2004

Impact of *Ceutorhynchus litura* Feeding on Root Carbohydrate Levels in Canada Thistle (*Cirsium arvense*)

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Published in *Weed Science* 52:4 (July–August, 2004), pp. 628–633; doi: 10.1614/WS-03-099R1.

Submitted June 26, 2003; approved December 18, 2003.

Impact of *Ceutorhynchus litura* Feeding on Root Carbohydrate Levels in Canada Thistle (*Cirsium arvense*)

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Abstract

Canada thistle is a serious perennial weed found throughout the northern regions of the United States and Canada. The weevil, *Ceutorhynchus litura* (F.), was first released in Canada in 1965 as a potential biological control agent for Canada thistle; however, its impact as a control agent has been sporadic. The objective of this study was to characterize *C. litura* impacts on the carbohydrate profile in Canada thistle roots through the growing season and to evaluate the potential for this biological control agent in causing stress to Canada thistle. Field plots, infested with *C. litura*, were established and extensively sampled for *C. litura* infestations. By sampling *C. litura*—damaged and undamaged Canada thistle shoots—roots through the season, we were able to establish the profile of free sugars and fructans in the roots and compare these levels to the presence and extent of insect damage. Levels of all free sugars and fructans were consistently found to be depressed in roots from *C. litura*—damaged shoots early in the summer during and shortly after the larval feeding period. *Ceutorhynchus litura* feeding in Canada thistle shoots appears to disrupt the movement of photoassimilates from leaves to roots. Late-season levels of free sugars and fructans indicate that roots do recover from these depressed levels, and in several instances, significant overcompensation occurred in the damaged roots. Measurement of free sugars and fructans to identify sublethal impacts of control tactics may allow the strategic combining of complementary tactics to maximize the impact of stresses on Canada thistle.

Nomenclature: Canada thistle, *Cirsium arvense* (L.) Scop. CIRAR; *Ceutorhynchus litura* (F.), Coleoptera: Curculionidae

Keywords: biological control, carbohydrates, fructans

Canada thistle is a troublesome perennial weed found throughout the northern part of the United States (Donald 1990). Since Canada thistle's introduction into the United States in the late 1700s, it has spread dramatically, and researchers have shown that it causes greater crop losses than any other perennial broadleaf weed in the north central region of the United States (Behrens and Elakkad 1981).

The weevil, *Ceutorhynchus litura* (F.), was first released in Canada in 1965 as a potential biological control agent for Canada thistle (Peschken and Beecher 1973). Numerous releases in the United States began in 1971 (Rees 1990). The life history of the weevil has been described by several researchers (Peschken and Beecher 1973; Zwölfer and Harris 1966). Adults overwinter in the soil and emerge in early spring. Females feed on Canada thistle rosettes and deposit their eggs within feeding cavities on the leaves. After stem elongation, emerging larvae migrate toward the main vein of the leaf and tunnel inside the stem. Larval feeding results in significant damage to the inner stem cavity and secondary infection by plant pathogens (Rees 1990). Mature third instar larvae burrow out of the plant near ground level in June and July and pupate in the soil.

Studies on the effectiveness of *C. litura* for biological control of Canada thistle have shown mixed results. Early studies in Canada by Peschken and Beecher (1973) indicated some promise, with reductions in thistle density apparently related to *C. litura* infestations. However, later studies indicated that *C. litura* had little impact on Canada thistle density (Peschken and Wilkinson 1981). In studies conducted in Montana, *C. litura* populations increased over time and reached levels of 5.8 to 7.5 larvae per Canada thistle stem (Rees 1990). This level of infestation caused increased overwintering mortality to infested plant stems. However, new shoots generally developed from underground roots and replenished the Canada thistle population the following spring. Peschken and Derby (1992) demonstrated that roots of attacked shoots had reduced nonstructural carbohydrate levels but only for a short time near the end of the mining period. They concluded that *C. litura* has little effect on Canada thistle when other stresses are limited and suggested that the increased impacts on the thistle seen by Rees (1990) may be due to additional drought stresses after the insect mining period.

Plant stresses such as mowing, defoliation, or herbicides have been shown to affect free sugars and fructans in plant roots (Van Den Ende et al. 2001; Wilson and Michiels 2003). Concentrations of fructans and free sugars in plant roots also change in response to seasonal changes in the environment and other plant stresses (Tworkoski 1992; Wilson et al. 2001). Canada thistle has been shown to produce fructans as reserve polysaccharides (Hodgson 1968; Ozer and Koch 1977; Wilson and Michiels 2003). Peschken and Derby (1992) demonstrated that *C. litura* causes changes in root nonstructural carbohydrate levels, but the functional significance of this is not known. Wilson and Michiels (2003) suggested that the impact on fructan levels of stresses can be used to evaluate control measures.

The positive results obtained by Peschken and Beecher (1973) and Rees (1990), the extensive degradation of the stem by larval mines, and our observations of dieback of heavily infested stems in field situations warrant a more extensive investigation of the physiological impact of *C. litura* on Canada thistle. The objective of this study was to characterize

C. litura impacts on the carbohydrate profile in Canada thistle roots through the growing season and to evaluate the potential for this biological control agent in causing stress to Canada thistle.

Materials and Methods

Site Description

Field experiments were conducted on a pasture naturally infested with Canada thistle, located approximately 1 km east of Scottsbluff, Nebraska. The long-term average annual precipitation for this area is 414 mm, 73% of which occurs between April 1 and August 31. The average annual temperature and frost-free period over the past 30 yr were 9.6 C and 138 d, respectively. The location had not been grazed by cattle for several years. The soil at the site is a Gering loam (Mesic Aquic Ustifluvents), with pH 8 and 2% organic matter. The density of Canada thistle averaged 20 shoots m^{-2}, and plants were uniformly distributed across the experimental site.

Experimental Procedure

Ceutorhynchus litura adults were placed on Canada thistle plants in the study area in the spring of 1993. Weevils and thistles were not disturbed, and the population was allowed to increase the next 7 yr producing a relatively high and uniform infestation across the area. The experimental design was a randomized complete block with four replications in 2000 and six replications of each treatment in 2001. Individual plots were 4.5 m wide by 7.6 m long. The experiment consisted of two treatments: (1) plots treated with a systemic insecticide (carbofuran[1]) at 1.1 kg ha21 to reduce the percentage of infested Canada thistle stems and (2) untreated plots that remained naturally infested with *C. litura*. Plots were treated on May 10, 2000 and April 25, 2001.

On each sampling date, approximately 20 to 30 Canada thistle shoots and roots from each plot were dug from the soil to a depth of 20 cm. Sampling dates were May 30, July 5, August 1, and September 11 in 2000 and June 5, July 13, and August 22 in 2001. Roots from each shoot sampled were cut off at the soil line and returned to the laboratory for analysis. Roots were washed with water and allowed to quickly air-dry in the laboratory before analysis. Each Canada thistle shoot was split and examined for the presence of *C. litura* mining, and the length of mines was measured. Results from the May 30, 2000, sampling indicated that the insecticide treatment did not provide a clear separation of damage between infested and noninfested stems because there were minimal differences in infestation level between the two paired plots. Therefore, for the remaining sampling dates in 2000, thistle shoots and associated roots within each plot were separated into two categories: (1) severely mined and damaged stems and (2) lightly damaged or undamaged stems. In 2001, shoots and associated roots were separated into (1) mined and damaged stems and (2) undamaged stems.

All root sections within each sample (5 to 20) were homogenized[2] into a single sample. A fraction of root extract was immediately analyzed for soluble dry matter with a refractometer.[3] The total sugar content of the sample was estimated from the refractometer reading by the procedure developed by Van Waes et al. (1998). A 0.3-g sample of extract was

diluted with high-performance liquid chromatography–grade water to a final volume of 100 ml. Ten milliliters of the diluted sample was filtered with a 0.45-mm filter[4] and analyzed by high-performance anion-exchange chromatography–pulsed amperometric detector with a Dionex 500 system[5] on a CarboPac PA-100 anion-exchange column[6] with a CarboPac PA-100 guard column. Output was quantified by a pulsed amperometric detector equipped with a gold electrode (potentials: E_1, +0.10 V; E_2, –2.00 V; E_3, +0.60 V; E_4, –0.10 V). The flow rate of eluent was 1 ml min^{-1}. The eluent conditions were stabilized for 6 min with 98% eluent A (100 mM NaOH) and 2% eluent B (100 mM NaOH, 600 mM NaOAc). Data collection began with 98% A and 2% B for 7 min, a curved gradient for 17 min changing from 98% A and 2% B to 85% A and 15% B, and a programmed gradient for 55 min changing from 85% A and 15% B to 25% A and 75% B, and a 5 min cleanup with 2% A and 98% B. Quantification was performed on the peak areas with the external standard method for glucose, fructose, sucrose, 1-kestose, 1-nystose, and 1-fructofuranosyl-nystose. Glucose, sucrose, fructose, 1-kestose, 1-nystose, and 1-fructofuranosyl-nystose were reported in milligram per gram of fresh weight.

Data were analyzed by using a split-plot analysis of variance, with the main plots being the insecticide treatments and the split plots being the damaged or undamaged samples within the main plots (SAS 1999). Mean comparisons were made using Fisher's Protected LSD at the 0.05 level of significance. Pearson correlation coefficients were determined between various carbohydrate variables and shoot tunnel length (SAS 1999).

Results and Discussion

Air Temperatures and Rainfall

Average air temperatures during the 5-mo period from May to September 2000 (18.3 C) were 1.5 C cooler than those during the 5-mo period from May to September 2001 (19.8 C). Rainfall during the 5-mo periods was 143 and 186 mm for 2000 and 2001, respectively. In relation to the long-term average, air temperatures during the 5-mo period were approximately 0.8 C cooler in 2000 and 0.9 C warmer in 2001. Rainfall for the 5-mo period in both 2000 and 2001 was below the long-term average for the region of 249 mm. Even though subtle environmental differences were observed, growing conditions for the 5-mo period in 2000 and 2001 were similar.

Ceutorhynchus litura *Populations*

Ceutorhynchus litura larval tunneling was observed from mid-May into late June. The extent of larval tunneling in infested shoots was readily evident for the remainder of the season. In the spring of 2000, *C. litura* infested 82% of all the shoots across the study area. The insecticide treatment was apparently applied too late in 2000 to reduce the number of infested shoots because no differences in infestation levels were observed between the treated and untreated plots. Across all the sampling dates, the untreated plots averaged 77.3% (± 7.2; SEM) infested shoots and the treated plots averaged 61.9% (± 7.2) infested shoots. Tunnel lengths for the damaged shoots from the untreated and treated plots did not differ ($P > 0.05$), averaging 9.9 cm (± 1.5) and 5.9 cm (± 1.5), respectively, across all sampling dates. Tunnel lengths in 2000 for the undamaged and lightly damaged shoots

also did not differ between the untreated and treated plots, averaging 0.8 cm (± 0.19) and 0.4 cm (± 0.19), respectively. However, on all sampling dates, there were differences in tunnel lengths between the insect-damaged and undamaged or lightly damaged Canada thistle shoots.

The insecticide treatment in 2001 was applied 15 d earlier than in 2000 and provided more consistent control of *C. litura*. Across the three sampling dates, the average *C. litura* infestation differed from 66.3% (± 4.7) for the untreated plots to 24.3% (± 4.7) for the insecticide-treated plots. Tunnel lengths for the damaged shoots from the untreated and treated plots did not differ, averaging 8.4 cm (± 1.0) and 6.0 cm (± 1.0), respectively. In 2001, shoots were separated with respect to the presence or absence of damage, so none of the undamaged shoots had signs of *C. litura* feeding. Establishment of weevil-free plots is difficult because, at the time of adult activity (early spring), the thistle shoots are small and often covered by dried vegetation, which hinders penetration of the insecticide.

In the spring of 2002, infestation estimates between the treated and untreated plots were made to determine any residual effects of the differences during the study. No differences were seen between the untreated and insecticide-treated plots in insect tunnel length, percent infested shoots, or thistle density. The final thistle density across the four initial repetitions used in 2000 averaged 15 shoots m^{-2} compared with the initial density of 20 shoots m^{-2}.

Influence of **C. litura** *on Root Carbohydrates*

The results of the analysis for free sugars are shown in figures 1–3. The results of the May 30, 2000, sampling are not included because sampling on that date did not result in *C. litura* infestation differences between the samples, and sampling modifications, as indicated previously, were incorporated. Glucose levels in Canada thistle roots not damaged by *C. litura* were higher during the June and early July sampling each year (fig. 1). By late July (2000), no differences were seen between the damaged and undamaged roots, but by late August (2001) and September (2000), glucose levels were elevated in the damaged roots; however, this reversal in glucose levels was significant only in September 2000. Fructose levels from June through September were similar to those observed for glucose, with reduced levels in damaged plant roots early in the summer and increased levels late in the summer (fig. 2). The impact of *C. litura* on sucrose levels was less dramatic (fig. 3), but in each year, the early-July sampling did demonstrate depressed sucrose levels for the *C. litura*–damaged roots, and no reversal of this change was evident later in the summer.

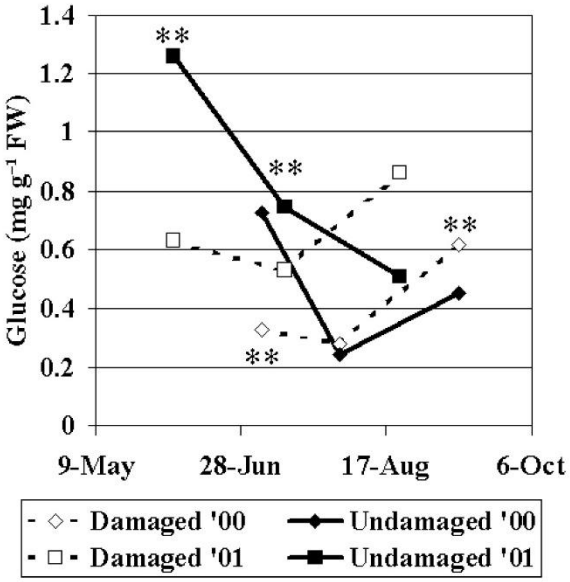

Figure 1. Glucose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

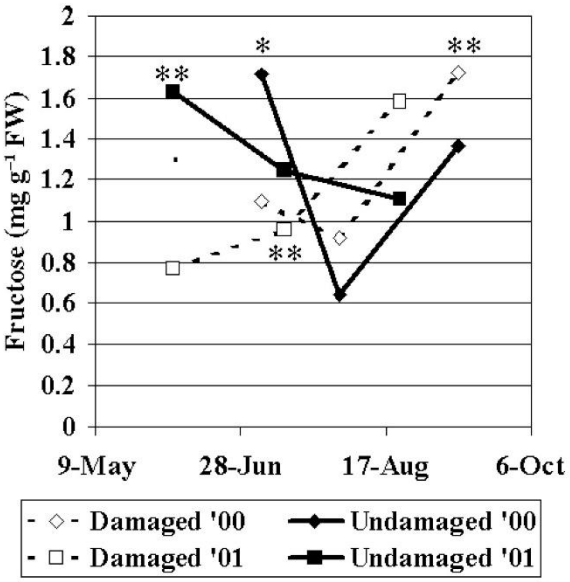

Figure 2. Fructose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged by *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

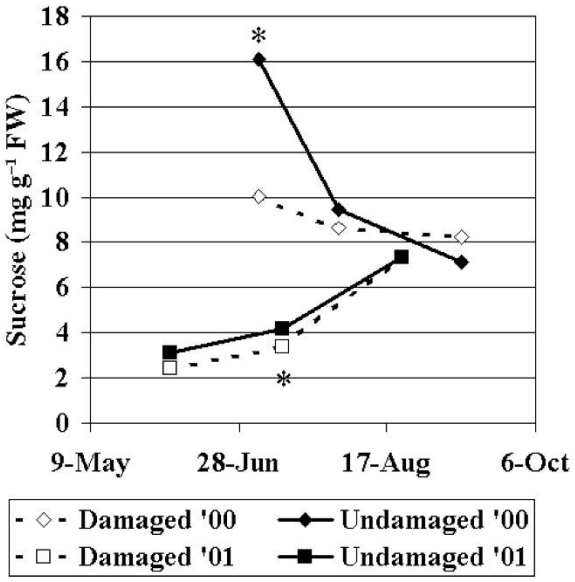

Figure 3. Sucrose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged by *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

Fructan levels through the season were similar to levels of free sugars (fig. 4–6). Levels of 1-kestose in the undamaged plant roots were elevated for the June and early-July sampling dates, but the August (2001) and September (2000) sampling showed no differences between damaged and undamaged roots (fig. 4). Levels of 1-nystose were reduced in the damaged plant roots for the June 2000 and early-July 2001 sampling dates (fig. 5), but again a significant reversal was seen in 2001, where the *C. litura*–damaged plant roots had elevated levels of 1-nystose in late August. Levels of 1-fructofuranosyl-nystose were similar to those of 1-nystose, with reduced levels in the damaged plant roots early in the summer and a reversal of these levels in late summer (August 2001; fig. 6).

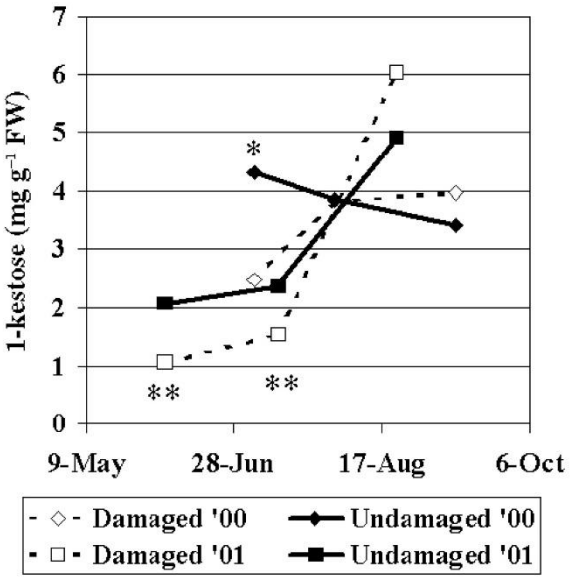

Figure 4. 1-Kestose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged by *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

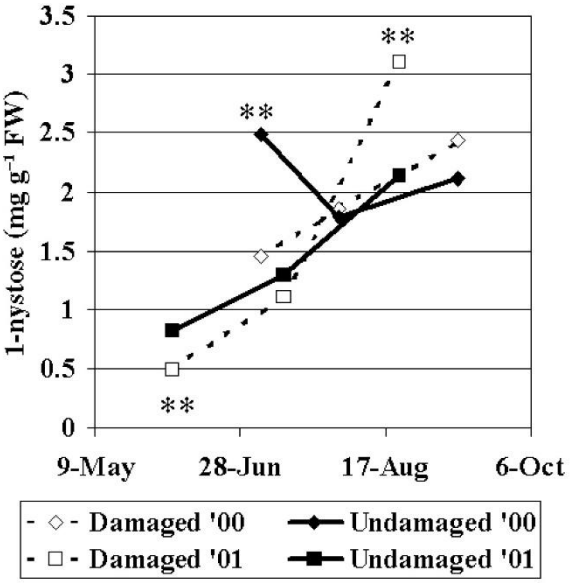

Figure 5. 1-Nystose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged by *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

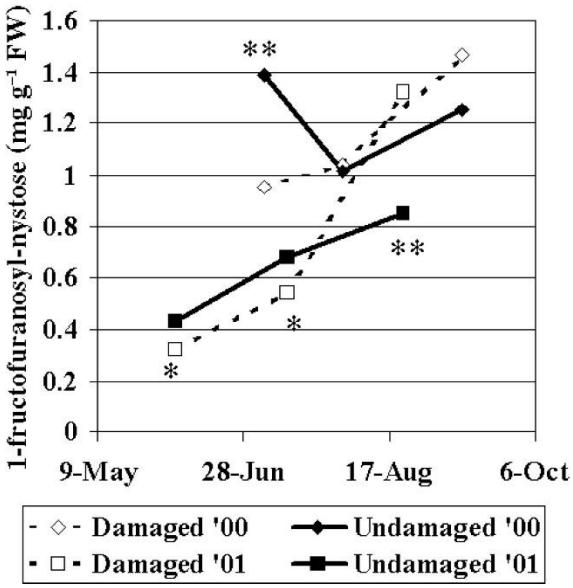

Figure 6. 1-Fructofuranosyl-nystose levels (mg g^{-1} fresh weight) in Canada thistle roots damaged and not damaged by *Ceutorhynchus litura*. Significant differences for comparisons between treatments within sampling date denoted by * ($P < 0.05$) or ** ($P < 0.01$).

We observed live larvae in tunnels only during late-May and early-June sampling. Levels of all free sugars and fructans were consistently found to be depressed in *C. litura*–damaged plant roots early in the summer during and shortly after the larval feeding period (i.e., through early July). It appears that *C. litura* feeding in Canada thistle shoots disrupts the movement of photoassimilates from leaves to roots. Later-season levels of free sugars indicate that roots do recover from these depressed levels, and, in several instances, significant overcompensation occurred in the damaged roots because they had elevated sugar and fructan levels.

The response of Canada thistle to *C. litura* damage is also demonstrated by the correlations between sugar and fructan levels and the extent of *C. litura* tunneling shown in table 1. Significant negative correlations between sugar levels and tunneling were seen in several instances for the June and early-July samples, indicating that greater *C. litura* damage (increased tunnel length) resulted in lower sugar levels in roots. The late-August and September sampling showed a reversal in this relationship, with several of the correlations being positive.

Table 1. Correlation between *Ceutorhynchus litura* tunneling in Canada thistle stems and various sugars present in the roots

	Pearson correlation coefficients (*r*)					
Sugars	July 5, 2000 (*n* = 16)	August 1, 2000 (*n* = 16)	September 11, 2000 (*n* = 16)	June 5, 2001 (*n* = 21)	July 13, 2001 (*n* = 22)	August 22, 2001 (*n* = 23)
Glucose	–0.34	0.23	0.44	–0.44*	–0.48*	0.31
Fructose	–0.43	0.14	0.42	–0.61**	–0.38	0.15
Sucrose	–0.54*	0.04	0.21	–0.52*	–0.36	–0.06
1-Kestose	–0.49*	0.05	0.16	–0.66**	–0.48*	0.42*
1-Nystose	–0.58*	0.15	0.21	–0.48*	–0.19	0.62**
1-Fructofuranosyl-nystose	–0.64**	–0.02	0.27	–0.36	–0.37	0.72**

* $P < 0.05$; ** $P < 0.01$

Tworkoski (1992) showed that, under field conditions, more photoassimilate moves to the roots of Canada thistle at the bolt than at bud, flower, or postflower stages. Canada thistle in Nebraska normally begins to bolt in late May and early June; therefore, during the period of active insect feeding, and according to the observations of Tworkoski (1992), photoassimilate should be moving from leaves to roots during this period. Our data indicate that late-spring (May–June) weevil feeding on Canada thistle reduces the levels of free sugars and fructans in infested roots.

Peschken and Derby (1992) showed reduced total carbohydrates during the actual feeding period. Our study demonstrates similar results and showed that both free sugars and fructans combine to produce this reduction. Peschken and Derby (1992) observed that plants recovered after feeding, with no late-season differences in carbohydrate levels. Our study indicates that roots from damaged stems actually may overcompensate and produce greater quantities of free sugars and fructans later in the summer and allow Canada thistle to recover from *C. litura* feeding stress that occurred earlier in the season.

Carbohydrate profile data suggest that *C. litura* feeding during May and June may significantly weaken Canada thistle roots by reducing carbohydrate reserves. But because the weevils stop feeding, infested shoots continue to grow and respond to early-season injury by storing more sugars in their roots to recover from early-season insect stress. A complicating factor in discussing assimilate partitioning in Canada thistle is the extensive horizontal root system of the plant. However, the effect of this extensive root system on the mobilization of sugars among root sections is not clear. Donald (1994) indicated that the physiological connections between all shoots and roots of the same plant have not been measured directly. However, Beuerman et al. (1984) demonstrated a degree of connectedness using apparent movement of glyphosate to untreated areas of the same plant. Whatever the degree of connection between Canada thistle roots and shoots, it is clear that *C. litura* damage to an extensive number of shoots of a plant will affect the overall photosynthate balance of the plant by creating a seasonal sink in the damaged roots. In our study, this sink was compensated for, and in some cases, overcompensation occurred. The source of the sugars and fructans mobilized to damaged roots is not known but may come from

either the damaged shoot or adjoining roots. From this, it is clear that to affect overall plant shoot density, high infestations of *C. litura* would be needed, as indicated by Rees (1990).

Measurement of the impact of various stresses on thistle, such as the impact on free sugars and fructans, may allow researchers to identify sublethal impacts of various control actions on Canada thistle. By taking advantage of this early-season damage from *C. litura* feeding and introducing additional stresses later in the season, such as mowing (R. G. Wilson, unpublished), additional phytophagous insects, plant pathogens, or herbicides (Wilson and Michiels 2003), improved management systems for Canada thistle may be developed. These later-season stresses may complement the early-season impact of *C. litura*, prevent late-season plant recovery, and maximize the potential for control.

There are few examples of using multiple stresses in weed management. Hoeft et al. (2001) combined cultural and biological methods on Canada thistle with no evidence for synergy in control. However, Ang et al. (1994) demonstrated that combining defoliation and plant competition did result in reduced biomass in Canada thistle. The full potential of combining control methods will likely be realized only with a more complete understanding of the true impacts of the individual components of control. For Canada thistle, the measurement of the impact of biological control components on free sugars and fructans may provide a tool to logically combine control tactics for optimal effects on the plant.

Sources of Materials

1. Carbofuran, FMC Corporation, Agricultural Products Group, 1735 Market Street, Philadelphia, PA 19103.
2. Juicer, Omega Products Inc., 6291 Lyters Lane, P.O. Box 4523, Harrisburg, PA 17111.
3. Refractometer, Atago, 32-10 Honcho, Itabashi-Ku, Tokyo 173, Japan.
4. Filter, Advantec MFS Inc., 6691 Owens Drive, Pleasanton, CA 94588.
5. Dionex 500 System, Dionex Corporation, P.O. Box 3603, Sunnyvale, CA 94088-3603.
6. CarboPac PA-100, Dionex Corporation, P.O. Box 3603, Sunnyvale, CA 94088-3603.

Acknowledgments – Contribution of the University of Nebraska Agricultural Research Division, Journal Paper 14179. We thank Ms. Lori Howlett and Ms. Trisha Neilson for their assistance with high-performance anion-exchange chromatography analysis of Canada thistle.

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