

Like herbivores, parasitic plants are limited by host nitrogen content

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Abstract Herbivores generally benefit from increased plant nitrogen content, because the nitrogen content of animals is much higher than that of plants. Consequently, high plant nitrogen alleviates the profound stoichiometric imbalance that herbivores face in their diets. Parasitic plants provide the opportunity to test this generalization for consumers across kingdoms. We fertilized two microhabitats in a California salt marsh that were dominated by *Salicornia virginica* or a mixture of *S. virginica* and *Jaumea carnosa*. The nitrogen content of both host plants and of the holoparasite *Cuscuta salina* (dodder) increased in fertilized plots in both microhabitats. *Cuscuta* preferred to attack *Jaumea*, although *Jaumea* had lower nitrogen content than *Salicornia*. When host nitrogen content was altered by fertilizing plots, however, the percent cover of the parasite doubled. Although parasitic plants and their hosts have similar tissue nitrogen contents, suggesting no stoichiometric imbalance between host and consumer, parasitic plants do not feed on host tissue, but on host xylem and phloem, which are very low

in nitrogen. Consequently, parasitic plants face the same dietary stoichiometric constraints as do herbivores, and both herbivores and holoparasitic plants may respond positively to increases in host nitrogen status.

Keywords *Cuscuta* · Fertilization experiment · Nitrogen · Parasitic plant

Introduction

Perhaps the most difficult problem facing ecologists is the apparent lack of generality in an idiosyncratic world. Life is extraordinarily diverse, and ecologists cannot study every species and their interactions—instead, ecologists necessarily seek general ecological principles. However, the unique features of each species make robust generalizations elusive. Nowhere is generality more elusive than in the study of plant-herbivore interactions, where the diversity of herbivore feeding modes and life histories mandates that theories must be examined separately for different herbivore guilds (Koricheva et al. 1998; Huberty and Denno 2004), for marine and terrestrial herbivores (Hay and Steinberg 1992), and for large versus small herbivores (Hay et al. 1988). On the other hand, students of plant-herbivore interactions have the rare opportunity to test for generality at the level of the kingdom by comparing herbivores with parasitic plants.

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One of the more robust generalizations of plant-herbivore theory is that herbivores eat nutritionally inadequate food (White 1978, 1984). Since animals have much lower tissue C:N ratios than plants, herbivores must eat an excess of carbon-rich plant material in order to obtain sufficient nitrogen. As a result of this dietary imbalance, plant nitrogen content is thought to play a central role in mediating herbivore feeding choices and population dynamics (Mattson 1980; Sterner and Elser 2002), and fertilization of host plants typically improves performance of herbivores (Waring and Cobb 1992).

Are parasitic plants also limited by host nitrogen content? Parasitic plants are the analogue to herbivores within the plant kingdom, obtaining some (hemiparasites) or all (holoparasites) of their nutrition from their host plants (Press and Graves 1995). Some aspects of the interactions between parasitic plants and host plants appear to parallel those between herbivores and host plants (Govier and Harper 1965; Atsatt 1977). Both parasitic plants and herbivores display host preferences, reduce host biomass, and alter host allocation patterns, modify plant community structure and dynamics, and mediate interactions between host plants and other organisms (Pennings and Callaway 2002). In other cases, differences between parasitic plants and herbivores in mobility, hormonal, and elemental composition and resource capture lead to differences in their interactions with their hosts (Pennings and Callaway 2002). In particular, in striking contrast to herbivores, parasitic plants do not have a large nutrient imbalance with their hosts, because both consumers and hosts are plants. Parasitic plants may have tissue nitrogen contents somewhat higher or lower than their hosts (Ehleringer et al. 1986), but these differences are modest compared with the order of magnitude differences that occur between plants and animals (Sterner and Elser 2002). On the other hand, many plant species are N-limited, and there is correlative evidence that parasitic plants perform better on host species with high nitrogen content (Watkinson and Gibson 1988; Seel et al. 1993; Matthies 1996), although exceptions to this pattern are common (Kelly 1990; Marvier 1996). In the laboratory, *Cuscuta* is more likely to accept (Kelly 1992) and grows better on host plants provided with more nitrogen (Jeschke and Hilpert 1997). In the field, the performance of hemiparasitic plants has been shown to improve following

fertilization of the host (Marshall et al. 1994), and the performance of holoparasites has been shown to improve following thinning of hosts, which presumably increased the availability of a wide variety of resources (Bickford et al. 2005). To the best of our knowledge, however, the impact of host nitrogen status on the performance of holoparasitic plants has not been experimentally addressed in the field.

Here, we report experimental manipulations of host plant nitrogen content in two microhabitats in a California salt marsh. Results indicate that the performance of a holoparasitic plant, *Cuscuta salina*, was strongly affected by host nitrogen content. These results suggest another important parallel between herbivores and parasitic plants—performance of both is mediated by host nitrogen content—and have important management implications for communities containing parasitic plants.

Methods

We worked at the Carpinteria Salt Marsh (34°24' N, 119°31'30" W) in southern California, USA (Callaway et al. 1990; Pennings and Callaway 1992, 1996). At this site, the holoparasite *Cuscuta salina* (hereafter, *Cuscuta*) preferentially attacks *Salicornia virginica* (hereafter, *Salicornia*) and *Jaumea carnosa* (hereafter, *Jaumea*) (Pennings and Callaway 1996). In marsh zones containing suitable host species and abiotic conditions, *Cuscuta* has strong impacts on plant community composition, increasing the abundance of species that it does not preferentially attack at the expense of those that it does preferentially attack (Pennings and Callaway 1996, 2002; Callaway and Pennings 1998).

In April of 1999, we delineated 0.5 × 0.5 m plots in the deep-*Salicornia* zone (sensu Pennings and Callaway 1996): 20 in microhabitats dominated by *Salicornia* and 20 in microhabitats dominated by mixtures of *Jaumea* and *Salicornia*. Every other plot in each zone was fertilized once (April, 1999) or twice (April and November, 2000–2003) each year, by burying five 21 g fertilizer pellets (20-10-5 N-P-K, Forestry Suppliers, Inc.) to a depth of 10 cm in each plot. This application rate (168 g/m²/y after 1999) was similar to or lower than those used in other salt marsh studies (Valiela et al. 1985; Emery et al. 2001; Pennings et al. 2002) and never produced signs of

burning. Control plots were similarly disturbed but fertilizer was not added. Cover of *Cuscuta* did not differ between treatments at the start of the experiment.

Percent cover of *Cuscuta* was determined in September 2003 using a 0.5×0.5 m quadrat divided by monofilament line into 100 cells. The quadrat was placed over each plot, and *Cuscuta* was scored as present or absent in each cell. Cover of *Salicornia* and *Jaumea* was determined in the same manner. Samples of *Cuscuta* (live stems), *Salicornia* (terminal portions of green stems) and *Jaumea* (mature leaves) were collected from each plot, in which each species was present on 9 May, 2003. Samples were taken from four different locations in each plot and pooled. Samples were dried at 60°C to a constant weight, frozen, ground, and analyzed for total nitrogen content using an elemental analyzer at the Marine Science Institute Analytical Lab at the University of California, Santa Barbara. Stable isotopes of carbon and nitrogen in plant tissue were analyzed on a Thermo-Finnigan Delta-Plus Advantage mass spectrometer coupled with a Costech Model 4010 elemental analyzer via a Finnigan ConFlo III continuous flow interface. Stable isotope values reported are relative to air for nitrogen and VPDB for carbon.

Results

Fertilization increased the nitrogen content of *Salicornia*, *Jaumea*, and *Cuscuta* in both microhabitats (Fig. 1). In stands of pure *Salicornia*, the nitrogen content of *Cuscuta* closely paralleled that of *Salicornia*. In mixtures of *Salicornia* and *Jaumea*, the nitrogen content of *Salicornia* was greater than that of *Jaumea*, but the nitrogen content of *Cuscuta* was closer to that of *Jaumea*.

Fertilization led to lower $\delta^{14}\text{N}$ values of *Salicornia* and *Jaumea* without altering $\delta^{13}\text{C}$ values (Fig. 2). *Salicornia* was more depleted in C than *Jaumea*, allowing the two hosts to be distinguished isotopically. In mixtures of *Salicornia* and *Jaumea*, *Cuscuta* $\delta^{13}\text{C}$ values were close to those of *Jaumea*, indicating (in agreement with total N data) that *Cuscuta* obtained most of its nutrition from *Jaumea*.

Cuscuta was over twice as abundant in fertilized than control plots, regardless of the vegetation mixture (Fig. 3). Fertilization did not affect cover

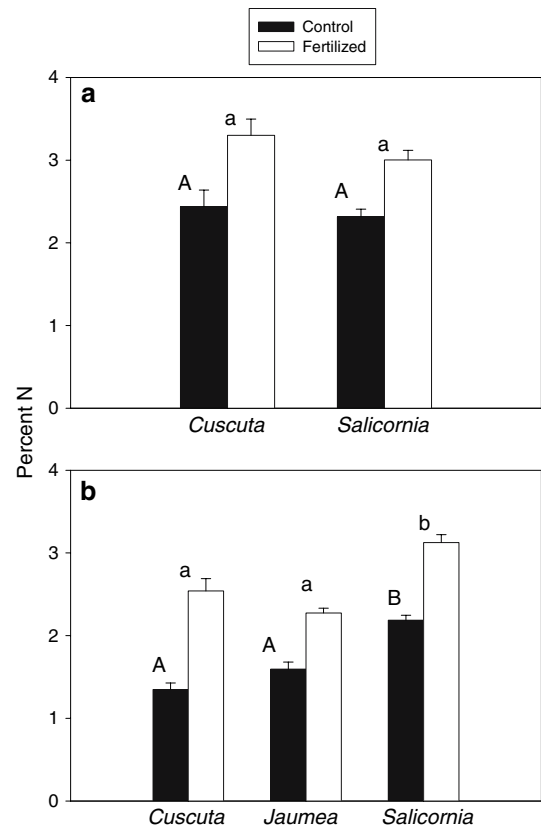


Fig. 1 Nitrogen content (% dry mass \pm 1 SE) of parasite and host plants in control and fertilized treatments in (A) a microhabitat dominated by *Salicornia*, and (B) a microhabitat dominated by both *Jaumea* and *Salicornia*. $N = 10$ plots/treatment/vegetation mixture. Fertilization increased nitrogen content of each plant in each zone (2-sample t-tests, $P < 0.05$ in each case). Letters above columns within each type of bars indicate significant differences among plant species within a particular microhabitat and fertilization treatment (Tukey test following ANOVA treating plots as blocks)

of *Salicornia* or *Jaumea* in either microhabitat ($P > 0.50$ in all cases; data not shown).

Discussion

The idea that interactions between herbivores and plants might serve as models for understanding interactions between parasitic plants and their hosts was proposed over 40 years ago (Govier and Harper 1965; Atsatt 1977). Research in the decades following these seminal publications identified some robust parallels between the two types of consumers, but also a number of differences that are related to

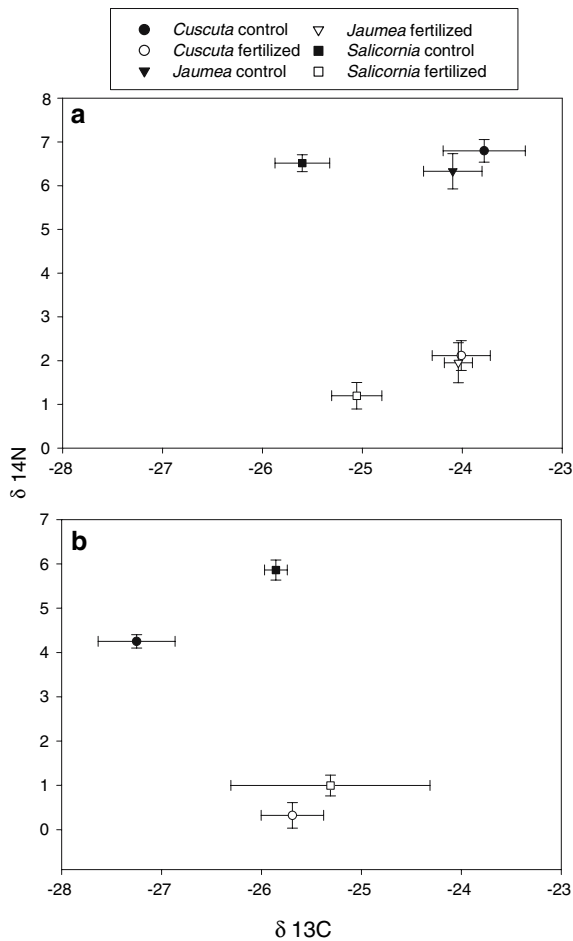


Fig. 2 Carbon (C) and nitrogen (N) isotopic signatures of *Cuscuta salina*, *Jaumea carnosa* and *Salicornia virginica* in control and fertilized plots. (A) microhabitat dominated by both *Jaumea* and *Salicornia*. (B) microhabitat dominated by *Salicornia*. $N = 10$ plots/treatment/vegetation mixture; data are means \pm 1 SE

differences between parasitic plants and herbivores in mobility, hormonal composition and resource capture (Pennings and Callaway 2002). Our results here argue for an additional parallel: both herbivores and holoparasitic plants benefit from an enhanced nitrogen supply to their hosts. A number of correlative studies suggested that parasitic plants perform better on host species with high nitrogen content (Watkinson and Gibson 1988; Seel et al. 1993; Matthies 1996), but exceptions to this pattern were also common (Kelly 1990; Marvier 1996). Many of these correlative studies (both pro and con) suffered from comparing relatively few species of hosts, and from the inherent weaknesses of the correlative

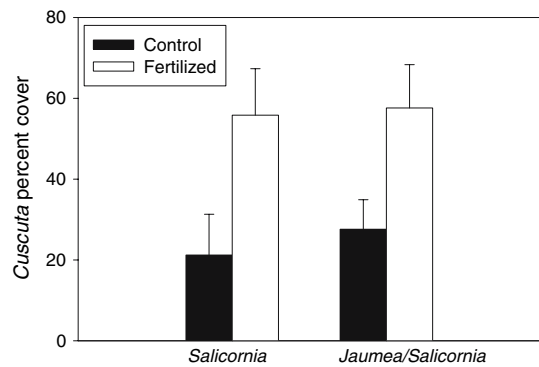


Fig. 3 Percent cover of *Cuscuta* in control and fertilized treatments in a microhabitat dominated by *Salicornia* and a microhabitat dominated by both *Jaumea* and *Salicornia*. $N = 10$ plots/treatment/vegetation mixture. Data are means \pm 1 SE. ANOVA results: fertilization, $F_{1,36} = 9.82$, $P = 0.003$; vegetation mixture, $F_{1,36} = 0.44$, $P = 0.51$; interaction, $F_{1,36} = 0.24$, $P = 0.63$

approach, because many host traits that might affect parasite performance vary among host species, but typically were unmeasured. Experimental studies (Kelly 1992; Marshall et al. 1994; Jeschke and Hilpert 1997; this article) offer a more powerful way to address the effect of host nitrogen on the performance of parasitic plants, and clearly indicate that parasite performance increases with increasing host nitrogen.

This study reveals the potential pitfalls of the correlative approach. *Salicornia* was higher in nitrogen than *Jaumea*, but *Cuscuta* foraging behavior (Pennings and Callaway 2002), nitrogen content (Fig. 1) and isotopic signature (Fig. 2) all indicated that *Jaumea* was the preferred host. This result, however, does not mean that host nitrogen content is unimportant to *Cuscuta*, but rather that other, unmeasured traits of the host were more important than nitrogen content in this particular comparison. The importance of host nitrogen content when all other factors are equal was clearly revealed in the fertilization experiment, where *Cuscuta* doubled in abundance in the fertilization treatment due to an experimental increase of host nitrogen content. We did not harvest to measure the biomass of the host plants, because the experiment was ongoing; however, there were no visually obvious differences in biomass of the host plants in the fertilized and control plots. It is possible that host plant biomass increased slightly without affecting percent cover values (such an increase with fertilization would be expected, and

will be measured at a future date when the experiment is terminated), but based on our observations host biomass certainly did not double. In sum, parasite biomass increased proportionately much more than host biomass did (if host biomass changed at all).

At first glance, it would be reasonable to conclude that stoichiometric theory (Sterner and Elser 2002) would predict that increasing plant nitrogen content would have no effects on parasitic plants. Parasitic plants have similar tissue nitrogen levels as their hosts (Ehleringer et al. 1986), and thus should (in theory) not suffer from the nutritional imbalance that renders nitrogen of great importance to herbivores (White 1978). Holoparasites, however, do not feed on plant tissue, but rather extract nutrition from host xylem and phloem (Press and Graves 1995), and in doing so are more analogous to aphids and other sucking insects than to leaf-chewing herbivores. The xylem and phloem saps of plants are an order of magnitude lower in nitrogen content than leaf tissue (Mattson 1980). Thus, parasitic plants do in fact face the same nutritional constraint faced by herbivores: their diet is severely deficient in nitrogen compared to the composition of their own tissues. Sucking insects solve this problem by processing vast quantities of plant sap, excreting excess carbon-rich material as “honeydew” (Van Hook et al. 1980). Similarly, parasitic plants often maintain very high transpiration rates (Pennings and Callaway 2002), and this may be an adaptation to process large amounts of host sap in order to extract sufficient nitrogen.

Our finding suggests that fertilization of its host benefited *Cuscuta* does not necessarily extrapolate to hemiparasites such as mistletoe and *Striga* spp., which parasitize the vascular system of their host plants but obtain much of their carbon through their own photosynthesis. Hemiparasitic plants may compete for light with their hosts, and consequently are usually limited to relatively nutrient-poor, low-biomass habitats (Matthies 1995; Smith 2000). For hemiparasitic plants, increased nutrient availability is likely to result in increased competition for light with their host plants, and this negative effect would likely counteract the benefit of increased nutrient availability, although it is possible that this effect could be partially offset by an increase in hemiparasite photosynthetic rates. In accordance with this hypothesis, one strategy for controlling crop infections by

hemiparasitic plants is to increase use of fertilizer, but the mechanisms of control (competition versus other effects of nitrogen) are not clearly understood (Eplee and Norris 1995).

Human activities are changing the global nitrogen cycle and making nitrogen more available in most natural communities (Vitousek et al. 1997). This creates various management issues, including the concern that herbivores may feed more aggressively on plants with higher nitrogen content. In the same way, increased nitrogen availability in ecosystems is likely to benefit holoparasitic plants and lead to more vigorous infections. In theory, increased nitrogen availability might instead make the host more vigorous, and thus better able to tolerate or resist the parasite, but we did not observe that result. In either case, because holoparasitic plants can have strong effects on community structure (Pennings and Callaway 2002), the impacts of increased nitrogen availability on plant communities that contain holoparasites may differ from those predicted solely by the effects of nitrogen on the host plants.

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