, unambiguous evidence of feeding by this pyralid (S.M. Louda, personal observation).

Introduced Biocontrol Agent. The flowerhead weevil (*Rhinocyllus conicus* Froel.) was introduced into the USA from Europe in 1969, and into Nebraska in 1972, to limit seed production by invasive Eurasian *Carduus* spp. thistles (Kok and Surles 1975, Rees 1977, 1991, Surles and Kok 1978, McCarty and Lamp 1982). Redistribution of *R. conicus* within the USA continues (e.g., Boldt and Jackman 1993). Although *R. conicus* is common in inflorescences of thistles in Europe, it is out-competed by other weevils (Zwoelfer 1978). Overwintered adults of *R. conicus* congregate on thistles early in spring to mate and oviposit (Rees 1982). Development to adult stage takes 6 - 9 weeks (Rees 1982). *Rhinocyllus conicus* oviposition and larval development overlaps most with that of *P. culta*.

So far, the weevil has been reared from flowerheads of a third of the native *Cirsium* spp. in California (Pemberton *et al.* 1985, Goeden and Ricker 1986a,b, 1987a,b; Turner *et al.* 1987; Turner and Herr 1996; Palmisano and Fox 1997), from half of the six native thistle species in Rocky Mountain National Park, Colorado (*C. centaureae* (Rydb.) K. Schum., *C. scopulorum* (Greene) Cockll., and *C. undulatum*) (Louda *et al.* 1997), from *C. undulatum* in Colorado, Montana, Nebraska and South Dakota (Rees 1977, 1991, Louda *et al.* 1997), and from *C. canescens* in Nebraska and Wyoming (Louda *et al.* 1997, S.M. Louda, unpublished data). Furthermore, numbers on native thistles appear to be growing in all populations where data are available (Louda *et al.* 1997).

Although feeding was recorded on some European species of *Cirsium* and on test plants of wavyleaf thistle (*C. undulatum*), significant damage to the North American species of *Cirsium* by *R. conicus* was not predicted. The pre- and early post-release preference and performance tests (e.g., Ward *et al.* 1974; Rees 1977, 1978, 1991; Surles and Kok 1977; Kok 1979; Zwoelfer and Harris 1984) were interpreted as suggesting that significant impact would be unlikely. A strong preference for *Carduus* spp., and slower larval development to smaller adult sizes on *Cirsium* spp. than on *Carduus* spp., were expected to select against major use of native North American *Cirsium* spp. (Zwoelfer and Harris 1984; see Gassmann and Louda 2000).

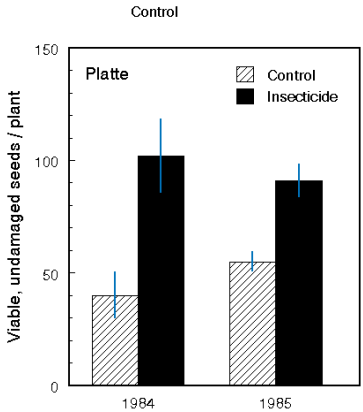

Fig. 1. Average number (± S.E.) of the number of viable, undamaged seeds released by Platte thistle plants, *Cirsium canescens* Nutt.in two treatments, insecticide-in-water or water-only hand-sprayed onto individual developing inflorescences, in each of two years at Arapaho Prairie, Arthur County, Nebraska USA (Data from Louda and Potvin 1995).

Results

Native Thistle Dynamics in Prairie. Studies conducted prior to the first observation of *Rhinocyllus conicus* in 1993 demonstrated that wide-spread use of inflorescences of these thistles by native insects limits seed reproduction (e.g., Fig. 1; Louda *et al.* 1990, 1992, S.M. Louda, T. Tesar and J. Burger, unpublished data). In addition, demographic data for Platte thistle from two insecticide exclusion experiments demonstrated that population density (Fig. 2A) and lifetime individual fitness (Fig. 2B) were limited by seed

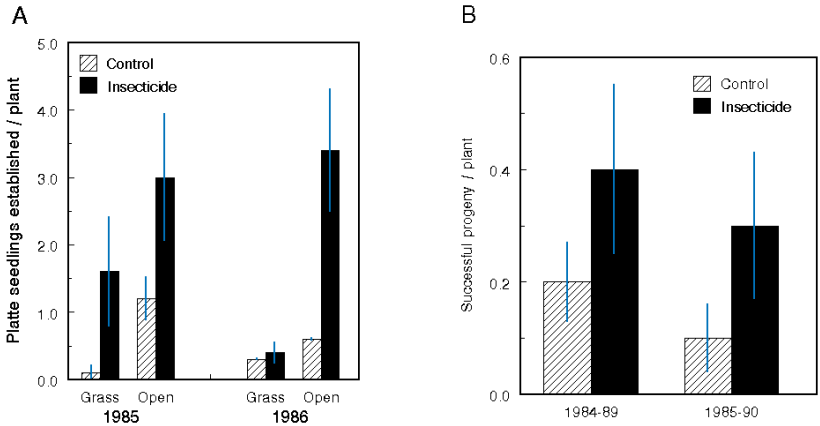

Fig. 2. (A) Average number (± S.E.) of seedlings that established within the grass stand or within semi-open disturbances, called "blowouts" within each of two treatments, insecticide-in-water or water-only hand-sprayed onto individual developing inflorescences, in each of two years at Arapaho Prairie, Arthur County, Nebraska USA (Adapted from Louda and Potvin 1995); (B) average number (± S.E.) of progeny surviving to reproduce, which is a measure of lifetime reproductive success for a monocarpic plant, for each plant by 1989 or 1990 within the insecticide exclusion vs. water-only treatments in experiments initiated in 1984 and 1985 at Arapaho Prairie, Arthur County, Nebraska USA (Data from Louda and Potvin 1995).

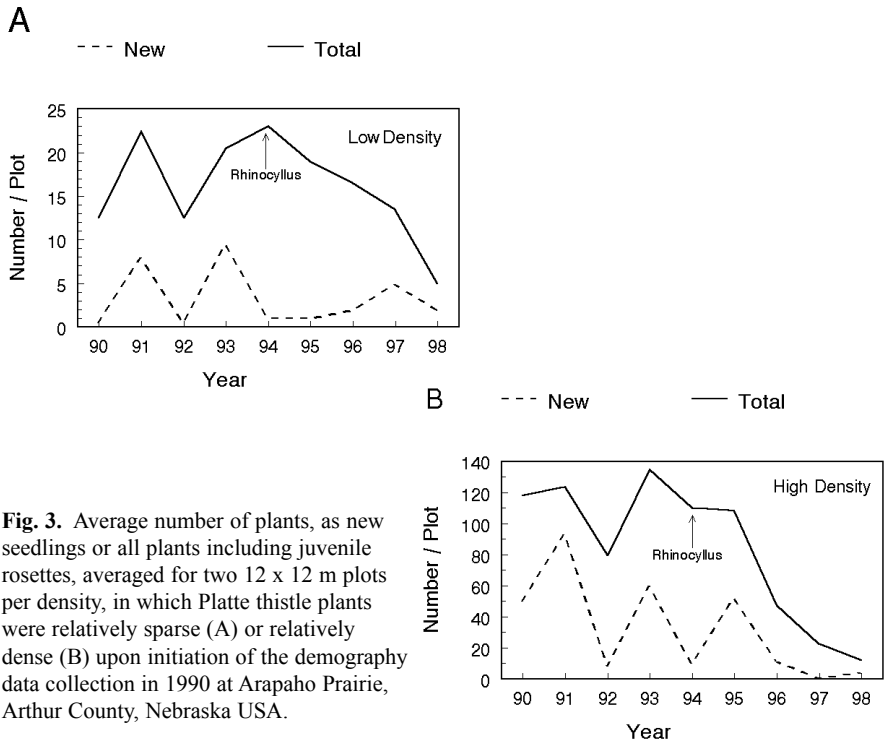

Fig. 3. Average number of plants, as new seedlings or all plants including juvenile rosettes, averaged for two 12 x 12 m plots per density, in which Platte thistle plants were relatively sparse (A) or relatively dense (B) upon initiation of the demography data collection in 1990 at Arapaho Prairie, Arthur County, Nebraska USA.

density and that these population traits were suppressed by the native inflorescence-feeding insect herbivores (Louda and Potvin 1995). Comparable data for wavyleaf thistle are more variable, perhaps because of this plant's iterocarpic reproductive strategy, but they also suggest that native insects can limit the number of seeds released into the seed rain (S.M. Louda, T. Tesar and J. Burger, unpublished data). In addition, demography plots were established in 1990, three years prior to the host range expansion by *Rhinocyllus conicus*. These document an accelerated population decline of Platte thistle correlated with the weevil population built up, in plots that were sparse (Fig. 3A) as well as ones that were dense initially (Fig. 3B; S.M. Louda, unpublished data). Together, the accumulated information from these studies demonstrates the dependence of native thistle populations on seed reproduction, especially the early-flowering Platte thistle. Our studies also provide background data with which to evaluate the direct effect of *Rhinocyllus conicus* on the fitness and population dynamics of both Platte and wavyleaf thistles in native prairie.

Establishment, Population Growth, and Impact of *Rhinocyllus* on Native Thistles. Intensive studies of *Cirsium canescens* (Platte thistle), and its relatives in sand prairie including at one of our sites, The Nature Conservancy's Arapaho Prairie, were initiated in 1976-1979 by W.O. Lamp (Lamp and McCarty 1979, 1981, 1982a,b,c, Lamp 1980). These have been continued annually since 1984 by our group. In addition, we recently discovered that similar interactions are being documented in several national parks (Louda *et al.* 1997, 1998). These longterm observations, complemented with exper-

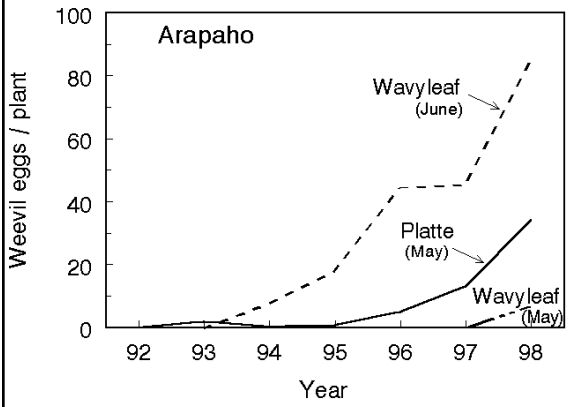

Fig. 4. Average number of *Rhinocyllus conicus* eggs observed per plant for wavyleaf thistle (*Cirsium undulatum*) or Platte thistle (*C. anescens*) in either late May or late June, 1990 -1998; the values for Platte thistle in June were lower than those presented for May observations and so are left out for clarity, at Arapaho Prairie, Arthur County, Nebraska USA. It is clear that comparative data for the two species taken in May seriously underestimates the oviposition activity of *R. conicus* on wavyleaf, but not on Platte thistle.

iments to test the underlying relationships, have been focused primarily on the population dynamics of Platte thistle, so they provide the justification for our quantitative examination of this species' response to the host range expansion by *R. conicus*. The evidence demonstrates the importance of seed reproduction for regeneration and fitness by Platte thistle, and it documents the occurrence and influence of native inflorescence-feeding insects on seed production prior to the host range expansion (e.g., Louda and Potvin 1995).

We are, thus, in the unique position of being able to quantitatively evaluate the impact of adding another insect to the inflorescence guild of these native thistles. Despite the long interval since original release within the state and the on-going, long-term research on thistle-insect interactions at our sites, the first documented occurrence of *R. conicus* on both native thistles only occurred in 1993 (Louda *et al.* 1997, 1998; Louda 1998b, 2000), more than 20 years after introduction. Interestingly, the host range expansion occurred in the absence of musk thistle. There are no *Carduus* spp., and never have been, in our sand prairie sites 50 km into the Sand Hills.

Once the weevil appeared, its numbers increased dramatically, initially documented on Platte thistle (Louda 1998b), but also occurring on wavyleaf thistle later in each season (Fig. 4). Feeding by *R. conicus* was associated with a 5-fold reduction in the number of viable seeds released by Platte thistle by 1996 (Fig. 5; Louda *et al.* 1997, 1998, Louda 2000). The weevil is also reducing seed production by wavyleaf thistle plants (34% of the heads censused at Arapaho Prairie were attacked in 1997: Louda 2000); however, the impact of this feeding is more difficult to predict because of the greater variability in successful seed production by this iterocarpic species, perhaps allowed by the option of vegetative (clonal) persistence (S.M. Louda, unpublished data). Evidence of activity and numbers of weevils on the native thistles, though variable, have remained high (Fig. 4; Louda 2000; S.M. Louda and A.E. Arnett, unpublished data).

The data demonstrating the importance of seed reproduction for density, population growth and lifetime fitness for Platte thistle, documented prior to the weevil buildup at our sites, leads to the inference that the added seed reduction imposed by *R. conicus* will

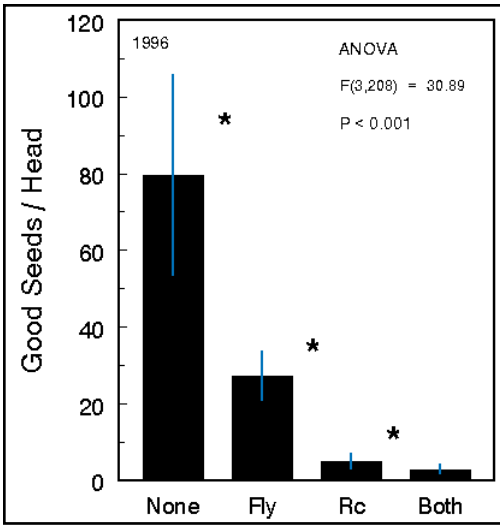

Fig. 5. Average number of viable seeds (± S.E.) produced per head for Platte thistle (*Cirsium canescens*) in sandhills prairie for flowerheads with: neither flies nor weevils, or native flies, or *Rhinocyllus conicus*, or both (ANOVA: $F_{3,208} = 30.89$, $P < 0.001$) (From Louda 2000).

severely decrease Platte thistle density, fitness, and population growth. In fact, the demography plot data provide direct support for this inference (Fig. 3). Thus, the evidence from this system is not consistent with the prediction of no significant damage by *R. conicus* to nontarget species.

Further, the phylogenetic relationship between Platte thistle and the federally-listed Pitcher's thistle, *C. pitcheri* [Torr.] Torrey and Gray (Johnson and Iltis 1964), as well as the ecological similarities between these closely related thistles (Louda 1994), leads to another inference. Platte thistle is considered the most likely candidate for the progenitor species of Pitcher's thistle (Johnson and Iltis 1963, Loveless and Hamrick 1988), which is now threatened in its dune habitat around the Great Lakes (Pavlovik *et al.* 1992) and fed upon by many of the same native insects (Stanforth *et al.* 1997, Bevill *et al.* 1999). If the more common species can be used as a model to understand the potential role of interactions in the population dynamics of the rare species, as was suggested prior to evidence for the host range expansion (Louda 1994, Louda and McEachern 1995), then it is likely that *R. conicus* will also have a similar impact on the threatened Pitcher's thistle if the weevil becomes established around the Great Lakes. We are in the process of testing key aspects of this hypothesis in the laboratory (S.M. Louda, A.E. Arnett, A. McClay, unpublished data).

Differential Susceptibility Among Thistles to *Rhinocyllus conicus*. Several plausible mechanisms could explain the higher demographic vulnerability of Platte thistle, compared to wavyleaf thistle, observed to date (Louda 1998b, 2000). Two hypotheses, which are not mutually exclusive, appear most relevant here. The first hypothesis is that Platte thistle is more chemically attractive or suitable than wavyleaf thistle for *R. concius*. We have no data yet with which to evaluate this hypothesis. However, the field data make it clear that both are acceptable hosts, and increasing use of wavyleaf thistle by *R. conicus* (Fig. 4) argues against the primacy of this explanation. The second hypothesis is that phenological synchrony between insect and host plant resource is critical to quantitative

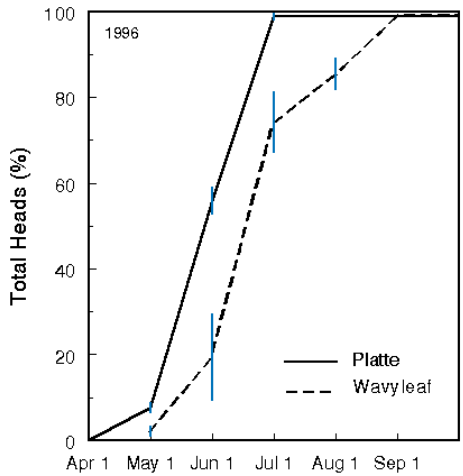

Fig. 6. The flowering phenology of Platte thistle (*Cirsium canescens*) and wavyleaf thistle (*C. undulatum*) at Arapaho Prairie, Arthur County, Nebraska USA, demonstrating the 2 - 3 wk lag in flowering by wavyleaf thistle, relative to Platte thistle, presented as a percent of final number of heads initiated by each census data in 1996 (From Louda 1998b)

effects (e.g., Klein 1986, Zwoelfer and Romstock-Volkl 1991, Louda 1998b).

The data available lend some support to the phenological synchrony hypothesis. First, peak flowering in Platte thistle is several weeks before that of wavyleaf thistle (Fig. 6). Thus, the occurrence of adult weevils that are mating and ovipositing tends to be more synchronous with the flowering of Platte thistle than with the flowering of wavyleaf thistle (Louda 2000). Second, a higher proportion of the total number of flower heads initiated are available during the weevil's oviposition period on Platte thistle (83%) than on wavyleaf thistle (58%) (Louda 2000). And, third, Platte thistle has only one lifetime flowering episode, consistent with its monocarpic life history, whereas wavyleaf thistle may "spread the risk," and increase the chance of having at least some seeds escape, with its multiple flowering episodes over several years.

Evidence for Indirect Effects of *Rhinocyllus conicus* on Native Insects. The observational evidence for an inverse relationship between the numbers of the earliest tephritid, *Paracantha culta*, and those of *R. conicus,* is suggestive, but variable (Fig. 7A). The effect of adding *Rhinocyllus* to the flowerhead guild of Platte thistle may be the cause of a decrease, particularly in poor flowering years (Louda *et al.* 1997, 1998, Louda 2000). In addition, if true, another type of indirect effect is possible. Since this fly has a second generation each year on later-flowering native thistles (Lamp 1980), reduction in the number of flies in the first generation could lead to a temporally-displaced increase in seed production and weediness by later-flowering, presently innocuous native thistles.

We hypothesized that the weevil occurrence caused decreased performance and lower numbers of *P. culta*. Three experiments have been initiated to provide the data with which to evaluate this hypothesis: 1) manipulation of weevil densities, 2) tests of fly behavioral response to naturally-deposited weevil eggs, and 3) alteration of the exposure sequence of the fly, vs. the weevil, to flowerheads. We have preliminary results from the first two experiments to share; however, the third experiment was only initiated in 1999.

Our test to document oviposition and development of *Paracantha culta* in the presence or absence of *Rhinocyllus conicus* was done in 1997-1998 by augmenting egg deposition using sleeve cages, or preventing larval entry into the head by regular removal of

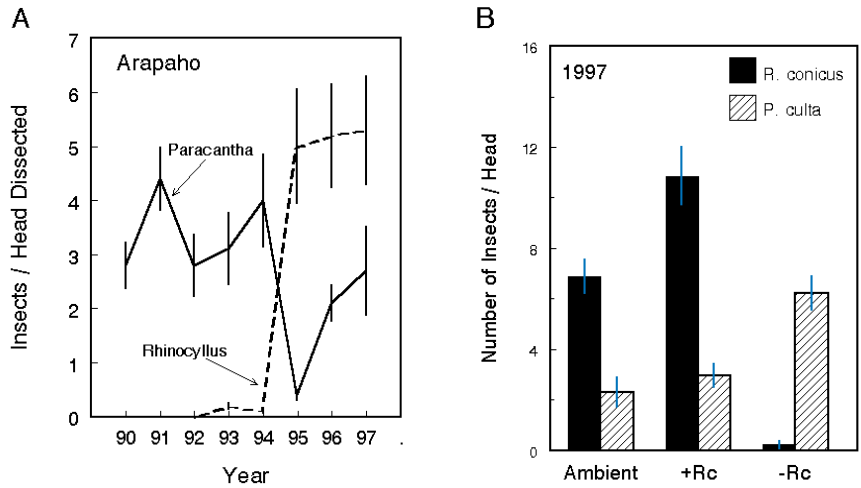

Fig. 7. (A) Number of the native tephritid fly, *Paracantha culta*, and of *Rhinocyllus conicus* within flowerheads dissected for each Platte thistle (*Cirsium canescens*) census plants, 1990 - 1997 at Arapaho Prairie Preserve, and (B) average number (± S.E.) of *P. culta* and of *R. conicus* in an experiment done in 1997 in the field at Arapaho Prairie, Arthur County, Nebraska USA, with three treatments, including: flowerheads with naturally-occurring eggs of *R. conicus* (= "Ambient" loads), with *R. conicus* eggs added by confining a mated pair of adult weevils on the head for 5 d (= "+Rc), or with any naturally-laid *R. conicus* eggs removed every other day to provide flowerheads with no or little interaction with *R. conicus.* (Louda and Arnett, in review)

newly lain eggs (S.M. Louda and A.E. Arnett, unpublished data). Preliminary analyses of our experimental data strongly support a causal role for *R. conicus* in the negative trend in fly numbers; the numbers of *P. culta* were significantly lower in the treatment with *R. conicus* (Fig. 7B, S. M. Louda and A.E. Arnett, unpublished data). The reduction in the number of flies emerging successfully in the presence of *R. conicus* could be caused by resource competition, aggressive interference within the heads, or reduced fly preference for flower heads with *Rhinocyllus* eggs. We found that the total number of flies emerging increased significantly with flower head size (ANOVA: $F_{1,115}$) = 39.04, $P < 0.001$), providing some support for the resource limitation hypothesis (Fig. 7B). However, our behavioral studies have also provided support for the role of fly oviposition behavior. Less time was spent (Fig. 8), and fewer observations of oviposition attempts were observed in the lab, on Platte flowerheads that had *Rhinocyllus* eggs compared to similar-sized heads with no eggs (Fig. 8; Arnett and Louda 2000).

Effectiveness of criteria used to determine probability of significant non-target effects? The two main criteria currently used to evaluate ecological risk for potential biological control of weeds agents are host plant specificity and larval performance (Marohasy 1998) on test plants chosen by phylogenetic relationship to the target plant (McEvoy 1996, Thomas and Willis 1998). The underlying assumption is that these criteria are both necessary and sufficient to quantify ecological risk. However, since *R. coni-*

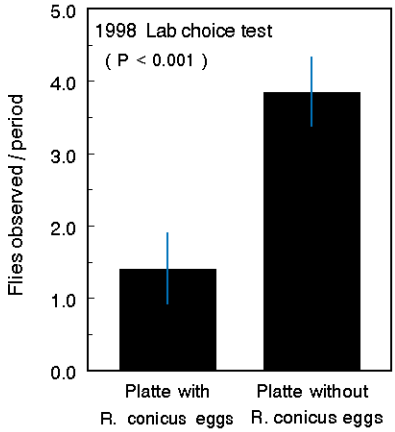

Fig. 8. Average number (±S.E.) of *Paracantha culta* flies observed per observation period (3 one hr periods per day over a week) on a flower head, either with *Rhinocyllus conicus* eggs or without, run in the laboratory in June 1998.

cus showed strong oviposition preference for *Carduus* spp. over *Cirsium* spp. and since larvae were larger as well, the unexpectedly severe impact of *R. conicus* on Platte thistle reopens the question of the adequacy of these criteria to predict risk (Howarth 1983, 1991; Secord and Kareiva 1996; Simberloff and Stiling 1996; Arnett and Louda 2000). Specifically, Zwoelfer and Harris (1984) argued that these criteria, plus the small populations of native *Cirsium* thistles in North America and poor synchronization of the weevil with their flowering, would prevent significant negative ecological consequences. They concluded (p. 59) that it was "unlikely that the native thistles will be adopted as a prime host."

Two main hypotheses could explain the magnitude of the ecological effects of *R. conicus,* given the pre-release results. First, relative preference or performance of *R. conicus* could have changed significantly since introduction. Or, second, the weevils have an impact on native plants despite stronger preference for, and higher performance on, their traditional host species. In order to evaluate these alternatives, we repeated the original

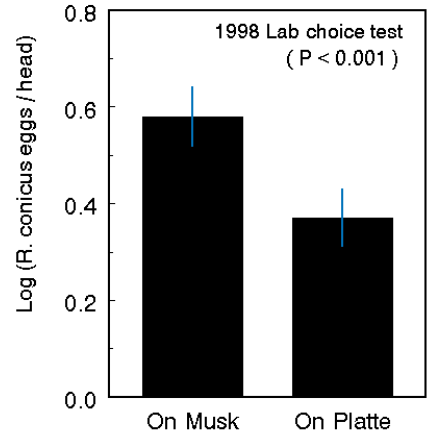

Fig. 9. Average number (±S.E.) of eggs, log-transformed, laid by a pair of *Rhinocyllus conicus* weevils onto either a musk thistle flower head or a Platte thistle flower head of the same diameter over 4 d in a laboratory choice experiment carried out in 30 x 30 x 30 cm mesh screen cages at the Cedar Point Biological Station, Ogallala, Nebraska USA in June 1998. (Data from Arnett and Louda 2000)

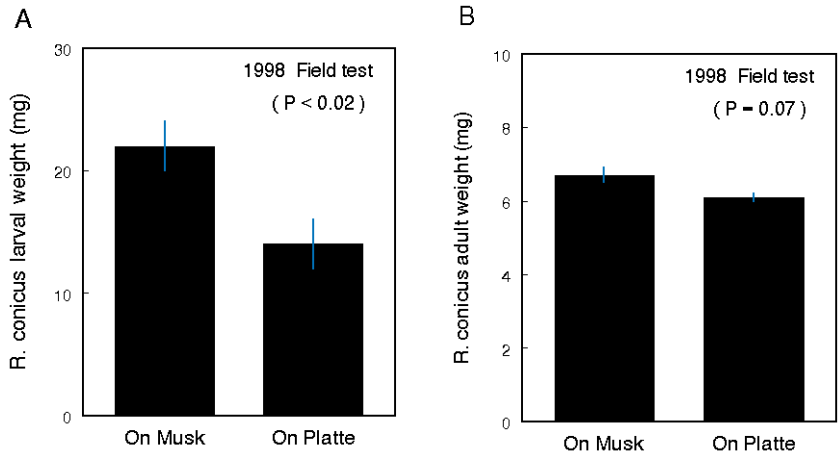

Fig. 10. Performance of *Rhinocyllus conicus* larvae and pupae on either musk thistle or on Platte thistle in Nebraska in June-July 1998 near Ogallala, Nebraska, both after rearing in mesh bags on flower heads on plants in situ in the field site. (A) average wet mass (±S.E.) of larvae after 10 d development in the head, and (B) average wet mass (±S.E.) of newly emerged adult weevil in August-September 1998 and B, average adult dry weight of *R. conicus* emerging from heads of Platte thistle, *C. canescens*, and from the exotic Musk thistle (*Carduus nutans* ssp. *leiophyllus* [Kartesz 1998]) in western Nebraska, 1998 (ANOVA, P > 0.10). (Data from Arnett and Louda 2000).

screening tests, using *R. conicus* naturalized in Nebraska.

In the first experiment, comparable to the initial laboratory screening test, we found that oviposition preference of *R. conicus* is still consistently greater for the naturalized exotic musk thistle, *Carduus nutans* subsp. *leiophyllous,* than for Platte thistle, *Cirsium canescens* (Fig. 9; Arnett and Louda 2000). Oviposition rates by naturalized *R. conicus* were significantly greater on the naturalized musk thistle than on the native Platte thistle, as would be expected from the original tests. For example, weevils laid eggs on musk thistle in 93% of the cages, but on the native Platte thistle in only 68% of the cages. And, significantly greater numbers of eggs were laid on musk thistle than on Platte thistle ($p << 0.001$). These results are similar to those reported originally. For example, Rees (1978) found that 98-99% *C. nutans* flower heads received eggs vs. only 22 - 41% of *C. undulatum* flower heads did; and, Zwoelfer and Harris (1984) reported more eggs were found on *C. nutans* plants than on any other plants.

In the second experiment, development and size of *R. conicus* larvae from standardized egg densities within mesh bags on plants in the field were greater on musk thistle than on native Platte thistle (Arnett and Louda 2000). Larval mass after 10 d was significantly greater on the musk thistle than on Platte thistle (Fig. 10A, $P = 0.02$), as would be expected based the earlier studies (Rees 1978, Zwoelfer and Harris 1984). Also, although we found a trend for greater adult mass for weevils raised on musk thistle than on Platte thistle (Fig. 10B), as expected, the difference was not quite statistically significant ($p = 0.07$). However, no differences in adult mass related to host plant were found in our previous

work with naturalized *R. conicus* (Louda 2000). In summary, Zwoelfer and Harris (1984) reported that larval mortality was 20x higher, larvae "were smaller", and body mass of newly emerging adults was 5.8% smaller on *Cirsium* spp. than on *Carduus nutans*. Our test results are consistent with these patterns.

Thus, the results of our experiments suggest rejection of the hypothesis that host preference or performance has changed enough to explain the ecological phenomena that we are documenting. Instead, the results provide support for the hypothesis that present criteria, while necessary, fall short of the ability to predict ecological impacts and environmental risk. Clearly, this case demonstrates that ecological consequences can be independent of adult preference and larval performance.

Discussion

We have three objectives in applying an experimental, field approach to the study of population interactions of insects with thistles. The first is to quantify the direct and indirect effects of insects, both native and exotic, on populations of native species in prairie, along with the mechanisms underlying these effects. The second objective is to test the predictive nature of the main criteria used to evaluate the probability of significant ecological risk of biocontrol agents of weeds. And, the third objective is to provide a basis for practical issues, by identifying the additional factors that would have improved the prediction of the documented ecological impacts on non-target plants, and their native inflorescence herbivores, in this case. This approach should lead to broader insights which have the potential for improving both the efficacy and the safety of biological control.

Our cumulative studies take advantage of both the intensive, experimental work done on the population dynamics and limitation of native thistles in prairie, and the host range expansion of *R. conicus* onto these thistles within our sites. Given these data and the extensive data base on *Rhinocyllus* biology, this system provides an excellent model for evaluating the ecological implications of host range expansion by this biocontrol insect. Furthermore, at least three suggestions emerge, based on the ecological principles underpinning both our approach and our findings, that can be used to help identify important factors on which to focus in the prediction of ecological risk (Louda 1998a, 2000).

First, we need to believe our data from the classical feeding and oviposition tests! Both "choice" and "no choice" tests provided valuable information that could have been followed up in this case (Gassmann and Louda 2000). We should assume that species accepted in feeding, and especially those that even partially support early larval development, need further assessment of potential ecological impact, as do their close relatives that may not have been tested initially.

Second, we should and can identify the more vulnerable nontarget species to evaluate, by using ecological traits, within the array of phylogenetically related species to be used for the "choice" and "no choice" feeding and oviposition preference tests and for tests for preference-performance relationships. This investment is likely to be our best protection, for both the field of biological control and for the environment, in the long run.

Third, our studies of this system provide clues to species that likely require further evaluation of the potential consequences of feeding and larval development. The most likely candidates for ecological side effects, at least in this system, were those plants, and their dependent smaller species, whose life histories were geared to provide phenologically- synchronized, required resources. If the herbivores on related, ecologically similar species are already resource-limited and thus limiting plant performance at least under

some conditions, then adding another consumer has measurable probability of having a significant ecological effect, based on this case history.

Fourth, additional, ecologically-defined criteria are needed to quantify the environmental risk associated with introductions for biological control. Our results demonstrate that the diet breadth and the preference/performance relationships did not lead to an accurate prediction of ecological risk in this case. Oviposition and significant numerical effects on Platte thistle by naturalized *R. conicus* occurred despite the persistence of stronger preference for, and higher performance on, naturalized *Carduus*. In this case, no preferred host plants co-occur with Platte thistle (*Cirsium canescens*) in the Sand Hills, so preference and performance are basically irrelevant. Interestingly, preference and performance will also become less and less relevant as the density of the preferred host plant declines, as is expected in a successful biocontrol program! Timing of the availability of thistle inflorescences was more likely the critical factor in vulnerability, combined with a short growing season and a monocarpic life history. In addition, since population densities of thistles are spatially and temporally dynamic, theory suggests the tests should place more emphasis on acceptance and development when the preferred host is rare or absent.

In sum, we conclude that *R. conicus* is having a significant impact on Platte thistle, and that it has the potential for reducing the contribution of seed reproduction in regeneration and population dynamics for wavyleaf thistles in our region. With hindsight, it is clear that the early feeding tests did suggest a potential for ecological impact that could have been investigated further to quantify risk (Gassmann and Louda 2000). Had ecological criteria, such as flowering phenology and guild interactions, been used in addition to phylogenetic relationship to establish the list of potential host plants to be tested, it is likely that early-heading species, such as Platte and Pitcher's thistles, would have been studied and better estimates of the potential impact on them determined.

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