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Author(s): Beth A. Newingham, Ragan M. Callaway and Hormoz BassiriRad

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Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory

Beth A. Newingham · Ragan M. Callaway ·
Hormoz BassiriRad

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Abstract *Centaurea maculosa*, an invasive North American plant species, shows a high degree of tolerance to the root-boring biocontrol herbivore, *Agapeta zoegana*. For example, infested individuals of *C. maculosa* often exhibit more rigorous growth and reproduction compared with their non-infested counterparts. Compensatory responses to aboveground herbivores often involve increases in leaf area and/or photosynthetic capacity, but considerably less is known about root system compensatory responses to belowground herbivory. We used a ^{15}N labeling approach to evaluate whether compensatory adjustments in N acquisition via changes in root morphology and/or physiological uptake capacity could explain the ability of *C. maculosa* to tolerate root herbivory. Root herbivory reduced whole plant N uptake by more than 30% and root uptake capacity by about 50%. Despite a marked reduction in N procurement, herbivory did not affect total biomass or shoot N status. Infested plants maintained shoot N status by shifting more of the acquired N from the root to the shoot. To our knowledge, shifting N allocation away from a root herbivore has not been reported and provides a plausible

mechanism for the host plant to overcome an otherwise devastating effect of a root herbivore-induced N deficit.

Keywords Allocation · Biocontrol · Nitrogen · Root herbivory · Root uptake capacity

Introduction

Compensatory adjustments in growth, shoot morphology and physiology have been shown to allow plants to cope with aboveground herbivores (Paige and Whitham 1987; Lennartsson et al. 1998; Agrawal 2000). These mechanisms include activating dormant meristems, increasing photosynthetic rates, using stored reserves, altering biomass and resource allocation patterns, and altering phenology or plant architecture (Polley and Detling 1989; Trumble et al. 1993; Baldwin et al. 1994; Strauss and Agrawal 1999; Tiffin 2000; Millard et al. 2001). However, much less is known about how plants respond to belowground herbivory. Belowground herbivores can affect plant growth (Karban 1980; Powell and Myers 1988), reproduction (Powell and Myers 1988; Maron 1998; Masters et al. 1993, 2001) density (Müller-Schärer and Brown 1995), nutrient status (Masters and Brown 1992; Masters 1995; Bardgett et al. 1999; Louahia et al. 2000), and species richness (Brown and Gange 1989), but very few studies have examined mechanisms of compensatory growth in response to root herbivory (Hunter 2001).

Belowground herbivores can have stronger impacts on plants than aboveground herbivores, but the effects are less recognized probably due to their invisibility (Brown and Gange 1990). Because of these strong effects, insect root herbivores have become widespread biocontrol agents used to combat invasive species (Hunter 2001; Blossey and

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B. A. Newingham · R. M. Callaway
Division of Biological Sciences, University of Montana,
Missoula, MT 59812, USA

H. BassiriRad
Department of Biological Sciences, University of Illinois
at Chicago, Chicago, IL 60607, USA

B. A. Newingham (✉)
School of Life Sciences, University of Nevada, Las Vegas,
NV 89154, USA
e-mail: newingha@unlv.nevada.edu

Hunt-Joshi 2003; Müller-Schärer and Schroeder 1993). For example, five of the 13 species of biocontrol insects introduced to North America to control the spread of *Centaurea maculosa* Lam. (spotted knapweed) attack the roots (Rosenthal et al. 1991; Sheley et al. 1998; Smith 2001; Story and Piper 2001).

C. maculosa is an invasive, perennial plant that was introduced from Europe to North America in the late 1800s (Dostal 1976; Sheley et al. 1998). *C. maculosa* often creates dense, almost monospecific stands and can result in declines in native species richness, diversity and cover (Tyser and Key 1988; Ortega and Pearson 2005). Müller-Schärer and Schroeder (1993) noted that despite extensive biocontrol efforts, *C. maculosa* still covered millions of hectares in North America and was still expanding its range. For example, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), a root-boring biocontrol for *C. maculosa*, may have local effects on *C. maculosa* density (Story et al. 2000), but *A. zoegana* appears to be ineffective at large scales. The lack of widespread negative effects may be explained by several studies showing that root herbivory does not always have negative effects on *C. maculosa* and does not necessarily reduce its competitive ability. Müller-Schärer (1991) and Steinger and Müller-Schärer (1992) found that root herbivory by *A. zoegana* did not reduce the growth of *C. maculosa* in its native environment in Europe, and in some cases, root herbivory caused overcompensation of root growth (Müller 1989) and increased survival (Müller-Schärer 1991). In North America, *A. zoegana* has been found to increase the reproductive output of *C. maculosa* (Ridenour and Callaway 2003). Callaway et al. (1999) found that root herbivory by *A. zoegana* and leaf damage by a generalist herbivore, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), may slightly increase the competitive ability of *C. maculosa*. Such increased competitive ability was demonstrated in the field by Thelen et al. (2005), who attributed the increased competitiveness to a higher allelopathic root exudation.

Currently, the potential mechanism for the strong compensatory responses of *C. maculosa* to root herbivory is not known. Studies of foliar herbivory on other species found that rapid increases in leaf area and/or photosynthetic rate appeared to explain compensatory responses (Hicks and Turkington 2000; Anten and Ackerly 2001; Pilon and Decker 2002). Therefore, it is reasonable to speculate that the observed compensatory responses in *C. maculosa* may be mediated through root physiological and/or morphological adjustments.

Given the limited soil N availability in North American habitats where *C. maculosa* occurs (Woodmanse et al. 1979), the compensatory response so often measured for *C. maculosa* when damaged by *A. zoegana* may involve some adjustment in root traits that increase N acquisition.

We conducted an experiment to determine whether physiological changes in N explain compensatory growth by *C. maculosa* to *A. zoegana*. Using an enriched ^{15}N -tracer approach, we examined biomass allocation to fine roots, N partitioning, and root physiological uptake capacity of N as potential mechanisms to explain this compensatory growth to root herbivory. We expected that root herbivory might induce similar types of compensatory responses that shoot herbivory has been shown to induce with respect to N uptake and allocation. More specifically, we hypothesized that root herbivory would: (1) increase biomass allocation to fine active roots, (2) increase N allocation to roots, and (3) increase root physiological N uptake capacity.

Materials and methods

Plant materials and growth conditions

The experiment was conducted in the Plant Growth Facility at the University of Illinois at Chicago. On 28 March 2002, we placed five *C. maculosa* seeds (collected from near Missoula, Montana) in 4-l pots in the greenhouse, which were filled with autoclaved, river-washed, fine sand. Two weeks after emergence, plants were thinned to three individuals per pot and were grown for an additional 6 weeks inside a temperature controlled, naturally lit greenhouse. Day and nighttime temperatures were set at 22 ± 2 and 18 ± 2 °C, respectively. On 6 June, plants were transferred outdoors where they grew under natural thermal and photo regimes for the remainder of the experiment. On 15 July, plants were further thinned to one individual per pot. Plants were watered with deionized water daily until free drainage and irrigated twice a week with 120 ml of either a low (2 mM) or high (10 mM) N fertilizer. Fertilizer was prepared by adding either 1 mM or 5 mM NH_4NO_3 to a 1/4 strength N-free Hoagland solution. Each N treatment had 30 replications.

Infestation of *A. zoegana*

Agapeta zoegana is an oligophagous, facultatively multi-voltine root feeder on *Centaurea* species, which was released in Canada in 1982 and Montana in 1984 (Rosenthal et al. 1991; Story et al. 1991). *A. zoegana* infestation rates in the field averaged from 1.2 to 2.0 larvae per attacked root in its native ranges of Austria and Hungary (Müller et al. 1988). A release site in Montana, reported approximately 1.5 larvae per attacked root (Story et al. 2000). Moth larvae overwinter in *C. maculosa* roots and emerge between June and September as adults. Adult *A. zoegana* females mate within 24 h of emerging and oviposit on soil and on the leaves and stems of *C. maculosa* the next day.

Larvae hatch after 7–10 days, crawl to the root crown, bore into the root, and feed on cortical tissue (Müller et al. 1988; Story et al. 2000). On 16 July, we collected several adult, female *A. zoegana* near Hamilton, Montana, in a *C. maculosa*-dominated community. Females were placed in paper cones and allowed to lay eggs for 4 days. Females were then removed from the paper cones and square sections of paper with eggs were cut out, mounted with a pin, and transferred to the University of Illinois at Chicago. We chose three females that produced at least 30 eggs. On 24 July, we placed one egg per female on each plant for a total of three eggs per plant. *A. zoegana* eggs were placed on 15 plants from each N treatment. Cages made of chicken wire and water-resistant paper were placed around the base of each plant, including non-infested plants to control for light and temperature. Cages were left on for 2 days to prevent larvae from escaping and increase chances of colonization. Larvae remained in *C. maculosa* roots for 11 weeks before ^{15}N labeling commenced.

^{15}N tracer, biomass N uptake, partitioning

On 1 October, 2 days before harvest, each pot received 120 ml of 2 mM $^{15}\text{NH}_4^{15}\text{NO}_3$ at an enrichment level of 10 atom%. The solution containing ^{15}N was injected into the sand using a long-needle syringe making sure that ^{15}N was distributed throughout the potting volume. Plants were harvested after 36 h of labeling and separated into shoots and roots. Harvested roots were examined for colonization rate by dissecting the taproots and carefully removing larvae, and then roots were further divided into fine and coarse roots. Fine roots were qualified as white or light brown roots that were less than 0.5 mm in diameter. Roots larger than 0.5 mm were qualified as coarse roots. The division between the fine and coarse root is particularly important in studies of nutrient uptake and when uptake rates are being expressed on a root biomass basis. The larger and darker roots are heavily suberized and as such are not involved in active ion uptake (BassiriRad et al. 1996). Root and shoot tissues were then oven-dried at 60°C to a constant mass.

All tissues were ground in a Wiley mill before they were analyzed for ^{14}N and ^{15}N at the University of California, Davis Molecular Structure Facility. Total ^{15}N gain for the 36-h labeling period was calculated using the following equation: (excess atom% ^{15}N in the plant tissue \times tissue N content)/excess atom% of the tracer. Physiological root N uptake capacity was determined by dividing the total ^{15}N gain of each plant by its fine root biomass per hour. Because biomass allocation to fine roots is an important indicator of morphological plasticity relevant to nutrient uptake, we estimated this fraction as the ratio of fine root biomass to total plant biomass and refer to it as fine root

ratio (FRR). We also calculated the whole-season N uptake and partitioning among fine roots, coarse roots, and shoots, using the product of average N concentration and biomass of each tissue.

Data analysis and statistical approaches

Data were analyzed using SPSS 11.0. All biomass and N parameters were analyzed separately with two-way ANOVAs using N and herbivory as fixed factors. Data were log transformed when necessary. Individual comparisons were made using Bonferroni corrections. *P*-values <0.05 were considered statistically significant and those *P*-values between 0.05 and 0.10 are noted. All reported values are mean \pm 1 SE.

Results

Infestation rate, total biomass, and biomass allocation

There was an average of 2.04 ± 0.16 *A. zoegana* larvae per infested plant whereas no larvae were detected in non-infested plants. Total plant biomass was not significantly affected by root herbivory regardless of N treatment (Table 1; *df* = 1, *F* < 0.0001, *P* = 0.99), but high N increased total biomass by an average of 80% across the herbivory treatments (Table 1; *df* = 1, *F* = 73.40, *P* < 0.0001). However, biomass partitioning was sensitive to both herbivory and N treatments. The high N treatment markedly increased biomass allocation to the shoot, i.e., the root to shoot ratio was 0.88 under low N and decreased to roughly 0.43 under the high N treatment (Fig. 1; *df* = 1, *F* = 111.01, *P* < 0.0001). Similarly, biomass partitioning to the shoot increased significantly in response to herbivory (*df* = 1, *F* = 13.95, *P* < 0.0001). Overall, coarse root biomass decreased by about 15% in response to herbivory (*df* = 1, *F* = 9.72, *P* = 0.003) under both N treatments. Biomass allocated to fine roots, as indicated by FRR, in the overall analysis was not affected by herbivory (*df* = 1, *F* = 0.69, *P* = 0.41); however, in pair-wise comparisons, FRR tended to decrease in response to herbivory in low N but not in high N treated plants (Table 1; *P* = 0.09).

N uptake capacity and N partitioning

The ^{15}N results indicated that root herbivory significantly reduced N uptake on a per plant basis, but this effect was more pronounced in the low N compared to the high N treatment (Table 1). More specifically, under low N supply, infested plants took up 33% less N compared with non-infested plants. Kinetics of root N uptake, as indicated by the root N uptake capacity, was not affected by N (*df* = 1,

Table 1 Total biomass, whole plant N uptake, root N uptake capacity, % total biomass allocated to the fine roots [fine root ratio (FRR)], and N partitioning responses of *Centaurea maculosa* to root herbivory at different N supply rates. Means \pm 1 SE ($n = 15$). Values

are compared for the herbivory effect within a N treatment and *different letters* denote significant differences ($P < 0.05$). *d.w.* Dry weight

Treatment	Total biomass (g)	N uptake ^a (mg plant ⁻¹)	Root N uptake capacity ^a (mg g ⁻¹ d.w. h ⁻¹)	FRR (%)	N transported to shoot ^a (% of total uptake)
Low N					
- Herbivore	13.42 \pm 0.62 a	394.3 \pm 13.2 a	1.02 \pm 0.11 a	9.62 \pm 0.32 a*	32.3 \pm 2.0 a
+ Herbivore	12.95 \pm 0.71 a	269.8 \pm 11.6 b	0.57 \pm 0.05 b	7.80 \pm 0.31 a	60.0 \pm 2.7 b
High N					
- Herbivore	20.98 \pm 1.32 a	801.8 \pm 42.6 a	0.81 \pm 0.09 a	6.30 \pm 0.57 a	49.0 \pm 3.9 a
+ Herbivore	21.13 \pm 1.17 a	784.9 \pm 31.3 a	0.72 \pm 0.08 a	6.53 \pm 0.86 a	60.4 \pm 3.3 b

* $P = 0.09$

^a N uptake, uptake capacity and transport rate to the shoot are calculated based on the ¹⁵N-tracer acquisition

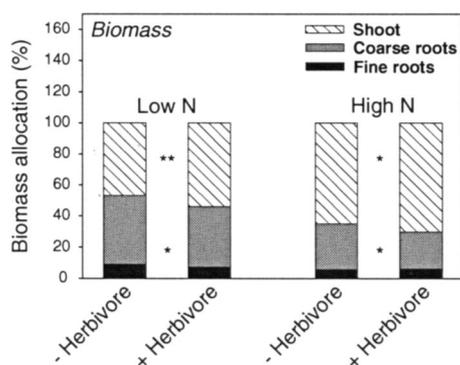


Fig. 1 The combined effects of root herbivory and soil N availability on biomass allocation among the shoot, coarse roots, and fine roots in *Centaurea maculosa*. Values are means ($n = 15$) and significant differences in response to herbivory are denoted with *asterisks*: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

$F = 0.14$, $P = 0.71$) but was significantly decreased by root herbivory ($df = 1$, $F = 10.58$, $P = 0.002$) depending on the N treatment. Root N uptake capacity was almost halved in response to root herbivory under low N but was not significantly affected by root herbivory under high N conditions (Table 1). We calculated the proportion of N that was translocated to the shoot during the 36 h of ¹⁵N incubation and found that regardless of the N treatment, root herbivory markedly stimulated the transfer of N to the shoot. During the ¹⁵N incubation period, infested plants grown under low N nearly doubled the amount of N transported to the shoot compared to non-infested plants. This root herbivory-induced shift in N allocation to the shoot was also significant in high N-treated plants, but to a lesser extent (Table 1).

A more detailed analysis of the short-term ¹⁵N partitioning among the internal pools clearly showed that coarse roots were a major compartment for N in non-infested plants, and that the coarse roots exhibited the most dynamic response to root herbivory (Fig. 2a; $df = 1$,

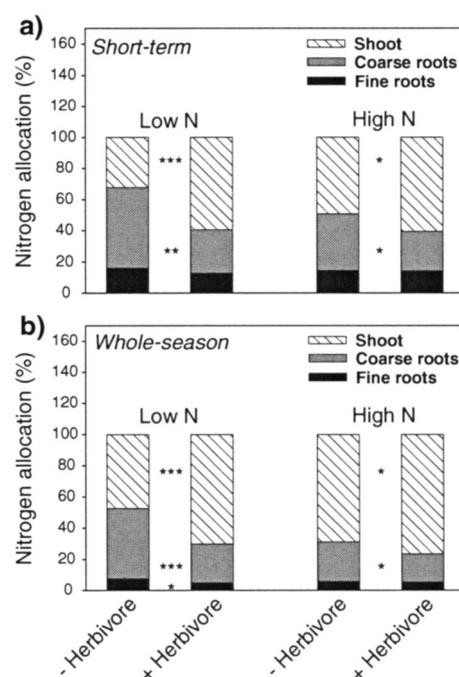


Fig. 2 The combined effects of root herbivory and soil N availability on **a** short-term allocation using enriched ¹⁵N and **b** whole-season N allocation among the shoot, coarse roots, and fine roots in *C. maculosa*. Values are means ($n = 15$) and significant differences in response to herbivory are denoted with *asterisks*: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

$F = 32.47$, $P < 0.0001$). For example, in non-infested plants, 51% and 36% of acquired N was allocated to the coarse roots in low N- and high N-treated plants, respectively. However, in infested plants, N allocation to the coarse roots dropped to 27% and 24% in low N- and high N-treated plants, respectively. This decrease in N allocation to coarse roots was accompanied by a significant increase in N allocation to the shoot in infested compared to non-infested plants ($df = 1$, $F = 38.94$, $P < 0.0001$). There

was little or no change in N partitioning to fine roots in response to herbivory ($df = 1$, $F = 0.60$, $P = 0.44$). Therefore, the short-term N uptake data indicate that root herbivory-induced N translocation to the shoot was almost exclusively at the cost of N that would have otherwise been partitioned to the coarse roots where *A. zoegana* larvae reside.

Patterns of whole-season N partitioning in response to herbivory were similar to those obtained from short-term ^{15}N labeling data. Like the short-term labeling experiments, herbivory significantly decreased whole-season N allocation to the coarse roots (Fig. 2b; $df = 1$, $F = 46.26$, $P < 0.0001$) but N allocation increased to the shoot ($df = 1$, $F = 39.35$, $P < 0.0001$). Whole-season N allocation responses were similar for low N- and high N-treated plants. Whole-season N allocation to fine roots was slightly lower in infested as opposed to non-infested plants ($df = 1$, $F = 4.34$, $P = 0.04$); however, this was only significant for low N-treated plants.

Tissue N concentration, which represents the balance between N acquisition, loss and partitioning over the entire season, showed that over all treatments root herbivory significantly reduced the N status of fine ($df = 1$, $F = 39.97$, $P < 0.0001$) and coarse ($df = 1$, $F = 64.25$, $P < 0.0001$) roots with no significant changes in shoot N concentration ($df = 1$, $F = 0.47$, $P = 0.50$) (Fig. 3). However, this effect was dependent on N availability, whereby root herbivory only significantly altered the N quality of above- and belowground tissues under low N supply and not high N supply.

Discussion

Previous studies have shown that *C. maculosa* plants infested by root biocontrols tend to either equally compensate (Müller-Schärer 1991; Steinger and Muller 1992) or overcompensate (Müller 1989; Ridenour and Callaway 2003) in biomass or reproduction in response to herbivory. Our results clearly indicated an equal compensatory growth

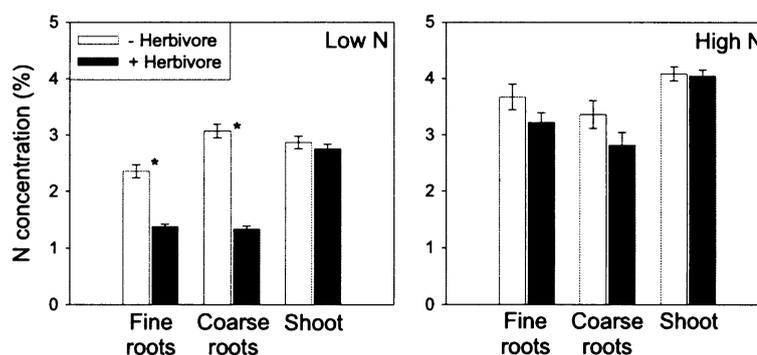
response as the infested plants were able to maintain their total biomass despite a significant loss of fine and coarse roots. Although reproductive output was not measured in this experiment, it is likely that an increase in biomass results in an increase in *C. maculosa* flower number ($R^2 = 0.80$; B. A. Newingham, unpublished data).

This compensatory growth response is surprising given the considerable reduction in the whole plant N uptake in response to herbivory and no adjustments in either root morphology or kinetics to help maintain N uptake. More specifically, under low fertility conditions whole plant N acquisition was reduced by more than 30%, and the infested plants were unable to exhibit an upward adjustment in either root morphology or kinetics to help maintain N uptake. In fact, under low N supply, root physiological N uptake capacity was down regulated by almost 50% and the biomass allocation to FRR decreased by 19% in response to herbivory. To our knowledge, this is the first report that has simultaneously assessed root morphological and physiological responses to a root herbivore.

Even with an overall marked reduction in plant N acquisition in response to root herbivory under low N supply, we found that infested *C. maculosa* plants were able to disproportionately shift N to the shoot. Under low N supply, the infested plants translocated almost twice as much N to the shoot when compared to undamaged plants. As a result, the infested plants maintained a similar N nutrition in their photosynthetic tissues, while fine and coarse root N content dropped significantly compared to the non-infested plants. Plants grown under high N supply did not show any significant adjustment in FRR or N uptake capacity in response to root herbivory. However, even in the high N-treated plants, the presence of the herbivore significantly stimulated N translocation to the shoot, though this was not as pronounced as for low N-treated plants.

We are unable to decipher the exact mechanism by which *A. zoegana* enhances N transport to the shoot in the host plant. Based on the present state of knowledge, however, it is well-established that active N (and most

Fig. 3 Effects of root herbivory and soil N availability on N concentration of various tissues in *C. maculosa*. Values are means ($n = 15$) and significant differences in response to herbivory are denoted with asterisks: * $P < 0.05$



other ions) uptake into the cortical cells and loading into the xylem are controlled separately (Glass 1989; Bassiri-Rad and Caldwell 1992). Therefore, we speculate that the presence of *A. zoegana* markedly stimulated xylem loading of N. Alternatively, because N can be retranslocated from shoot to root via phloem (Glass 1989), it is possible that root herbivory may have significantly reduced phloem transport of N back to the root. This is a strong possibility particularly if the shoot N content of infested plants is already incorporated into larger organic N compounds such as Rubisco. Large molecular weight nitrogenous compounds (larger than amino acids) are rarely loaded and transported in the phloem.

We found that N mass allocation to herbivory was not driven by changes in biomass. For example, under low N supply herbivory increased shoot biomass allocation by only 7% whereas shoot N mass allocation increased by 27%. This clearly indicates an uncoupling of biomass and N allocation in *C. maculosa*'s response to the root herbivore. This is not surprising because biomass and nutrients are not always allocated similarly, and a dynamic nutrient movement in plants has often been reported without a concomitant change in biomass (Abrahamson and Caswell 1982). We also believe that mechanical damage is an unlikely cause of N uptake and allocation responses in *C. maculosa* to the root herbivore. Given the similar level of infection in low N- and high N-treated plants, any mechanical damage effect should have been equally expressed regardless of the N status of the soil. In contrast, we found that herbivory generally had a significantly greater influence on N uptake and the allocation pattern of the low N- compared to high N-treated plants.

Internal reallocation of N and its importance to a plant's N budget has received some attention in studies of shoot herbivory. For example, it has been shown that moderate browsing of the shoot has little effect on N remobilization in birch saplings (Millard et al. 2001; Millett et al. 2005). In contrast, Baldwin et al. (1994) showed that leaf mechanical damage, as a surrogate for shoot herbivory, resulted in increased production and mobilization of nicotine (a secondary defense metabolite synthesized in the root) to the site of damage. As a highly nitrogenous compound, mobilization of nicotine to the site of the damage represents a significant translocation of N in the direction of the damage.

We believe this is the first report of herbivory-induced active N mobilization away from the site of the damage and the herbivore, which may have two consequences. First, remobilization to the shoots increases N supply to the photosynthetic tissues. We do not know how N is allocated within the leaves of infested plants, but given the well-established positive correlation between leaf N concentration and the photosynthetic rate (Field and Mooney 1986;

Evans 1989), it is reasonable to assume that this internal redistribution of N enables the infested plant to overcome an otherwise deleterious effect of N shortage on C acquisition. Second, N allocation away from the herbivore may result in N starvation of the insect. Although we do not know whether *A. zoegana* is N limited, many insects are strongly limited by N and this may be a plant's defense mechanism against the herbivore.

Interestingly, despite allocating N away from the roots, the shoot N status of infested *C. maculosa* did not rise above that of the control. Other studies have found either no change (Masters and Brown 1992) or an increase (Gange and Brown 1989) in total foliar N with root herbivory. Although Masters et al. (2001) suggested that an increase in seed predators with root-damaged plants was due to improved foliar N, N was not measured in their study. Since there was no difference in the shoot N status of *C. maculosa* infested by *A. zoegana*, we believe infested *C. maculosa* will be no more palatable or nutritionally attractive to leaf herbivores than their non-infested counterparts, unless there are other changes in leaf chemistry.

Both short-term ^{15}N labeling and whole-season N allocation data indicated that fine and coarse roots were a significant sink for N storage; however, the proportion of N allocated to roots was markedly larger in short- as opposed to long-term N allocation patterns across all treatments. These differences are not surprising and may simply represent the time scale over which the N uptake and partitioning data are integrated. More specifically, the ^{15}N experiments capture uptake and allocation patterns of N only in the later stages of the plant's growing season when shoot N demand is likely to be low; in contrast, whole-season N allocation integrates N partitioning over the entire growing season. Nevertheless, results from short and long-term N allocation pattern reported here indicates that coarse roots may be an important and dynamic storage organ in this plant and in its response to herbivory.

Our results support a large number of other studies that show physiological compensation to herbivory (Paige and Whitham 1987; Lennartsson et al. 1998; Agrawal 2000; Hicks and Turkington 2000) and suggest that it cannot be assumed that herbivory will always have negative effects on plants. Clearly, one must consider that the expression of any compensatory mechanism may be significantly modified by other biotic and abiotic stresses (Maschinski and Whitham 1989; Polley and Detling 1989). Our study shows, however, that neither compensatory growth nor N partitioning responses of the host plant depends on N availability in the soil. These results conflict with reports that N stress increased the negative effects of root herbivory on *C. maculosa* (Müller and Steinger 1990; Steinger and Müller-Schärer 1992) but are supported by other

studies that found compensatory responses were independent of N availability in Swingle citrumelo and sour orange (Borowicz et al. 2005).

Conclusion

To our knowledge, this is the first study that has simultaneously assessed root morphological and physiological responses to a root herbivore. However, it was the internal partitioning of N that offers the most plausible mechanism as to why *C. maculosa* has been highly resistant to the biocontrol agent *Agapeta zoegana*. Such mechanisms cannot be deciphered from broad assessments of biomass allocation alone. Even in studies where N concentrations of different tissues are assessed, without a clear knowledge of whole plant N uptake, it would be difficult to evaluate whether a significant shift in N allocation has occurred. Since this was a short-term experiment, long-term field experiments are important to verify if results scale up both in time and space and whether such a mechanism confers competitive and evolutionary advantages.

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