

RESEARCH ARTICLE

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Canada Thistle
Biological Control
Agents on Two South
Dakota Wildlife
Refuges

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ABSTRACT: We monitored populations of Canada thistle biocontrol agents *Cassida rubiginosa*, *Ceutorhynchus litura*, *Larinus* (= *Hadroplantus*) *planus*, *Urophora cardui*, *Orellia* (= *Terellia*) *ruficauda*, and *Rhinocyllus conicus* on Canada thistle (*Cirsium arvense*) at two national wildlife refuges in South Dakota from 1999 through 2003. *C. litura*, *U. cardui*, *O. ruficauda*, and *R. conicus* were present on both refuges. Agent populations were low except for *C. litura*, which was present in up to 90% of stems in some plots. *C. litura* infestation did not reduce thistle flowering, stem length, or over-winter survival. There was no change in thistle stem numbers over the study period and no difference in stem numbers in areas of high *C. litura* populations compared to areas of low *C. litura* populations. Our results suggest that insect biological control agents are inadequate for reduction of Canada thistle in southern South Dakota.

Index terms: biocontrol, Canada thistle, *Ceutorhynchus litura*, *Cirsium arvense*, *Rhinocyllus conicus*

INTRODUCTION

Many insect species have been released in attempts to control Canada thistle (*Cirsium arvense* (L.) Scop.) populations in South Dakota, including the Canada thistle tortoise beetle *Cassida rubiginosa* Muller (Coleoptera: Chrysomelidae), the Canada thistle stem weevil *Ceutorhynchus* (= *Hadroplantus*) *litura* (F.) (Coleoptera: Curculionidae), the Canada thistle bud weevil *Larinus planus* (F.) (Coleoptera: Curculionidae), and the Canada thistle gall fly *Urophora cardui* (L.) (Diptera: Tephritidae). In addition to these agents, the seedhead fly *Orellia* (= *Terellia*) *ruficauda* (F.) (Diptera: Tephritidae) is a seed predator of this weed (Lalonde and Roitberg 1992). The seedhead weevil, *Rhinocyllus conicus* (F.) (Coleoptera: Curculionidae), which was released as a biocontrol agent for musk thistle, also attacks Canada thistle (Youssef and Evans 1994).

The species listed attack Canada thistle in Europe and have been introduced accidentally or deliberately in the United States. They were promoted as biocontrol agents based on encouraging results in experimental situations. *U. cardui* caused stunted growth and reduced flowering of thistle (Peschken and Harris 1975, Forsyth and Watson 1985). Rees et al. (1996) observed that stems infested by *C. litura* generally did not survive the winter, and surviving infested plants produced fewer shoots than uninfested plants when regrowth began in the spring. *R. conicus* attacks on musk thistle (*Carduus nutans* L.) were successful in reducing weed infestations in many locations (Gassmann and Kok 2002). *O. ruficauda* reduced seed production of Canada thistle by 21.5% in

infested flowerheads in a study by Forsyth and Watson (1985): up to 70% of heads were infested.

Other early studies were discouraging. Peschken and Wilkinson (1981) observed that larval mining by *C. litura* did not cause noticeable reduction in Canada thistle populations in Ontario, where the agent had been established since 1967. Lalonde and Roitberg (1992) observed up to 36% infestation of Canada thistle flowerheads by *O. ruficauda* in British Columbia but concluded that fly infestations had little impact on total achene production. Liu et al. (2000) found high levels of *C. litura* infestation in South Dakota in 1997 and 1998, as well as smaller populations of *L. planus* and *U. cardui*, and observed that total nonstructural carbohydrates were reduced in thistle stems in areas where agents had been released. Few studies of Canada thistle biocontrol effectiveness have been done within the last decade (McClay 2002).

C. rubiginosa, *C. litura*, *L. planus*, and *U. cardui* were released repeatedly at Lacreek and Lake Andes national wildlife refuges in South Dakota by refuge staff between 1989 and 1996 according to refuge records. We do not know the size of all the releases, but for those having records, releases generally consisted of between 100 and 400 individuals released at each of two or three sites in a given year. Many other releases of these agents have occurred in South Dakota according to records of the United States Department of Agriculture's Animal and Plant Health Inspection Service, and these insects are widely available for purchase by individuals.

In 1998, we initiated a study of the effects of competitive plantings, nitrogen manipulation, and herbicide on Canada thistle and its biocontrol agents. We attempted to reduce thistle productivity in order to increase agent damage, but were generally unable to significantly decrease thistle biomass and stem number. We monitored agent populations, effects of experimental treatments on agents, and the effects of agents on thistle stem length, flowerhead number, and stem count in plots. We expected that stems infested with biocontrol agents would be shorter and have fewer flowerheads than non-infested stems, and that stem counts would decrease over time in areas highly infested with thistle and that they would remain stable in relatively uninfested areas.

STUDY AREAS AND METHODS

Study Areas

Four study sites (fields) were established: two at the Lake Andes and two at the Lacreek National Wildlife Refuges in South Dakota. Fields were chosen based on the uniformity of soil types and similarity in land management practices. The study areas are not cultivated and are dominated by alien plant species such as smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.) but also support a small proportion of native plants. Canada thistle is present at all sites. We collected preliminary data for two years to determine the level of thistle abundance and variability and the presence of biological control insects.

At Lake Andes National Wildlife Refuge, the study field was located at Trout Wildlife Management Area (TR). Soils are primarily Eakin-Ethan complex, deep, well drained silty clay loam and silty loam with medium fertility. Ethan soils are more calcareous, with the higher lime content tending to decrease fertility. A small area of ponded Worthing silty clay loam exists at the western edge of the field. The second field, Owens Bay Field (OB), was located south of Owens Bay. Soils are deep, well drained Highmore silty loams with moderate organic matter, medium fertility, and

slow runoff.

Both study fields at Lacreek National Wildlife Refuge are within the refuge proper. At Birdwalk Field (BW), minitire soils predominate: deep, saline, and loamy with a fluctuating water table. They are poorly drained with low to moderate fertility. The Loop Drive Field (LD) has similar soils.

Plant and Insect Sampling Methods

Following the establishment of 520 6-m x 2-m permanent plots within 12.2 m x 12.2 m buffers in 2000, we counted thistle stems in each permanent plot. Stem counts were made on six randomly selected 0.25-m x 0.25-m subplots in each plot each year thereafter.

Preliminary collections to document insect species presence were made in 1998 and 1999 at Lake Andes and Lacreek. We collected insects from 82 plots each in 2000, 2001, and 2002. Stem and insect collections were taken from buffer zones in late June or early July (summer) and late August or early September (fall). A summer collection was made in 2000, and both summer and fall collections were made in 2001, 2002, and 2003. In 2003, we collected thistle stems in 0.25-m x 0.25-m quadrats in 164 plots. To collect adult insects and spiders, we swept 24 sweeps in each plot using a 40 cm diameter sweep net, and collected all insects and spiders present in the net. The insects were killed with ethyl acetate and stored frozen. Adult insects larger than 1 mm in length were identified to family and subfamily level by Catherine Reed. Biocontrol agents were identified to species.

We collected 10 stems per sampled plot, measured the length of each (starting in 2001), counted flowerheads and buds and noted any damage, dissected the stems and flowerheads, and preserved all larvae in 80% ethanol. Stem length was measured as height of the plant from base of stem to base of topmost bud. Preserved larvae were identified to order and to family when possible by Catherine Reed and verified by John Luhman, Minnesota Department of Agriculture, St. Paul, Minnesota. Any

parasitized larvae were noted. Larvae from a subsample of stems in 2000 and 2002 were reared to adulthood by Anthony Cor-tilet, Minnesota Department of Agriculture, to verify identification of larvae. Voucher specimens are curated at the Insect Museum, Entomology Department, University of Minnesota, St. Paul, Minnesota.

Statistical Analysis

Data were normalized by arcsine or log transformations when necessary and possible. GLM models were used to study the effects of field, year, and agent infestation levels on stem count, stem length, and flowerhead number. Nonparametric t-tests were used to compare lengths and flowerhead numbers of infested and uninfested stems paired by field and date of collection. All analyses were performed using the JMP statistical analysis program (SAS Institute 2001).

RESULTS

Ceutorhynchus litura, *R. conicus*, *U. cardui*, and *O. ruficauda* were observed in most years on most fields (Table 1). We did not observe *C. rubiginosa* or *L. planus* at any site although these agents had been released in previous years. *C. litura* infestation was generally common among fields and consistent among years (Table 2). Significantly more stems were damaged by *C. litura* at Lacreek than at Lake Andes when means for all years were analyzed (Table 3) ($F = 4.17$, $DF = 27$, $p = 0.02$). *C. litura* damage did not differ among years ($F = 2.17$, $DF = 27$, $p = 0.15$). *Rhinocyllus conicus*, *U. cardui*, and *O. ruficauda* were present at lower densities and less consistently among years and fields. *O. ruficauda* larvae were highly parasitized in 2002, and no individuals of this species were observed on our sites in 2003. Up to 40% of stems, but usually many fewer, contained larvae (mostly Coleoptera: Mordellidae; Table 4). Total stem larvae excluding *C. litura* showed no trend with year and did not differ significantly among fields. With few exceptions, 5% or fewer flowerheads contained larvae.

We compared stem length, flowerhead

Table 1. Presence of agents and evidence of damage on fields in each year. Adults were collected in 1999, 2001 and 2002, adults were reared from larvae in stems and flowerheads in 2000 and 2002, and stem damage, larvae and galls were recorded in all years. Field abbreviations: Birdwalk BW; Loop Drive LD; Owens Bay OB; Trout TR. BW and LD fields are located at Lacreek, OB and TR fields are located at Lake Andes.

Year	<i>Ceutorhynchus litura</i>	<i>Rhinocyllus conicus</i>	<i>Orellia ruficauda</i>	<i>Urophora cardui</i>
1999	damage at all fields	larvae at OB	TR	OB and BW
2000	damage at all fields, adults at BW	adults at OB	TR and BW	OB and BW
2001	damage at all fields	adults at BW	all fields	BW
2002	damage at all fields, adults at BW and LD	larvae at all fields	all fields	BW
2003	damage at all fields, larvae at BW and LD	larvae at all fields	none observed	all fields

49, p t l = 0.87). Longer stems had more flowerheads, but *C. litura* damage did not affect flowerhead number independent of stem length, and *U. cardui* galling did not affect flowerhead number. Presence or number of stem and flowerhead larvae did not affect thistle stem length or flowerhead number.

We expected that changes in stem number per plot would occur during the course of the experiment in response to damage by *C. litura*. Between 2000 and 2001, there was a decrease in stem counts at BW and TR only (Table 6). There was no relationship between percent damage per plot and change in stem count per plot in either field. Only 30 of 84 plots sampled had damaged stems (10 plots BW, 2 at LD, 7 at OB and 11 at TR). Between 2001 and 2002, there was no change in stem counts at BW, a decrease at LD, and an increase at OB and TR. There was no relationship between summer 2001 damage and change in stem count from 2001 to 2002. For fall 2001 damage, there was a significant

number, and stem count among plots with varying degrees of agent infestation to identify any effects of agent populations on thistle populations. *C. litura* damaged stems averaged about 4 cm longer than undamaged stems from the same field,

plot, and date ($t = 3.8$, $DF = 223$, $p > |t| = 0.0002$; nonparametric t-test; Table 5). Comparison of 50 pairs of stems (one with a gall and one without), matched by field and date, indicated that *U. cardui* galling did not affect stem length ($t = 0.166$, $DF =$

Table 2. Mean stems per plot (\pm SE) and mean percent (\pm SE) of stems or flowerheads supporting agents. Stems were counted in six randomly selected 0.25-m x 0.25-m quadrats in all study plots.

Year	Field	Mean stems per plot	N stems dissected	Percent of stems with <i>C. litura</i> damage	Percent of stems with <i>U. cardui</i> galls	N flowerheads dissected	Percent of flowerheads with <i>R. conicus</i>	Percent of flowerheads with <i>O. ruficauda</i>
2000	BW	5.5 \pm 0.3	160	26.9 \pm 3.7	0.09 \pm 0.01	1230	0	15.2 \pm 3.0
	LD	2.6 \pm 0.3	80	68.0 \pm 4.9	0	821	0	11.1 \pm 2.1
	OB	1.5 \pm 0.1	80	37.5 \pm 9.2	0.07 \pm 0.02	256	0	2.6 \pm 1.0
	TR	4.9 \pm 0.3	160	67.5 \pm 4.5	0	2119	0	2.2 \pm 0.4
2001	BW	4.9 \pm 0.4	538	39.7 \pm 5.1	0.02 \pm 0.01	887	0.68 \pm 0.3	1.9 \pm 0.4
	LD	3.3 \pm 0.5	276	51.6 \pm 8.8	0	208	0	3.8 \pm 0.8
	OB	1.4 \pm 0.2	278	45.7 \pm 4.5	0	280	0	1.8 \pm 0.4
	TR	3.8 \pm 0.4	472	35.6 \pm 5.0	0	169	0	0
2002	BW	5.3 \pm 0.4	555	48.5 \pm 14.6	0.08 \pm 0.01	587	1.0 \pm 0.1	0.68 \pm 0.3
	LD	2.0 \pm 0.3	278	15.4 \pm 7.2	0	200	7.0 \pm 0.7	0.5 \pm 0.2
	OB	1.7 \pm 0.2	279	7.14 \pm 4.9	0	41	7.3 \pm 0.4	4.9 \pm 0.4
	TR	5.5 \pm 0.6	538	3.5 \pm 2.4	0	294	2.7 \pm 0.2	0.68 \pm 0.3
2003	BW	6.1 \pm 0.6	1094	55.5 \pm 3.2	0.07 \pm 0.01	4875	1.2 \pm 0.4	0
	LD	3.2 \pm 0.5	558	51.4 \pm 4.9	0.03 \pm 0.01	1783	1.0 \pm 0.5	0
	OB	2.0 \pm 0.4	559	15.7 \pm 15.7	0.05 \pm 0.01	880	1.8 \pm 1.4	0
	TR	4.8 \pm 0.5	1077	30.5 \pm 30.5	0.002 \pm 0.002	4599	3.5 \pm 1.0	0

Table 3. Mean percent (\pm SE) of *C. litura* damaged stems by field, all years combined.

Field	Percent damaged stems
BW	36.3 \pm 1.8
LD	39.6 \pm 2.7
OB	12.5 \pm 1.7
TR	17.5 \pm 1.6

relationship between damage and stem count only at OB. At that site, plots with more damaged stems in the fall of 2001 showed an increase in stem number from 1.4 stems per 0.25-m quadrat 2001 to 1.7 stems per quadrat in 2002, a 25% increase. For the model: difference in stem counts from 2001-2002 = damage in fall 2001, $r^2 = 0.43$, $DF = 13$, $F = 9.04$, $p < 0.012$. Between 2002 and 2003, stem counts did not change at BW and OB; they increased at LD and decreased at TR. There was no significant relationship between percent damaged stems in either collection date and change in stem count for any field.

Mean stem count per plot did not change significantly for the entire experiment or for any field between 2000 and 2003 when counts were paired by field and plot (Table 6). Of 519 plots that had stem counts for both 2000 and 2003, 157 had no thistle in either year, 56 had no thistle in 2000 and at least 1 stem per quadrat in 2003, 75 had at least 1 stem in 2000 and no thistle in 2003, and 231 quadrats had at least 1 thistle

Table 4. Mean percent (\pm SE) of stems containing at least one larva. Of 234 larvae that were identified to family, 88% were Coleoptera from 4 families: Mordellidae 68.8%, Curculionidae 9.4%, Languriidae 6.4% and Cerambycidae 3.4%. Coleoptera: Elateridae, Diptera: Tephritidae, and a few Lepidoptera larvae (Nymphalidae and Pyralidae) were also present in stems.

Field	Year			
	2000	2001	2002	2003
BW	7.4 \pm 1.8	3.0 \pm 0.7	6.6 \pm 1.4	2.0 \pm 0.3
LD	20.0 \pm 5.6	3.9 \pm 1.1	6.8 \pm 1.6	14.5 \pm 0.2
OB	12.5 \pm 3.0	3.6 \pm 1.4	0.7 \pm 0.5	9.5 \pm 2.4
TR	40.0 \pm 5.7	6.3 \pm 1.2	10.8 \pm 2.3	7.1 \pm 1.2

stem in both years. That is, of 519 plots, 165 showed an increased stem count, 163 a decreased stem count, and 191 no change in stem count. There was no relationship between mean *C. litura* damage and the tendency of individual plots to lose or gain thistle stems during the experiment.

No statistically significant trends in agent populations were observed during the experiment. Number of galls per stem differed among years and among fields, but galls were so rare that the differences are not expected to be biologically significant (Table 2). There was no effect of thistle density (measured in 2000) on *C. litura* damage in any year and no effect of density on number of galls per stem, number of larvae per flowerhead, or number of larvae per stem. Stem damage varied among years but no trend could be identified (Table 7).

Flowerhead numbers per stem were lower

in 2003 than in 2000 (Table 7), but the trend was only marginally significant. For the model: flowerheads/stem = year, $r^2 = 0.24$, $DF = 15$, $F = 4.34$, $p = 0.06$. Stems averaged 34.8 cm longer in 2003 than in 2001 based on means by field and date. This effect was significant for both collection dates and for all fields ($DF = 53$, t -ratio = 14.2, p $|t| < 0.0001$). There was no significant difference in stem length between 2001 and 2002 (stem length was not measured in 2000 or 1999).

DISCUSSION

Agent populations were low over the entire study at Lacreek and Lake Andes, except for *C. litura*, despite dense populations of their Canada thistle host and repeated releases of these agents since 1986. There was little change in mean thistle stem counts over four years in test plots; no ef-

Table 5. Number and mean (\pm SE) length of stems with and without *C. litura* damage, 2003. The increased sample size in 2003 allowed enough damaged stems for analysis on both dates and in all fields.

Field	Date of collection	No. stems without <i>Cl</i> damage	No. stems with <i>Cl</i> damage	Length undamaged stems (cm)	Length damaged stems (cm)
BW	6/24/03	284	270	71.3 \pm 1.5	85.5 \pm 1
BW	8/4/03	240	300	69.8 \pm 1.2	76.1 \pm 0.9
LD	6/24/03	171	108	58.0 \pm 1.6	70.4 \pm 1.9
LD	8/4/03	136	143	57.7 \pm 1.2	62.1 \pm 1.2
OB	6/27/03	274	5	42.6 \pm 0.8	43.3 \pm 4.1
OB	8/11/03	236	44	44.7 \pm 0.9	56.0 \pm 2
TR	6/27/03	556	3	57.4 \pm 0.9	79.7 \pm 11.2
TR	8/11/03	360	158	51.6 \pm 0.7	57.1 \pm 1.1

Table 6. Relationships between stem damage and changes in stem count from year to year. See text for details of analysis.

Years	Changes in stem count	Relationship between stem damage and change in stem count
2000-2001	decrease at BW and TR only	NS
2001-2002	decrease at LD, increase at OB and TR	summer NS; fall, cells with more damaged stems showed increased stem count at OB only
2002-2003	increase at LD, decrease at TR	NS both summer and fall
2000-2003	NS (pairwise comparisons of 519 plots at all fields)	NS

fect of year on either *C. litura* infestation or thistle stem count could be demonstrated statistically. Damage to stems by *C. litura* did not cause decreased stem counts in later years. Infestation levels were similar to those observed in South Dakota by Liu et al. (2000).

Harris (1997) suggested sequential monitoring of establishment, attack, and impact of biological control agents. He defined establishment as field survival for two years. By this criterion, *C. litura*, *O. ruficauda*, *R. conicus*, and *U. cardui* can be considered established in our study area. In contrast, we never observed *L. planus*, although this species was reported by Liu et al. (2000) from South Dakota sites. Unidentified weevil larvae in thistle buds may have belonged to this species but they were present in very low numbers. Usually fewer than 5% of buds contained larvae, and less than 25% of these larvae were Coleoptera. We did not collect *C. rubiginosa*, nor did Liu et al. (2000) find it in South Dakota, although this species has been reported from southern Minnesota (A. Cortilet, Minnesota Department of Agriculture, St. Paul Minnesota, pers. comm.).

Intensity of agent attack is defined as the proportion of resource exploited by the agent; attack levels of less than 20% are unlikely to result in significant impacts on the host population (Harris 1997). By this criterion, only *C. litura* can be expected to have any impact on Canada thistle (Table

2). Attack intensity is easily determined for *C. litura* and *U. Cardui*, which damage stems without destroying them, but it is more difficult to quantify for *R. conicus*, *O. ruficauda*, and *L. planus*, which may cause buds or flowerheads to abort and fall off stems before they can be sampled.

Loss of flowerheads from stems (observed as broken stem tips) in 2001 and 2002 probably caused the loss of some *R. conicus* and *O. ruficauda* larvae from the samples. Possibly flowerhead loss was related to infestation; if so, the apparent decrease in flowerhead number from 1999 to 2003 may be related to agent presence. *C. litura* and *U. cardui* infestation did not affect flowerhead number. Seed predators damaged flowerheads but their populations were low.

Stems showing *C. litura* damage tended to be a few centimeters longer than uninfested stems. This effect was observed by Pesch-

ken and Wilkinson (1981), who concluded that the *C. litura* mining itself stimulated stem growth. They demonstrated that the increased length was not caused by early emergence of attacked thistle stems. *C. litura* females oviposit early in the growing season when Canada thistle stems are approximately 5 cm long (Rees et al. 1996), suggesting that the increase in stem length is not due to preferential oviposition in taller plants. *U. cardui* gall presence did not affect stem length.

Once agent impact on individual plants has been documented, the next step is to determine whether the agent is causing declines in the target plant populations (Syrett et al. 2000). We measured population change on our sites as change in stem count among plots from year to year and observed that *C. litura* damage did not reduce thistle stem count in heavily agent-infested plots compared to lightly infested plots.

The long-term goals of these releases have been to reduce Canada thistle infestations on the wildlife refuges and to reduce thistle seed production, with the ultimate goals of allowing recovery of natural vegetation on the refuges and reducing the spread of Canada thistle onto cultivated areas. Biomass and thistle stem count have not decreased in areas of high *C. litura* density on our sites as of 2003.

Future Prospects

Agent populations did not appear to be increasing over the five years of this study, which started 14 years after initial releases and seven years after the last documented release of biocontrol agents. *O. ruficauda*

Table 7. Mean percent *C. litura* damaged stems (\pm SE) and mean number (\pm SE) of flowerheads per stem by year, all fields combined.

Year	N stems dissected	Percent damaged stems	Flowerheads per stem
2000	269	48.7 \pm 3.9	8.6 \pm 2.1
2001	538	33.1 \pm 2.1	1.1 \pm 0.2
2002	555	5.6 \pm 1.0	0.6 \pm 0.2
2003	1094	31.2 \pm 1.6	3.4 \pm 0.7

populations are not expected to increase to high levels due to oviposition behavior; they produce relatively few eggs and avoid laying additional eggs into already-infested flowerheads (Lalonde and Roitberg 1992). *O. ruficauda* populations may have been reduced by parasitism. Agent effectiveness did not seem to be increasing and there is no reason to expect this to occur.

R. conicus attacks a number of native thistles as well as Canada thistle and its target weed, musk thistle (Louda et al. 1997). Some of the agents released specifically against Canada thistle are known to attack native *Cirsium* species in laboratory tests, but so far none of these has been recovered from native *Cirsium* in the field (McClay 2002). While safety (i.e., fidelity to the target host) was given less importance in earlier criteria for the importation of biocontrol insects, current criteria give high weight to host specificity (Strong and Pemberton 2001). In uncultivated areas, such as wildlife refuges and rangelands where mechanical and chemical disturbances should be minimized, suppression of weeds by biological control agents remains an ideal, but existing Canada thistle biocontrol agents have not been effective. Canada thistle remains a major agricultural weed over its native range in Europe, suggesting that herbivores there do not control its populations (McClay 2002). Extensive studies have been made of possible European control agents, making it unlikely that new agents remain to be discovered there. Plans are being made to search for additional agents in China and Central Asia (McClay 2002), and research continues on methods to enhance agent populations and the damage they do to their hosts. We echo McEvoy and Coombs (1999) in urging that effectiveness be a primary consideration, along with safety, in the evaluation of potential new biocontrol insects. We do not advocate further releases or distribution in the northern Great Plains of the agents mentioned in this paper.

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