

Response of a Gall-Forming Guild (Hymenoptera: Cynipidae) to Stressed and Vigorous Prairie Roses

MARK A. WILLIAMS^{1,3} AND JAMES T. CRONIN^{2,4}

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ABSTRACT Two general hypotheses that describe the relationship between plant quality and host-plant preference of insect herbivores are the plant-stress and plant-vigor hypotheses. We examined the response of a gall-forming guild of insect herbivores associated with prairie rose, *Rosa arkansana* Porter (Rosaceae), to experimental manipulations of plant stress (addition of NaCl) and vigor (addition of nitrogen; NH_4NO_3). The most common members of the gall-forming guild on roses are *Diplolepis ignota* Osten Sacken, *D. nodulosa* Beutenmüller, and *D. rosaefolii* Cockerell (Hymenoptera: Cynipidae). The repeated application of nitrogen throughout the growing season to prairie plots resulted in significantly higher plant nitrogen levels and plant growth rates. Both low and high NaCl additions caused leaves to turn yellow and wilt, but reductions in rose growth rates or xylem water potentials with NaCl additions were not statistically significant. All three members of the cynipid guild responded similarly to nitrogen and NaCl additions to rose plots. Incidence of occurrence and density within a plot decreased with increasing nitrogen or NaCl, but the decline associated with increasing NaCl was not significant for any of the cynipids. Neither the plant-vigor hypothesis (higher abundance on fast-growing, vigorous plants) nor the plant-stress hypothesis (higher abundance on physiologically stressed plants) was supported by this study. For cynipids, there is growing evidence that larvae perform best on plant tissues low in nitrogen (less vigorous plants). Agricultural runoff, of which nitrogen is an important constituent, may be significantly altering cynipid distributions and their interactions with other members of the tall-grass prairie ecosystem.

KEY WORDS *Diplolepis* spp., plant-stress hypothesis, plant-vigor hypothesis, *Rosa arkansana*, tall-grass prairie

UNDERSTANDING THE MECHANISMS RESPONSIBLE for the heterogeneous distribution of insect herbivore populations among their host plants has long been a goal of ecologists. A substantial body of literature has implicated plant quality as a primary mechanism influencing herbivore distributions (e.g., Louda 1982, Thompson 1988a, Louda and Collinge 1992). Here, the basic premise is that herbivorous insects will choose to feed and lay eggs on plants that confer the greatest fitness to themselves and their progeny (Thompson 1988b, Fox and Lalonde 1993; but see Thompson 1991, Larsson and Strong 1992) and that natural selection will favor a tight linkage between host-plant preference and herbivore performance, especially for sedentary larvae (Craig et al. 1989, Larsson and Ekblom 1995, Abrahamson and Weis 1997). Two general hypotheses that describe the relationship between plant quality and host-plant preference are the plant-stress and plant-vigor hypotheses.

Proponents of the plant-stress hypothesis have argued that plants under physiological stress (e.g., reduced xylem water potential, stomatal conductance, or photosynthetic capacity) are more suitable as hosts and should accumulate higher abundances of herbivorous insects than nonstressed plants (White 1969, 1974, 1984, Mattson and Haack 1987, Larsson 1989, Price 1991, Pires and Price 2000). Stress is broadly defined as the adverse effects of abiotic or biotic factors on plant traits or performance (Hsiao 1973, Louda and Collinge 1992). Stressed plants must allocate more resources for repair and maintenance of vital physiological processes, and therefore, have less energy for defense (White 1969, 1974, 1984, Rhoades 1979, Mattson and Haack 1987). Stressed plants often exhibit reduced protein synthesis but have increased free amino acids in their tissues, thus offering a more nutritious food source for nitrogen-limited insects.

As one alternative to the plant-stress hypothesis, the plant-vigor hypothesis predicts that herbivores will prefer to oviposit and feed on the most vigorous plants in a population (Price 1991). Vigorous plants are defined by Price (1991) as those that have more rapid growth and larger size relative to conspecifics. Vigor seems to be related to plant age, phenology, and ar-

¹ Department of Biology, University of North Dakota, Grand Forks, ND 58202.

² Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.

³ Present address: Bureau of Land Management, 1300 N. Third Street, Rawlins, WY 82301.

⁴ E-mail: jcronin@lsu.edu.

chitecture (Rhoades 1984, Waring and Price 1990, Burnstein and Wool 1993, Roininen et al. 1993).

Support in favor of either of these hypotheses is equivocal, but recent surveys of the literature suggest that the response to stressed or vigorous plants is partially feeding-guild dependent. Price (1990, 1991) found that 84% of all insects that prefer vigorous plants fed endophytically as galling or mining insects. In contrast, Koricheva et al. (1998) found that increased herbivore preference and performance on stressed plants occurs most commonly among cambium feeders and sucking insects, but less commonly among galling and mining insects (but see Waring and Cobb 1992). However, even within feeding guilds, we might expect a variety of responses by herbivores to variations in plant quality. For example, natural selection may favor different responses by members of a feeding guild as a means to reduce interspecific competition (i.e., an ideal-free distribution; Fretwell and Lucas 1970, Milinski and Parker 1991, Tregenza 1995) or apparent competition (reduced predation by shared predators; Holt 1984, Holt and Lawton 1994, Bonsall and Hassell 1999). As a consequence, the response to plant quality by the members of the entire herbivore guild, i.e., the sum total of herbivores that feed in a specific way and on the same plant species, may exist as a continuum in which some insects distribute themselves on stressed plants, some on average plants, and some on vigorous plants (Price 1991, Koricheva et al. 1998).

An ideal system for addressing the response of herbivores to plant stress and vigor involves the insects associated with prairie rose, *Rosa arkansana* Porter (Rosaceae). A large assemblage of herbivores attacks this rose; the most dominant component is a guild of specialist cynipid gall wasps (Hymenoptera: Cynipidae; Shorthouse 1975, Williams 2001). The goal of this study was to determine how members of the gall-forming herbivore guild respond to field-plot manipulations designed to stress (NaCl [sodium chloride] additions) or invigorate (nitrogen additions) their host plant. We specifically tested the prediction by Price (1990, 1991) that gall insects, because of their endophytic lifestyle, should occur more commonly on nitrogen-treated vigorous plants than on NaCl-treated plants. We also evaluated whether members of the same guild responded differently to changes in plant quality, and whether the nitrogen and NaCl additions interacted to affect the distributions of guild members among roses.

Materials and Methods

Study System. This study was conducted in a tall-grass prairie (Oakville Prairie) located 19 km west of Grand Forks, ND (97°19'0" W, 47°55'4" N). The soil type at Oakville is Antler silty clay loam characterized by a neutral pH, highly variable salinity (4–16 mmhos/cm), and poor to moderate drainage (Soil Survey Staff for Grand Forks County 1963).

Rosa arkansana is found throughout the Great Plains of the United States, the lower Prairie Provinces in

Canada (Manitoba and Saskatchewan), and some areas in the southwestern United States (Great Plains Flora Association 1986). Roses propagate vegetatively through rhizome production and sexually through seed production (Great Plains Flora Association 1986). In Oakville Prairie, bud break begins in late April, with individual leaflets becoming visible in May (Williams 2001). Flowering occurs from June to August, and hips appear shortly thereafter. The maximum age of rose ramets was 4 yr, and 70% of the ramets were <2 yr old (Williams 2001).

The five cynipid wasps that induce galls on prairie rose are all members of the genus *Diplolepis*. *Diplolepis bicolor* Harris, *D. ignota* Osten Sacken, and *D. rosaeifolii* Cockerell are leaf galls (Shorthouse 1975, Shorthouse and Brooks 1998), whereas *D. nodulosa* Beutenmüller and *D. radicum* Osten Sacken attack stems and adventitious shoots, respectively (Shorthouse 1988, Brooks and Shorthouse 1997). All *Diplolepis* species are univoltine. Individuals overwinter as prepupae, emerge from galls as adults in the spring, and search for suitable oviposition sites among the fresh foliage of roses (Shorthouse 1993). Eggs are fully developed in females before they exit the gall, and adults live 3–5 d in the field (Kinsley 1920 Shorthouse 1993). *Diplolepis* larvae hatch 5–15 d after being laid, and their feeding action promotes a wounding response from the plant and causes gall formation (Shorthouse 1975, 1993). *Diplolepis* reproduction has been termed obligate homozygous automictic deuteroctoky (Stille and Dävring 1980). In this unusual type of reproduction, males are reproductively inactive, and unfertilized eggs can develop into either sex. To our knowledge, no manipulative experiments have been done using *Diplolepis*, and almost all information on their distribution has been obtained at the geographic range level or over the entire range of the species (see Shorthouse 1975; exception Caouette and Price 1989).

Experimental Design. To test the plant-vigor and plant-stress hypotheses, plant quality was manipulated through the addition of NaCl and nitrogen (in the form of ammonium nitrate [NH_4NO_3]) to experimental plots of roses. We selected these two factors because nitrogen is an important constituent of agricultural runoff, and salt intrusion from artesian sources affects a large area of prairie in Eastern North Dakota (Redmann 1972, Goldberg and Miller 1990, Bowdish and Stiling 1998, Levine et al. 1998).

The NaCl additions were expected to stress the roses (reviewed by Levitt 1980). Increased levels of salt can lower the water potential within the plant cells and cause a decrease in cell volume and loss of turgor, resulting in decreased cell growth, photosynthesis, and cellular respiration. Excess salt may also decrease the uptake of potassium, calcium, and nitrogen that are needed for growth and metabolism. Finally, elevated soil salinity can be toxic, altering the permeability of the plasma membrane, interfering with membrane transport functions, increasing protein hydrolysis, and disrupting nucleic acid metabolism.

Nitrogen additions to the soil were expected to invigorate the rose plants (even though NH_4NO_3 is also a salt). Nitrogen is used by plants not only in the structural component of cell walls but also in other vital components of the cell, including enzymes, chlorophyll, and nucleic acids (Mattson 1980, Shrader 1984, Moon and Stiling 2000). Nitrogen additions can stimulate cell division, cell elongation, and photosynthetic activity, which lead to higher biomass and amino acid and protein levels (Mattson 1980). Because plants are typically nitrogen limited, additions of nitrogen often result in increased plant growth (by definition, increasing plant vigor) (Hauck 1984).

Shortly after spring thaw (13 April 1999), we established 180 1-m^2 plots at Oakville Prairie. Plots were chosen to contain a minimum of 10 rose stems, and all plots were at least 1 m apart. To minimize differences among plots, we hand removed all *D. ignota* and *D. nodulosa* galls from the previous year from each plot. *D. rosaefolii* disappears from the plants before the onset of winter and thus could not be collected. Biases in their distribution were minimized by the random assignment of treatments to plots.

Plots were assigned at random to one of three NaCl levels (none, low, high) and three nitrogen levels (none [i.e., control], low, high) in a fully crossed factorial design. Each of the nine treatment combinations was replicated 20 times. The first treatment application took place on 26 April, well in advance of the emergence of rose herbivores. We added 1.4 and 0.7 kg of NaCl (99.99% pure) to high and low NaCl plots, respectively, and 0.7 and 0.35 kg of NH_4NO_3 (34-0-0) to high and low nitrogen plots, respectively. High NaCl treatments were based on the maximum amount of salt allowed for rose growth (Jackson 1960), with low NaCl additions being one-half that amount. High and low nitrogen treatments were triple and double the median ambient level of nitrogen (0.34%) found in the soil, respectively (Williams 2001). The resulting soil nitrogen levels were within the range found naturally at Oakville Prairie (Williams 2001). After this initial pulse, treatment amounts were reduced (0.7 kg high NaCl, 0.35 kg low NaCl, 0.2 kg high N, and 0.1 kg low N) to avoid adding toxic amounts and yet still maintain distinctions among treatment levels. Further applications were made on 3 June, 19 July, 3 August, and 17 August. The irregular intervals in the application of treatments were caused by the occurrence of frequent heavy rains that summer (National Climatic Data Center 2000).

Plant Response to Treatments. We examined the effects of nitrogen and NaCl additions on the following rose plant characters: tissue nitrogen levels, xylem water potential, growth rate (our measure of plant vigor [see Price 1991]; determined as the proportional change in plant height between two census dates), and fecundity (hip number, seeds/hip, seed mass). We note here that growth rate is positively correlated with the length of new rose shoots (unpublished data), a measure that is also commonly used as an indicator of plant vigor (e.g., Craig et al. 1986, Caouette and Price 1989, Inbar et al. 2001, Fritz et al. 2003). Because of the

large number of plots, measures of plant nitrogen and xylem water potential were obtained from five randomly chosen plots per NaCl-nitrogen treatment combination, whereas growth rates were obtained from 10 randomly chosen plots per treatment combination. Within each of the selected plots, growth rates were obtained from three randomly selected rose plants. Fecundity, however, was measured for all roses in all plots. The initial census of plant heights took place on 5 May. On three subsequent census dates (27 May, 28 June, 5 August), we sampled plots for rose heights, xylem potentials, and nitrogen levels. Different plots were chosen at each census date. Finally, on 10 September, all rose hips were collected, and the number of plants was counted from within each plot. Plot means were computed for all measurements and census dates.

To estimate plant nitrogen levels, leaves were collected, immediately placed on dry ice, and later stored in an ultracold freezer at -70°C . Samples were subsequently lyophilized (72 h) and ground in a Wiley Mill. Nitrogen effects on plants were assessed by determining the percent nitrogen (% dry mass) of leaves using an elemental analyzer (NA1500; Carlo Erba; CE Elantech, Lakewood, NJ 08701). Salinity effects on the plant were measured with a pressure chamber apparatus (PMS Instrument, Corvallis, Oregon 97333) according to the procedure outlined by Scholander et al. (1965). This apparatus provides a measure of the xylem water potential, which is indicative of plant stress levels (low water potential = high osmotic stress).

For rose fecundity, we determined the number of hips per plant, mean number of seeds per hip, and the average mass of a seed per plot. From all plots containing hips, five hips were randomly chosen, and the number of seeds was counted per hip. We dried the seeds in an oven for 72 h at 65°C and obtained the combined mass to the nearest 0.01 g. Mass per seed was determined by dividing the combined mass by the total number of seeds.

Herbivore Response to Treatments. We tested the effects of NaCl and nitrogen additions on the distribution of gall-forming herbivores among plots. It was impossible to observe herbivore ovipositions or infer ovipositions from traces left behind on the plant (e.g., ovipuncture scars or presence of eggs). As a result, we could not distinguish whether a heterogeneous distribution of herbivores among plots was because of differences in oviposition preference or larval survival. However, because changes in plant quality were detected before the emergence period of most of the cynipid herbivores, differential preference among plots was a possibility.

Only three of the five species of *Diplolepis* were common enough to be used in the analysis: two leaf galls, *D. ignota* and *D. rosaefolii*, and the stem galler *D. nodulosa*. Treatment effects on the number of individuals per plant per plot and the proportion of plots occupied were estimated at three times during the summer (11 June, 28 June, and 23 August; Table 1); for

Table 1. The cynipid gall formers found on prairie rose in northeastern North Dakota

Cynipid species	Feeding location	Occurrence on plants	References
<i>Diplolepis ignota</i>	Leaf	Early May	Shorthouse 1975, 1993
<i>D. nodulosa</i>	Stem, shoots	Late May	Brooks and Shorthouse 1997
<i>D. rosaeifolii</i>	Leaf	Early May	Shorthouse and Brooks 1998; Shorthouse 1975
<i>D. bicolor</i>	Leaf	Late April Early May	Shorthouse 1975
<i>D. radicum</i>	Shoots (roots)	Late May	Shorthouse 1988

analysis of insect density, we used the final count that was taken in late August.

Statistical Analysis. To determine the effects of nitrogen and NaCl additions on leaf nitrogen levels, we performed a three-way, full-factorial analysis of covariance (ANCOVA). Fixed factors in this model include NaCl, nitrogen, and census date. Because data for each census were collected from different subsets of plots, date was not a repeated measure. Plot rose density was used as the covariate to account for the possible effects of intraspecific interactions (e.g., competition) among plants. Effects of nitrogen and NaCl additions and rose density on rose growth rates, hips per plant, seeds per hip, and seed mass were analyzed using separate two-way, completely randomized, fixed-factor ANCOVAs. Normality and homogeneity of variances were confirmed through inspection of the graphical distribution of data and through the use of a Levene's test (Sokal and Rohlf 1995). Natural log (i.e., \ln) transformations were necessary to normalize distributions or homogenize variances among treatments for rose hip and seed densities. Multiple-comparison tests were performed using the Bonferroni method (Day and Quinn 1989).

For the analysis of treatment effects on herbivore density per plot, there were enough plots with no individuals/species that the distributions of herbivore densities could not be normalized. To circumvent this problem, we used logistic regression to analyze the effects of NaCl and nitrogen on the presence or absence of each herbivore species among plots (Trexler and Travis 1993, Hosmer and Lemeshow 2000). Here, the binomial dependent variable (presence/absence of an herbivore within a plot) was logit-transformed ($\ln [P/1 - P]$; where P = probability that a patch is occupied by a particular species of herbivore). A separate logistic regression was performed for each herbivore species. The factors were nitrogen, NaCl, nitrogen \times NaCl interaction, and the covariate was an index measure of plant biomass. The biomass index was defined as the product of plot-plant density and mean plot-plant height (cm). Because plant height was only measured in 10 of the 20 plots per treatment combination, only those plots were used in the analysis. Biomass was included in the analysis because nitrogen (and possibly NaCl) additions were expected to not only affect plant quality, but also the amount of

plant material available to herbivores. It was our intention to partition the effects of plant quality and biomass on herbivore abundance. Our initial analyses revealed that biomass did not significantly influence the proportion of plots occupied by any cynipid species ($P > 0.10$). Therefore, we omitted biomass from the analyses reported below and included all 20 plots per treatment combination. The significance level for each model component was determined with a G -test (Hosmer and Lemeshow 2000). Posthoc tests for differences among nitrogen or NaCl addition levels were performed by excluding one level from the model and testing for a significance difference between the other two levels with a G -test (Hosmer and Lemeshow 2000). To ensure that the type I error rate for all comparisons did not exceed 0.05, the critical level of α for each pairwise test was adjusted using the Bonferroni method (Sokal and Rohlf 1995).

To compliment the logistic-regression analyses based on species presence/absence, we also used a two-way ANCOVA for ranked data (Scheirer et al. 1976, Sokal and Rohlf 1995) to evaluate the effects of treatments on herbivore species density among plots. For the combined densities of all cynipids, the distribution of densities per plot was approximately normal; thus, the analyses of these data were amenable to parametric statistics. We used a two-way ANCOVA design similar to the one used for rose growth rates. For both types of analysis of variance (ANOVA; using ranks or means), we initially used the indexed biomass as a covariate (10 plots per treatment combination), found it to be an insignificant factor, and replaced it with \ln -rose density (20 plots per treatment combination).

Results

Plant Response to Treatments. Two to 3 d after the addition of nitrogen and NaCl to the plots, there were visible signs of treatment effects on the roses. Changes in plant appearance were evident by the yellowing and wilting of leaves in high NaCl-treated plots. On average, roses in fertilized plots had significantly higher nitrogen levels in their leaves (3.3%, high; 2.8%, low) than in control plots (1.8%; Fig. 1; Table 2). There was no significant difference between low and high nitrogen additions (Fig. 1). Among census dates, mean nitrogen levels were significantly higher in May (3.6%) than in June or August (2.0 and 2.2%, respectively; $F_{2,87} = 92.4$, $P < 0.001$). Treatments also interacted to affect plant-nitrogen levels (Table 2). In general, roses treated with both nitrogen and NaCl had higher nitrogen levels than plants treated with nitrogen alone (up to 18.5% higher).

Based on our late-summer census (5 August), xylem water potentials decreased from -1.1 mmhos/cm in control plots to -2.8 mmhos/cm in high NaCl plots. The 154% decrease in pressure (a decrease in pressure = increase in plant stress) was not statistically significant (Table 2; similar results were found for the previous two census dates). This unexpected result may have been a consequence of the above average

Table 3. Logistic-regression analyses results for the distribution (present or absent) of each of three species of cynipid gall-formers on prairie roses

Source of variation	df	<i>Diplolepis</i> species								
		<i>D. ignota</i>			<i>D. rosaeifolia</i>			<i>D. nodulosa</i>		
		G	P	Trend ^a	G	P	Trend ^a	G	P	Trend ^a
Nitrogen level	2	9.43	0.009	–	17.4	<0.001	–	2.66	0.260	–
NaCl level	2	5.18	0.075	–	3.62	0.160	–	5.63	0.059	–
Nitrogen × NaCl	4	3.23	0.520	n/a	10.0	0.040	n/a	2.01	0.730	n/a
Covariate										
<i>In</i> (prairie rose density)	1	2.12	0.150	+	0.013	0.910	–	0.804	0.730	–
Error	170									

^a Trends in herbivore occurrence with respect to each factor (interaction excluded [n/a]) are indicated by an increase (+) or a decrease (–).

exhibited a nonsignificant decline with increasing additions of NaCl. For all cynipids combined, nitrogen additions caused significant reductions in gall densities (nitrogen: $F_{2,164} = 19.36, P < 0.001$; Fig. 4). Mean gall densities in the low- and high-nitrogen plots were 61 and 64% lower than the control plots, respectively (both comparisons, $P < 0.001$). High-NaCl plots had gall densities 32% lower on average than control plots, but the NaCl effects only verged on significance ($F_{2,164} = 2.71, P = 0.07$). Finally, the combined density of cynipid galls was inversely related to rose density per plot ($F_{1,164} = 5.82, P < 0.017$).

Discussion

The plant-stress and plant-vigor hypotheses are two of the more widely recognized hypothesis invoked to explain heterogeneous distributions of herbivores among their host plants (e.g., White 1969, 1978, Levitt 1980, Mattson and Haack 1987, Price 1991, Preszler and Price 1995). In both cases, the emphasis is on bottom-up processes (i.e., host-plant quality). A recent survey of the literature has suggested that there are feeding-guild-specific differences in response to plant quality (Koricheva et al. 1998; see also Larsson 1989, Price 1990, 1991). The most notable pattern is that sedentary, internal feeders tend to support the vigor hypothesis, whereas cambium feeders and sucking insects lend more support to the stress hypothesis.

Our early-season treatment applications of NaCl and nitrogen elicited physiological changes in the roses that predated the emergence times of all insects reported in this study. Nitrogen additions to plots resulted in significantly higher leaf nitrogen levels (Fig. 1) and an increase in plant growth rate (Fig. 2). Plants with high growth rates are, by definition, vigorous plants (Price 1991). Supplemental soil nitrogen was primarily allocated to vegetative growth, not to sexual reproduction; nitrogen additions did not increase hip or seed production or mass/seed (Table 2). In contrast to nitrogen additions, NaCl additions had no measurable effect on the rose physiological characters listed in Table 2. However, roses in NaCl-treated plots had yellow and wilted leaves, and there

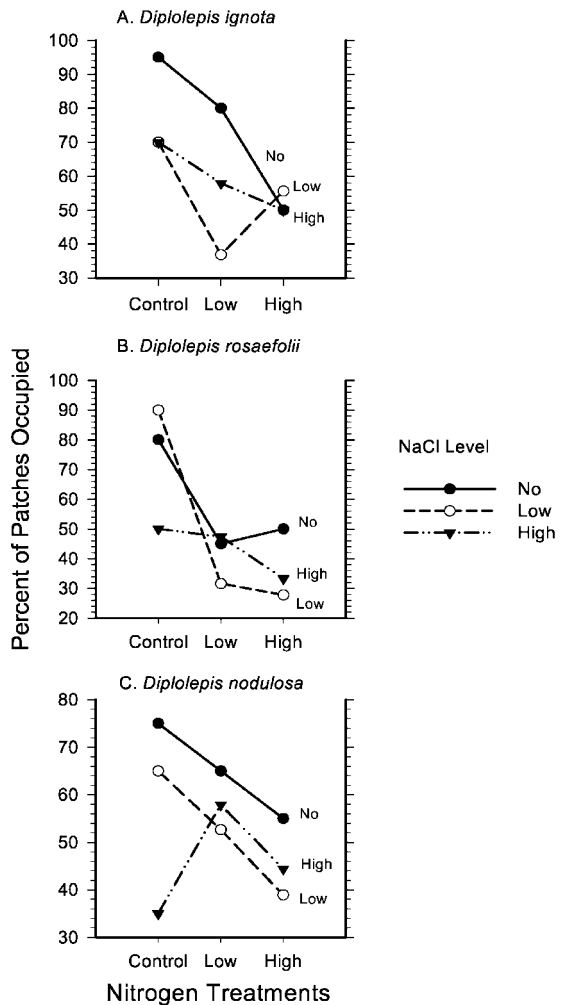


Fig. 3. Nitrogen and NaCl treatment effects on the distribution of *Diplolepis ignota* (A), *D. rosaeifolia* (B), and *D. nodulosa* (C). The y-axis represents the percentage of plots inhabited by at least one gall of the focal species. Results of separate logistic-regression analyses for the effect of nitrogen and NaCl are reported in Table 3.

Table 4. Nonparametric analysis of covariance results for densities of galls formed by three species of cynipids on prairie roses

Source of variation	df	<i>Diplolepis</i> species								
		<i>D. ignota</i>			<i>D. rosaefolii</i>			<i>D. nodulosa</i>		
		H	P	Trend ^a	H	P	Trend ^a	H	P	Trend ^a
Nitrogen level	2	23.7	<0.001	–	22.69	<0.001	–	2.73	0.256	–
NaCl level	2	2.73	0.255	–	1.91	0.385	–	5.15	0.076	–
Nitrogen × NaCl	4	7.43	0.115	n/a	7.96	0.093	n/a	3.63	0.485	n/a
Covariate										
<i>In</i> (prairie rose density)	1	0.384	0.535	+	0.82	0.365	–	0.70	0.402	–
Error	170									

^a Trends in herbivore occurrence with respect to each factor (interaction excluded [n/a]) are indicated by an increase (+) or a decrease (–).

were nonsignificant trends toward lower growth rates (Fig. 2) and xylem water potentials (increased salt stress) between control and high-NaCl plots (33 and 154% reductions, respectively). Much higher than normal levels of rainfall during the summer of 1999 (National Climatic Data Center 2000) may have mitigated the effects of our NaCl treatments on roses, particularly with regard to osmotic stress (Levitt 1980). However, NaCl-induced stress could have been manifest in other ways besides growth rates and water potentials. For example, NaCl additions may have altered amino acid ratios or levels of defensive compounds, sugars, carbohydrates, or proteins (e.g., Hsiao 1973, Levitt 1980, Brodbeck and Strong 1987, Rossi et al. 1996).

The three cynipid gall wasps displayed similar responses to nitrogen and NaCl treatments (Figs. 3 and 4; Tables 3 and 4). For two of three *Diplolepis* species, and all three species combined, incidence and density among plots declined significantly with increasing nitrogen additions. On average, combined densities were 64% lower in the high-nitrogen compared with control plots. NaCl effects on cynipid incidence and density were universally nonsignificant (probably be-

cause of the lack of a rose physiological response to NaCl); however, it is interesting to note that all tests exhibited a negative effect of NaCl on cynipid abundance. In fact, for all cynipids combined, gall densities were 32% lower in the high NaCl than control plots. In summary, the distribution of *Diplolepis* species among prairie rose plants clearly does not support the plant-vigor hypothesis or the plant-stress hypothesis (despite the lack of statistically significant effects, the response to NaCl was in the opposite direction of predictions).

Our results contradict the findings of most other studies of gall insects, which found that distributions were biased in favor of vigorous host plants (e.g., Caouette and Price 1989, Preszler and Price 1995, Rossi and Stiling 1998, Fritz et al. 2000, Pires and Price 2000; but see Eliason and Potter 2000). We offer two possible explanations for the differences between our rose cynipids and other gall insects.

One possibility is that cynipids avoid nitrogen-rich roses because their offspring are more at risk to attack by natural enemies than when present on nitrogen-poor roses. This is a reasonable hypothesis because roses in nitrogen-rich plots had higher growth rates and thus were taller (see Results); consequently, cynipid galls on those roses may be more visible or apparent to searching natural enemies (e.g., Price et al. 1980, Price 1986, Hare 1992). Higher plant growth rates may also promote the growth of larger galls, and larger galls are known to represent a more profitable resource for those natural enemies that can gain access to them (e.g., Abrahamson et al. 1989, Weis 1993). For example, Forkner and Hunter (2000) found that arthropod natural enemies were more abundant on fertilized than unfertilized oaks (see also Hunter and Price 1992, Abrams 1993, Stiling and Rossi 1997, Moon et al. 2000). We did not measure gall sizes in our study, but we did examine natural enemy induced mortality rates among the 180 plots (unpublished data). Each rose cynipid has a rich community of arthropod natural enemies, including inquiline cynipid predators, parasitoids, and hyperparasitoids (Williams 2001; see also Shorthouse 1973, 1975, Brooks and Shorthouse 1997; Shorthouse and Brooks 1998). However, our study revealed no relationship between nitrogen and salt treatments and the proportions of cynipid galls attacked by natural enemies. These results in the ni-

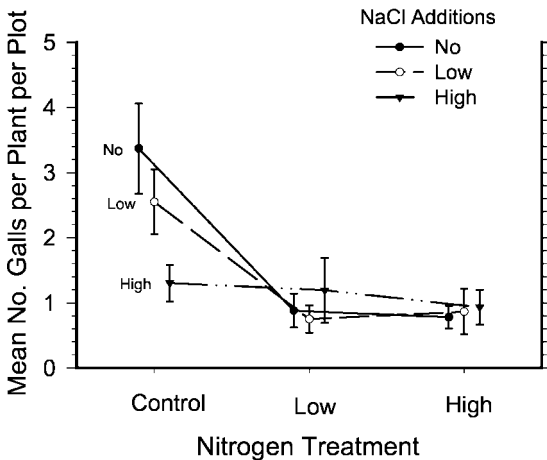


Fig. 4. The effect of nitrogen and NaCl treatments on the density (numbers per plant per plot) of all three gall cynipid species combined. Marginal least-square means ± SEM are reported.

trogen-treated plots contradict the prediction that increased primary productivity, brought about by fertilization, will strengthen the effect of top-down forces on herbivore populations (Price et al. 1980, Oksanen et al. 1981, Hunter and Price 1992, Strong 1992). Thus, our results do not support this explanation.

A second explanation is that rose cynipids simply perform poorly on plants with high nitrogen. Recent studies with other cynipid species point to this possibility. Hartley (1998), Schönrogge et al. (2000), and St. John and Shorthouse (2000) found that plant tissues of *Rosa* species have lower levels of nitrogen and protein within galled than nongalled tissues of the same plant. In fact, mature rose galls were found to contain less than one-half of the protein present in nongalled tissue. Another study by Hartley and Lawton (1992) found that survivorship of the cynipids *Neuroterus quercus-baccarum* L. and *Andricus lignicola* Hartig on *Quercus robur* L. was negatively correlated with gall nitrogen content. Cynipid galls on fertilized oaks manipulated plants to receive a lower proportion of the available nitrogen in gall tissue. Results from these studies suggest that some cynipid galls do not function as nutrient sinks, as is the case in other noncynipid gall systems (e.g., McCrea et al. 1985). Instead, cynipids may exclude the buildup of nitrogen in gall tissue because of detrimental consequences to their survival. The cause for the negative effects on gall insect survivorship is currently unknown, but it may be that an increase in nitrogen ions and organic acids in plant cells can decrease osmotic potential in the plants and interfere with early gall development (P. W. Price, personal communication).

Fertilized and stressed roses may also represent poor-quality hosts because increased nitrogen or NaCl uptake can lead to highly unbalanced amino acid profiles, large concentrations of organic acids, or increases in plant defensive chemistry (Rhoades 1979, Brodbeck et al. 1990, Rossi et al. 1996). Diets of unbalanced amino acids have been shown to be detrimental or even toxic to insects (Reese 1979, Brodbeck et al. 1990). Some insect species are capable of detecting and avoiding plants with these chemical imbalances (Sôgawa 1982). If roses grown in a high nitrogen and NaCl environment represent poor quality hosts, the low density or occurrence of cynipids on those plants may be caused by female *Diplolepis* preferentially ovipositing on nontreated plants or to random oviposition among plants but proportionately greater larval mortality on treated compared with nontreated plants. We currently lack the data to distinguish between these two possibilities.

Today, tall-grass prairie exists as island fragments surrounded by agricultural lands and is at risk to contamination by runoff (Samson and Knopf 1994, 1996). Nitrogen, a significant component of agricultural runoff, can influence both plant community structure and plant-herbivore interactions (Goldberg and Miller 1990, Bowdish and Stiling 1998, Levine et al. 1998). Therefore, understanding how plants and herbivores respond to contamination is important. In this study, insects responded to small-scale contamination by

having reduced preference or survival on invigorated plants. An increase in aggregation of prairie rose herbivores onto plants unaffected by runoff may have a tremendous impact on the entire food web associated with roses. For example, herbivory concentrated onto a small subset of the roses might lead to reduced plant fitness, increased interspecific competition among the herbivore assemblage, and higher predation and parasitism of the herbivores by natural enemies that forage in a density-dependent fashion.

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References Cited

- Abrahamson, W. G., and A. E. Weis. 1997. The evolutionary ecology of a tritrophic-level interaction: goldenrod, the stem gallmaker and its natural enemies. Princeton University Press, Princeton, NJ.
- Abrahamson, W. G., J. F. McCrea, and A. E. Weis. 1989. Variation in selection pressures on the goldenrod gall fly and the competitive interactions of its natural enemies. *Oecologia* (Berl.) 79: 15–22.
- Abrams, P. A. 1993. Effects of increased productivity on the abundance of trophic levels. *Am. Nat.* 141: 351–371.
- Bonsall, M. B., and M. P. Hassell. 1999. Parasitoid-mediated effects: apparent competition and the persistence of host-parasitoid assemblages. *Res. Pop. Ecol.* 41: 59–68.
- Bowdish, T. F., and P. Stiling. 1998. The influence of salt and nitrogen on herbivore abundance: direct and indirect effects. *Oecologia* (Berl.) 113: 400–405.
- Brodbeck, B., and D. R. Strong. 1987. Amino acid nutrition of herbivorous insects and stress to host plants, pp. 347–364. *In* P. Barbosa and J. C. Schultz (eds.), *Insect outbreaks*. Academic, Inc., San Diego, CA.
- Brodbeck, B. V., R. F. Mizell, W. J. French, P. C. Anderson, and J. H. Aldrich. 1990. Amino acids as determinants of host preference for the xylem feeding leafhopper, *Homalodisca coagulata* (Homoptera: Cicadellidae). *Oecologia* (Berl.) 83: 338–345.
- Brooks, S. E., and J. D. Shorthouse. 1997. Biology of the rose stem galler *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and its associated component community in central Ontario. *Can. Entomol.* 129: 1121–1140.
- Burnstein, M., and D. Wool. 1993. Gall aphids do not select optimal galling sites (*Symynthurodes betae*: Pemphigidae). *Ecol. Entomol.* 18: 155–164.
- Caouette, M. R., and P. W. Price. 1989. Growth of Arizona rose and attack and establishment of gall wasps, *Diplolepis fusiformans* and *D. spinosa*. *Environ. Entomol.* 18: 822–828.
- Craig, T. P., P. W. Price, and J. K. Itami. 1986. Resource regulation by a stem-galling sawfly on the arroyo willow. *Ecology*. 67: 419–425.
- Craig, T. P., J. K. Itami, and P. W. Price. 1989. A strong relationship between oviposition preference and larval

- performance in a shoot-galling sawfly. *Ecology*. 70: 1691–1699.
- Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecol. Mon.* 59: 433–463.
- Eliason, E., and D. A. Potter. 2000. Budburst phenology, plant vigor, and host genotype effects on the leaf-galling generation of *Callirhytis cornigera* (Hymenoptera: Cynipidae). *Environ. Entomol.* 29: 1199–1207.
- Forkner, R. E., and M. D. Hunter. 2000. What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*. 81: 1588–1600.
- Fox, C. W., and R. G. Lalonde. 1993. Host confusion and the evolution of insect diet breadths. *Oikos*. 67: 577–581.
- Fretwell, S. D., and H. L. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheor.* 19: 16–36.
- Fritz, R. S., B. A. Crabb, and C. G. Hochwender. 2000. Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. *Oikos*. 89: 555–563.
- Fritz, R. S., B. A. Crabb, and C. G. Hochwender. 2003. Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. *Oikos*. 102: 601–613.
- Goldberg, D. E., and T. E. Miller. 1990. Effects of different resource additions on species diversity in an annual plant community. *Ecology*. 71: 213–225.
- Great Plains Flora Association. 1986. *Flora of the Great Plains*. University Press of Kansas, Lawrence, KS.
- Hare, J. D. 1992. Effects of plant variation on herbivore-natural enemy interactions, pp. 278–298. *In* R. S. Fritz and E. L. Simms (eds.), *Plant resistance to herbivores and pathogens: ecology, evolution, and genetics*. University of Chicago Press, Chicago, IL.
- Hartley, S. E. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia (Berl.)*. 113: 492–501.
- Hartley, S. E., and J. H. Lawton. 1992. Host-plant manipulation by gall-insects: a test of the nutrition hypothesis. *J. Anim. Ecol.* 61: 113–119.
- Hauck, R. D. 1984. Nitrogen in crop production. American Society of Agronomy, Madison, WI.
- Holt, R. D. 1984. Spatial heterogeneity, indirect interactions, and the coexistence of prey species. *Am. Nat.* 124: 377–406.
- Holt, R. D., and J. H. Lawton. 1994. The ecological consequences of shared natural enemies. *Annu. Rev. Ecol. Syst.* 25: 495–520.
- Hosmer, D. W., and Lemeshow, S. 2000. *Applied logistic regression*. Wiley, Inc., New York.
- Hsiao, T. C. 1973. Plant responses to water stress. *Annu. Rev. Plant Physiol.* 24: 519–570.
- Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottomup and topdown forces in natural communities. *Ecology*. 73: 724–732.
- Inbar, M., H. Doostdar, and R. T. Mayer. 2001. Suitability of stressed and vigorous plants to various insect herbivores. *Oikos*. 94: 228–235.
- Jackson, M. L. 1960. *Soil chemical analysis*. Prentice-Hall, Englewood Cliffs, NJ.
- Kinsley, A. C. 1920. Life histories of American Cynipidae. *Bull. Am. Mus. Nat. Hist.* 42: 319–357.
- Koricheva, J., S. Larsson, and E. Haukioja. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. *Annu. Rev. Entomol.* 43: 195–216.
- Larsson, S. 1989. Stressful times for the plant stress-insect performance hypothesis. *Oikos*. 56: 277–283.
- Larsson, S., and D. R. Strong. 1992. Ovipositional choice and larval survival of *Dasineura marginemtorquens* (Diptera: Cecidomyiidae) on resistant and susceptible *Salix viminalis*. *Ecol. Entomol.* 17: 227–232.
- Larsson, S., and B. Ekbom. 1995. Ovipositional mistakes in herbivorous insects: confusion or a step towards a new host giant. *Oikos*. 72: 155–160.
- Levine, J. M., S. D. Hacker, C.D.G. Harley, and M. D. Bertress. 1998. Nitrogen effects on an interaction chain in a salt marsh community. *Oecologia (Berl.)*. 117: 266–272.
- Levitt, J. 1980. *Responses of plants to environmental stresses*. Academic, Inc., New York.
- Louda, S. M. 1982. Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecol. Mon.* 52: 25–41.
- Louda, S. M., and S. K. Collinge. 1992. Plant resistance to insect herbivores: a field test of the environmental stress hypothesis. *Ecology*. 73: 153–169.
- Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11: 119–161.
- Mattson, W. J., and R. A. Haack. 1987. The role of drought stress in provoking outbreaks of phytophagous insects. *In* P. Barbosa and J. C. Schultz (eds.), *Insect outbreaks*. Academic, Inc., San Diego, CA.
- McCrea, K. D., W. G. Abrahamson, and A. E. Weis. 1985. Goldenrod ball gall effects on *Solidago altissima*: ¹⁴C translocation and growth. *Ecology*. 66: 1902–1907.
- Milinski, M., and G. A. Parker. 1991. Competition for resources, pp. 137–168. *In* J. R. Krebs and N. B. Davies (eds.), *Behavioral ecology, an evolutionary approach*. Blackwell, Boston, MA.
- Moon, D. C., and P. Stiling. 2000. Relative importance of abiotically induced direct and indirect effects on a salt marsh herbivore. *Ecology*. 81: 470–481.
- Moon, D. C., A. M. Rossi, and P. Stiling. 2000. The effects of abiotically induced changes in host plant quality (and morphology) on a salt marsh plant hopper and its parasitoid. *Ecol. Entomol.* 25: 325–331.
- National Climatic Data Center. 2000. Monthly mean precipitation levels. Weather Data, Grand Forks, ND.
- Oksanen, L., S. D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation ecosystems in gradients of primary productivity. *Am. Nat.* 118: 240–261.
- Pires, C.S.S., and P. W. Price. 2000. Patterns of host plant growth and attack and establishment of gall-inducing wasp (Hymenoptera: Cynipidae). *Environ. Entomol.* 29: 49–54.
- Preszler, R. W., and P. W. Price. 1995. A test of plant-vigor, plant-stress, and plant-genotype effects on leaf-miner oviposition and performance. *Oikos*. 74: 485–492.
- Price, P. W. 1986. Ecological aspects of host plant resistance and biological control: interactions among three trophic levels, pp. 11–30. *In* D. J. Boethel and R. D. Eikenbary (eds.), *Interactions of plant resistance and parasitoids and predators of insects*. Wiley, New York.
- Price, P. W. 1990. Evaluating the role of natural enemies in latent and eruptive species: new approaches in life table construction, pp. 221–232. *In* A. D. Watt, S. R. Leather, M. D. Hunter, and N.A.C. Kidd LTD, *Population dynamics of forest insects*. Intercept, Andover, United Kingdom.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos*. 62: 244–51.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Evol.* 11: 41–65.

- Redmann, R. E. 1972. Plant communities and soils of an eastern North Dakota prairie. *Bull. Torrey Bot. Club.* 99: 65–76.
- Reese, J. C. 1979. Interactions of allelochemicals with nutrients in herbivore food, pp. 309–320. *In* G. A. Rosenthal and D. H. Janzen (eds.), *Herbivores: their interactions with secondary plant metabolites*. Academic, Inc., New York.
- Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores, pp. 3–54. *In* G. A. Rosenthal and D. H. Janzen (eds.), *Herbivores: their interactions with secondary plant metabolites*. Academic, Inc., New York.
- Rhoades, D. F. 1984. Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. *Am. Nat.* 125: 205–238.
- Roininen, H., P. W. Price, and J. Tahvanainen. 1993. Colonization and extinction in a population of the shoot-galling sawfly, *Euuira amerinae*. *Oikos*. 68: 448–454.
- Rossi, A. M., and P. Stiling. 1998. The interaction of plant clone and abiotic factors on a gall-making midge. *Oecologia* (Berl.). 116: 170–176.
- Rossi, A. M., B. V. Brodbeck, and D. R. Strong. 1996. Response of xylem-feeding leafhopper to host plant species and plant quality. *J. Chem. Ecol.* 22: 653–671.
- Samson, F., and F. Knopf. 1994. Prairie conservation in North America. *BioScience*. 44: 653–671.
- Samson, F., and F. Knopf. 1996. Prairie conservation: preserving North America's most endangered ecosystem. Island Press, Washington, DC.
- Scheirer, C. J., W. S. Ray, and N. Hare. 1976. The analysis of ranked data derived from completely randomized factorial Designs. *Biometrics*. 32: 429–434.
- Scholander, P. F., E. D. Bradstreet, M. T. Hammell, and E. A. Hemmingen. 1965. Sap pressure in vascular plants. *Science*. 148: 339–346.
- Schönrogge, K., L. J. Harper, and C. P. Lichtenstein. 2000. The protein content of tissues in cynipid galls (Hymenoptera: Cynipidae): similarities between cynipid galls and seeds. *Plant Cell Environ.* 23: 215–222.
- Shorthouse, J. D. 1973. The insect community associated with rose galls of *Diplolepis polita* (Cynipidae, Hymenoptera). *Quaest. Entomol.* 9: 55–98.
- Shorthouse, J. D. 1975. The roles of insect inhabitants in six *Diplolepis* rose leaf galls of western Canada. PhD thesis, University of Saskatchewan, Saskatoon, Saskatchewan.
- Shorthouse, J. D. 1988. Occurrence of two gall wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) on the domestic shrub rose, *Rosa rugosa* Thunb. (Rosaceae). *Can. Entomol.* 120: 727–737.
- Shorthouse, J. D. 1993. Adaptations of galls wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) and the role of gall anatomy in Cynipid systematics. *Mem. Entomol. Soc. Can.* 165: 139–163.
- Shorthouse, J. D., and S. E. Brooks. 1998. Biology of the galler *Diplolepis rosaefolii* (Hymenoptera: Cynipidae), its associated component community, and host shift to the shrub rose *Thérèse bugnet*. *Can. Entomol.* 130: 357–366.
- Shrader, L. E. 1984. Functions and transformations of nitrogen in higher plants, pp. 55–65. *In* R. D. Hauck (ed.), *Nitrogen in crop production*. American Society of Agronomy, Madison, WI.
- Sôgawa, K. 1982. The rice brown planthopper: feeding physiology and host plant interactions. *Annu. Rev. Entomol.* 27: 49–73.
- Soil Survey Staff for Grand Forks County. 1963. General soil map. North Dakota Agriculture Experimental Station, Fargo, ND.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. W. H. Freeman and Company, New York.
- Stiling, P., and A. M. Rossi. 1997. Experimental manipulations of top down and bottom up factors in a tri-trophic system. *Ecology*. 78: 1602–1606.
- St. John, M. G., and J. D. Shorthouse. 2000. Allocation patterns of organic nitrogen and mineral nutrients within stem galls of *Diplolepis spinosa* and *Diplolepis trifurca* (Hymenoptera: Cynipidae) on wild roses (Rosaceae). *Can. Entomol.* 132: 635–648.
- Stille, B., and L. Dävring. 1980. Meiosis and reproduction strategy in the parthenogenic gall wasp *Diplolepis rosae* (L.) (Hymenoptera: Cynipidae). *Hereditas*. 92: 353–362.
- Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology*. 73: 747–754.
- Thompson, J. N. 1988a. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. Exp. Appl.* 47: 3–14.
- Thompson, J. N. 1988b. Variation in interspecific interactions. *Annu. Rev. Ecol. Syst.* 19: 65–87.
- Thompson, J. N. 1991. Evolution of oviposition behavior and host preference in Lepidoptera. *Annu. Rev. Entomol.* 36: 65–89.
- Tregenza, T. 1995. Building on the ideal free distribution. *Adv. Ecol. Res.* 26: 253–307.
- Trexler, J. C., and J. Travis. 1993. Nontraditional regression analyses. *Ecology*. 74: 1629–1637.
- Waring, G. L., and P. W. Price. 1990. Plant water stress and gall formation (Cecidomyiidae: Asphondylia spp.) on creosote bush. *Eco. Entomol.* 15: 87–95.
- Waring, G. L., and N. S. Cobb. 1992. The impact of plant stress on herbivore population dynamics. *In* E. A. Bernays (ed.), *Plant-insect interactions*, pp. 167–226. CRC, Boca Raton, FL.
- Weis, A. E. 1993. Host gall size predicts hosts quality for the parasitoid *Eurytoma gigantea* (Hymenoptera: Eurytomidae), but can the parasitoid tell? *J. Insect Behav.* 6: 591–602.
- White, T.C.R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology*. 50: 905–909.
- White, T.C.R. 1974. A hypothesis to explain outbreaks of looper caterpillars with special reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in New Zealand. *Oecologia* (Berl.). 33: 71–86.
- White, T.C.R. 1978. The importance of a relative shortage of food in animal ecology. *Oecologia* (Berl.). 33: 71–86.
- White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* (Berl.). 63: 90–105.
- Williams, M. A. 2001. Herbivore assemblage response to stressed and vigorous prairie roses. MS thesis, University of North Dakota, Grand Forks, ND.

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