



Inter-annual variation in above- and belowground herbivory on a native, annual legume

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Abstract

The relative importance of subterranean versus aboveground insect damage to plants is not well understood. In particular, the simultaneous effects of above- and belowground herbivory, and the importance of highly variable abiotic factors such as rainfall, have received little attention in diverse natural ecosystems. We investigated the influence of both above- and belowground herbivory on *Lupinus nanus* (Fabaceae), an annual plant native to coastal California. A number of insect species damage *L. nanus* aboveground, and a weevil larva consumes nodules belowground. To manipulate herbivory in the field, we employed a combination of insecticides and simulated herbivory during two different years. In 1997, simulated belowground damage reduced *L. nanus* survival, and insecticide application to roots increased seed production and seed mass. By contrast, in 1998, only aboveground folivory significantly reduced *L. nanus* reproduction, and, in combination, above- and belowground insecticides did not affect flower or seed number relative to controls. A growth chamber experiment conducted in the absence of herbivory revealed that the aboveground insecticide marginally reduced flower production and the belowground insecticide marginally increased flower production compared to controls; these non-target effects made our field experiments for aboveground herbivory conservative. Finally, ambient levels of herbivory differed among years (1997, 1998, and 2000), which varied greatly in rainfall due to the effects of El Niño. The results suggest that the impacts of herbivores are temporally variable and that abiotic factors, particularly those related to large-scale changes in weather patterns, may be more important than insect herbivory to *L. nanus* in some years.

Introduction

Determining the conditions under which herbivory reduces plant fitness is critical for understanding the importance of herbivores in the dynamics of plant communities. The effects of herbivory may depend on both the biotic and abiotic context in which damage occurs (e.g., Karban (1989)). For example, herbivores may have greater effects on plants under conditions of low plant competition (e.g., Parmesan (2000)) or when predators and parasitoids of herbivores are absent (reviewed in Schmitz et al. (2000) and Halaj and Wise (2001)). In addition, abiotic factors can have

significant impacts on herbivore populations, potentially altering herbivores' effects on plants (e.g., McKillup and McKillup (1997) and Polis et al. (1997)).

One biotic condition that may affect the importance of a single herbivore species is the presence of other herbivores (Faeth 1986; Harrison and Karban 1986; Strauss 1991; Hougén-Eitzman and Rausher 1994; Denno et al. 1995; Wise and Sacchi 1996; Scheu et al. 1999; Denno et al. 2000; Hufbauer and Root 2002). For example, in some cases, the effects of aboveground herbivores on plant fitness depend on the presence of belowground damage (Gange and

Brown 1989; Masters et al. 1993, 2001). Specifically, some evidence supports the prediction that belowground herbivory causes a stress response in plants, increasing aboveground tissue quality and thus having a positive impact on the performance of aboveground herbivores (Masters et al. 1993, 2001). Specific predictions such as these have rarely been tested. Indeed, the simultaneous effects of above- and belowground herbivores on plants have been studied in relatively few natural systems (e.g., Moran and Whitham (1990) and Masters and Brown (1992), Masters (1995a, 1995b), Strong et al. (1995), Masters and Brown (1997), Maron (1998), Masters et al. (2001)). In fact, despite strong evidence that herbivores consuming aboveground tissues can have major impacts on plants by directly altering plant allocation patterns, biomass, survival, and reproduction (Marquis 1992; Trumble et al. 1993), belowground herbivory, particularly by insects, largely has been neglected by researchers relative to aboveground herbivory (van der Putten et al. 2001).

A second biotic condition that may influence the importance of herbivores is the complexity of the surrounding community of plants, consumers, and their natural enemies. Current understanding of the impact of subterranean, insect herbivores on plant fitness is limited mostly to agricultural studies and weed biocontrol investigations (reviewed in Andersen (1987) and Brown and Gange (1990)). This work has found considerable, negative effects of belowground herbivores on plant growth (Müller-Schärer 1991; Hopkins et al. 1993; Nötzold et al. 1998), seed production (Sheppard et al. 1995), mortality (Pomerinke et al. (1995), but see Callaway et al. (1999)), and establishment (Godfrey et al. 1986); however, the impacts of belowground damage might be less substantial in more complex and speciose natural systems than in agroecosystems and systems heavily impacted by introduced weeds (e.g., Strong (1992) and Polis and Strong (1996)). Thus, for a more complete understanding of the importance of herbivory on plants, studies of herbivory in the context of diverse natural systems are necessary.

Interactions among complex communities of herbivore species may change dramatically with environmental conditions, such as water or nutrient availability. For example, the addition of water to *Capsella bursa-pastoris* plants offset the positive effect of root-feeding by beetle larvae on aphid weight and growth rate (Gange and Brown 1989). Also, in two *Hypericum* species the combined stresses of drought condi-

tions, mite damage, and aphid infestation were greater than the added effects of each individual stress on the plants (Willis et al. 1993). To determine the environments in which damage by multiple herbivore species reduces plant fitness, long-term studies that assess the relative importance of herbivory under different conditions are essential. To date, most investigation and discussion of the impacts of above- and belowground herbivory have neglected the potentially important implications of seeing these interactions as being dynamic over longer time scales (van der Putten et al. 2001).

We investigated the effects of both above- and belowground herbivory on lifetime reproduction of *Lupinus nanus* (Fabaceae), a native, annual plant occurring in the diverse natural grasslands of coastal California. A number of foliage-feeding insects attack *L. nanus* aboveground, and a weevil consumes the nodules that house this legume's symbiotic bacteria belowground. We manipulated damage in field experiments, using insecticides and simulated herbivory, and examined effects on plant survival and reproduction for two years. To evaluate the non-target impacts of the insecticides used, we conducted a growth chamber experiment investigating the direct effects of two insecticides on *L. nanus* in the absence of herbivores. Finally, we assessed variation in natural damage levels, plant survival, and reproduction for three years that differed in abiotic conditions due in part to the effects of El Niño. We addressed the following specific questions: (1) Does belowground herbivory reduce *L. nanus* survival or reproduction? (2) What are the combined influences of above- and belowground herbivory on *L. nanus* survival and reproduction? (3) Do the insecticides used to manipulate herbivory have non-target effects? (4) How does temporal variation in the impact of herbivores on *L. nanus* relate to variation in abiotic conditions?

Methods

Study site and system

We conducted observations and experiments at the University of California-Davis Bodega Marine Reserve (BMR), a 147-hectare biological preserve located on the Pacific Coast in Bodega Bay, CA, USA (38°19' N, 123°04' W). The coastal grassland at BMR supports a diverse plant community, densely populated by the perennial bush lupine, *Lupinus ar-*

boreus L. (Barbour 1973). *Lupinus nanus* Douglas ex Benth. (Fabaceae), a native, annual plant, grows in abundance on low reaches of the grassland at BMR. *Lupinus nanus* is self-compatible but outcrosses at intermediate rates (Károly 1994) that depend on pollinator abundance (Horovitz and Thorp 1970; Horovitz and Harding 1972). Typically, *L. nanus* seedlings emerge in late December, plants flower from early April until mid-May, and fruits have fully dehisced by mid-June.

Herbivorous insects attack both above- and below-ground tissues of *L. nanus*. Aboveground, phytophagous insects include *Platyrepia virginalis* Boisduval (Lepidoptera: Arctiidae), and less commonly *Philaenus spumarius* L. (Homoptera: Cercopidae) and aphids (Homoptera: Aphididae, unidentified species), among others. *Lupinus nanus* plants are also damaged by snails and slugs and occasionally eaten by gophers (*Thomomys bottae* Eydoux and Gervais). The principal belowground herbivore of *L. nanus* at BMR is the larval stage of a nodule-feeding weevil, *Sitona* sp. (*S. californicus* Fahraeus or *S. hispidula* Fabricius). In cultivated legumes such as alfalfa, white clover, and vetch, *Sitona* are common belowground pests (Nielsen 1990; Hower et al. 1995; Murray et al. 1996). *Sitona* sp. larvae have damaged the nodules of up to 50% of naturally occurring *L. nanus* plants at some sites at BMR (Hoeksema, unpublished data). In this system, nodule-feeding *Sitona* sp. not only damage plant tissue, but also could disrupt the mutualism between *L. nanus* and their nitrogen-fixing bacteria by consuming the nodules that encase symbiotic bacteria.

Does belowground herbivory reduce L. nanus survival or reproduction?

During March 25–31, 1997, we applied three treatments to alter belowground herbivory: simulated enhanced nodule damage, a control, and insecticide to reduce nodule damage. These three treatments were meant to represent a gradient from high nodule damage to low nodule damage. At the time treatments were implemented, plants were at an early stage of vegetative growth, typically having fewer than six leaves and no stem branching; however, nodule development and subsequent damage by *Sitona* larvae was already extensive (see below). Natural damage by *Sitona* larvae varies greatly over the course of the *L. nanus* growing season (see Results for 1998 below), but late March appears to represent the typical

time of onset of significant damage. We simulated enhanced nodule damage by clipping off two nodules after exposing the roots at the base of plants to a depth of 5 cm (which was approximately half of the maximum depth of the root system). The nodules removed were usually at a depth of 1–3 centimeters, where most nodules were observed to occur at this developmental stage of the plant. Soil was replaced following clipping. *Sitona* larvae often consume entire nodules on the roots of *L. nanus* (Hoeksema and Rudgers, personal observations), so clipping nodules reasonably mimicked the natural pattern of damage. However, induced plant responses to damage may be herbivore specific (reviewed in Karban and Baldwin (1997)) and would not be simulated by this treatment. At the time the treatments were applied, *L. nanus* plants had an average of 5.5 nodules, and thus the denodulation treatment (removal of two nodules) represented a removal of approximately 36.5% of each plant's nodules, in addition to ambient levels of damage. Naturally occurring plants at BMR experienced a mean of $17.6 \pm 4.6\%$ SE nodules damaged per plant ($n = 25$) at the time treatments were applied; therefore, simulated denodulation (in addition to natural damage) approximately tripled the amount of belowground damage. For the insecticide treatment, we exposed the roots as in the denodulation treatment, sprayed roots with the insecticide, Dursban (0.5 mL active ingredient/L H₂O, Dow Elanco Corporation, Midland, MI), and replaced the soil. Care was taken to avoid contaminating aboveground tissues. Dursban, a non-systemic insecticide, has been used successfully in other studies of belowground insect herbivory (Brown and Gange 1989a, 1989b; Maron 1998, 2001). The active ingredient is chlorpyrifos, which has no known negative effects on nitrogen-fixing bacteria, nitrifying and denitrifying bacteria, fungal abundance (Pozo et al. 1995), earthworms (Clements et al. 1986), spiders (Clements et al. 1988), or nodule size and number (Revellin et al. 1992). Finally, control plants received disturbance treatments, with similar root exposure and soil replacement, but no additional water. While it is possible that the insecticide increased water availability to *L. nanus*, the amount of water used to apply the insecticide (approx. 1 mL) was minimal, representing less than one-tenth of a percent of total rainfall during the growing season. Treatments were only applied once, and it is not known how long this particular formulation of Dursban persisted in the soil around the nodules of plants.

In 1997, we randomly chose 111 plants occurring in the BMR grassland community and assigned them at random to one of three treatments: denodulation, insecticide, or control ($n = 37$). Plants were chosen by selecting the closest plant at 1 m intervals along a 111 m transect through the grassland. We did not destructively sample plants; thus, the effectiveness of the insecticide on belowground damage could not be evaluated in 1997 (but see 1998). To estimate lifetime reproduction, we determined the proportion of plants surviving to set seeds, and for the subset of plants that reproduced we assessed flower production, seed production, and mean seed mass. All *L. nanus* flowers that do not develop into fruits leave a visible pedicel scar that allows determination of total flower production even after some flowers have senesced. On 11 May 1997, we covered immature fruits with mesh bags (Agryl row cover, PlastiTech, St-Rémi, Quebec, Canada) to prevent seed loss. Fruits were collected as they matured, and seeds were counted and weighed. In order to minimize the direct effects of human visitation on herbivory and plant success (see Cahill et al. (2001)), every effort was made to minimize handling of plants during visitation in this experiment and others (see below). Furthermore, no treatment received more frequent visitation than any other, such that any direct effects of visitation should have been similar across treatments.

The initial hypothesis was that *L. nanus* survival and reproduction would increase from the denodulation treatment to the control treatment, and be highest in the insecticide treatment. We used logistic regression to analyze the effects of treatments on whether a plant survived to produce seeds (Proc Genmod, SAS Institute, Inc. (1999)), and likelihood ratio (LR) tests with X^2 statistics were reported (Stokes et al. 1995). Because distributions of reproduction responses were extremely non-normal, and non-normality could not be substantially reduced through transformations, we used distribution-free randomization tests (with 10,000 iterations) to evaluate the treatments (Edgington 1987; Manly 1991). We used a randomization test equivalent of ANOVA for the number of flowers produced and a MANOVA equivalent for seed production and seed mass, including only those plants that survived to produce seeds. When the effect of insecticide was statistically significant at $P < 0.05$ in the multivariate test, we decomposed effects on individual responses with univariate analyses. We used planned contrasts to compare the three treatments when the effect of insecticide was significant.

What are the combined effects of above- and belowground herbivory on L. nanus survival and reproduction?

In 1998, we reduced insect damage to plants with insecticides that targeted either above- or belowground herbivores. Sevin (2.1 mL active ingredient/L H_2O , Union Carbide Corporation, Danbury, CT) targeted aboveground damage, and Dursban reduced belowground damage; neither insecticide has systemic action. Water served as a control spray for both above- and belowground treatments. The active ingredient in Sevin, carbaryl, kills a number of herbivorous insects and is relatively short-lived with a half-life of 3–4 d (Kuhr and Dorough 1976). In a 2×2 factorial design, treatments included Control (water above and belowground), Sevin (Sevin above, water below), Dursban (water above, Dursban below), and Both (Sevin above, Dursban below). Sevin was sprayed to saturate aboveground tissues, and approximately 3.5 mL of Dursban was applied directly to the soil beneath each plant, taking care to keep spray from the leaves and stems. Because of its relatively short half-life, it is unlikely that Sevin had a significant direct impact on belowground herbivores, even if small amounts were washed into the soil during rainfall events. We chose to apply Sevin because it has been used successfully to reduce herbivory on a related species, *L. arboreus* (Maron 1998). Treatments were applied beginning 6 March 1998 and every 10–14 d thereafter until fruits matured. Because this period is longer than the half-life of Sevin, it is possible that more effective control of folivores would have been possible with a more persistent pesticide.

As in 1997, we randomly chose naturally occurring plants by choosing the nearest plant at 1 m intervals along eight transects (25 m long) in two adjacent parcels (treated as blocks in statistical analyses) of the BMR grassland. The transects did not overlap the areas treated in 1997. In total, 200 plants were chosen, and individual plants were assigned at random to one of the four treatments ($n = 50$). In a third parcel of the BMR grassland, we also randomly selected (every 1 m along a single 50 m transect) fifty plants for assessment of the impact of Dursban on nodule damage throughout the season. These additional plants were all either treated with Dursban (water above, Dursban below) or as Controls (water above and below) ($n = 25$), and they were destructively harvested throughout the season and scored for nodule damage.

We measured a number of response variables to evaluate the effectiveness of the insecticides. To assess aboveground herbivory, we estimated leaf damage to the nearest $\frac{1}{4}$ of a leaflet for all leaflets per plant and calculated total percentage damage. Leaf damage was surveyed four times: once prior to insecticide application (6 March) and monthly thereafter. We averaged leaf damage per plant over the three post-treatment census dates for the analysis. To assess treatment effects on nodule number and damage, we destructively collected two plants from the separate Dursban experiment each month throughout the season. The nodules of harvested plants were inspected under a microscope and classified as damaged (when a characteristic *Sitona* sp. hole, frass, or larva was present) or undamaged. The proportion of nodules damaged per plant was used in the analysis. We combined destructive harvest data with estimates of nodule damage on all plants that died during the course of the season to determine the effectiveness of Dursban in reducing nodule damage and to ask whether Sevin had non-target effects on nodule damage or the number of nodules per plant.

We employed analysis of variance to determine the effects of treatments on herbivory. For all analyses, block and transect (nested within block) were included as random factors to control for spatial variability. When proportion or percentage data were analyzed, we applied angular transformations to obtain normally distributed residuals. For percentage leaf damage, we used an ANCOVA model with pre-treatment leaf damage as a covariate (Proc GLM, SAS Institute, Inc. (1999)). A separate, multivariate analysis tested effects on nodule number and the proportion of nodules damaged with pre-treatment number of leaves (an estimate of plant size, log-transformed) as a covariate (MANCOVA, Proc GLM, SAS Institute, Inc. (1999)). For all multivariate analyses we present Pillai's Trace statistic following Scheiner (2001).

We assessed survival and lifetime reproduction for *L. nanus* plants. Plants were examined every 10–14 d to determine the dates and proximate causes of death. Survival analysis tested differences among treatments in the lifespan of plants, including the effect of transect and the covariate, pre-treatment number of leaves (log-transformed) (Proc Glimmod and Phreg for Cox regression analysis, SAS Institute, Inc. (1999), see Allison (1995)); we report Wald X^2 statistics for hypothesis tests. We assessed flower number over two censuses during peak flower production (30 April and 29 May 1998). Seeds were collected after

bagging plants with mesh prior to pod dehiscence (see 1997), then counted and weighed. Flower number and seed production were log-transformed for normality and evaluated with MANCOVA including the covariate, pre-treatment number of leaves (log-transformed). When effects in the model were significant, univariate tests were conducted to partition effects for individual responses. A separate ANOVA tested effects on seed mass, including only the subset of plants that survived to produce seeds.

Do the insecticides have non-target effects?

As the herbivory reduction treatments depended on the use of insecticides, it was important to determine whether the insecticides had non-target effects on *L. nanus*. We tested the direct impacts of the insecticides in the absence of herbivores at the controlled environment facility (growth chambers) of the University of California, Davis, CA. Using data collected at BMR (V. Chow, University of California-Davis BMR, unpublished data), we programmed the growth chambers every 2 wks to reflect the mean photosynthetically active radiation (PAR), minimum (night) and maximum (midday) air temperature, and minimum and maximum relative humidity for the corresponding two wks of the 1998 growing season at BMR. In 1998, we collected four seeds from each of 21 naturally occurring plants at BMR. One seed per family was assigned at random to a Control, Sevin, Dursban, or Both treatment, as described in the 1998 experiment ($n = 21$). Seeds were scarified with a razor blade and germinated on moist filter paper. Seedlings were planted in sterilized, University of California Sunshine Mix #1, (70–80% peat with perlite, dolomitic limestone, gypsum, and a wetting agent) in the growth chamber on 8 May 1998. When seedlings were planted, we inoculated pots with *L. nanus* symbiotic bacteria using a mixture of water plus ground nodules that were collected from multiple *L. nanus* plants at BMR. Plants were watered every 2–3 d with a dilute fertilizer solution (0.0061% nitrogen, 0.012% soluble potash, 0.0056% phosphoric acid, 0.0031% magnesium, 0.0040% sulfur, and 0.0059% calcium) and sprayed every 10–14 d beginning 27 July until the fruits had developed (17 September). Plants began flowering in early September (corresponding to early April at BMR), and flowers were subsequently hand-pollinated flowers every 2–3 d using a randomly determined sire from the same maternal family.

Flower production, seed number, and mean seed mass were determined as in 1997.

To evaluate the effects of the insecticides on survival to reproduction, we used logistic regression (Proc Genmod, SAS Institute, Inc. (1999)), and we analyzed flower and seed production data with MANOVA (SAS Institute, Inc. 1999). Response variables were log-transformed for normality. When effects in the multivariate model were significant, we then examined the univariate models. For seed mass, we evaluated only the subset of plants producing seeds with a separate ANOVA. Family identity was included in the models as a random effect.

How does temporal variation in the impact of herbivores on L. nanus relate to variation in abiotic conditions?

Rainfall and temperature during three *L. nanus* generations (1997, 1998, 2000) were obtained from a permanent weather station at BMR. We compared total rainfall (mm) per year with the 33-year mean at BMR to assess temporal variation in this abiotic factor. To document differences among years in the severity of winter temperatures, we report mean minimum temperatures (°C) for the month of January calculated from daily minimums, as well as the 5-year average of these minimums for comparison.

In 2000, we assessed an additional *L. nanus* generation for ambient levels of herbivory, rates of survival, and reproductive output. Five haphazardly positioned 1 m × 2 m plots were established at BMR, and all *L. nanus* individuals were monitored from 8 January until final fruit dehiscence on 11 June 2000. These plots did not overlap previously treated areas at BMR. To estimate leaf damage, we scored damage per leaflet at monthly intervals, as in 1998. To determine levels of nodule damage, we destructively sampled plants from a randomly chosen half of each plot every month, which preserved one half of each plot for survival and reproduction censuses. On harvested plants, we counted nodules and calculated the proportion of nodules damaged per plant (see 1998). We conducted monthly censuses of all individuals in the undisturbed half of each plot. In May, we bagged fruits on 5 randomly chosen plants/plot that had survived to set seed (see 1997). Mature seeds were counted and weighed. In both 1998 and 2000, all plants were visited every 10–14 days.

We compared data from 2000 with data from control plants during 1997 and 1998 to document tempo-

ral variation in herbivory and *L. nanus* survival and reproduction. We assessed variation in survival to reproduction across years using logistic regression with planned contrasts (Proc Genmod, SAS Institute, Inc. (1999)). Because the 1997 experiment was not initiated until late March, for all years we used conditional survival data including only those plants that were alive in late March. We also used ANOVA to test differences among years for three additional response variables: (1) percentage leaf damage (1998 and 2000 only) (2) proportion of nodules with *Sitona* sp. damage and (3) final seed production for plants that survived to reproduce (Proc GLM, SAS Institute, Inc. (1999)). Data could not be combined into a single analysis because the same plants were not represented across years and experiments. Therefore, to adjust for multiple comparisons, we Bonferroni-corrected p-values from the ANOVAs.

Results

Does belowground herbivory reduce L. nanus survival or reproduction?

The 1997 treatments represented a range of nodule damage, from low (insecticide) to high (denodulation). Nodule damage was substantially enhanced through clipping. Denodulated plants had on average 54.1% of nodules damaged (clipped nodules + natural damage; see Methods for details). By contrast, ambient levels of damage in 1997 were 17.6% of per plant. In 1997 we did not obtain measures of the effects of the insecticide treatment on nodule damage. However, the 1998 destructive harvests demonstrated that Dursban significantly reduced nodule damage (see below) even after a single treatment application (ANOVA, Dursban effect, $F_{1,18} = 4.64$, $P = 0.0458$). Ambient levels of nodule damage did not significantly differ between 1997 and 1998 (Figure 5b). Therefore, we made the assumption that Dursban had similar effects in both years.

Enhanced herbivory (denodulation) reduced *L. nanus* survival, whereas reduced herbivory (insecticide) increased seed production and seed mass in 1997. More control plants survived to reproduce (73% survived) than did denodulated plants (42% survived; $X^2 = 4.15$, $P = 0.04$), but differences between denodulated plants and insecticide-treated plants (61% survived) were not significant ($X^2 = 1.91$, $P = 0.17$). Control plants did not differ in survival from insecti-

cide-treated plants ($X^2 = 1.05$, $P = 0.31$). Denodulated plants on average produced almost 50% fewer flowers than insecticide-treated plants (Figure 1a); however, the effect of damage was not statistically significant (randomization test, Model $F_{2,104} = 1.05$, $P = 0.36$). Despite the lack of significant effects on flower number, belowground herbivory strongly influenced seed number and seed mass combined (randomization test (MANOVA) $F_{2,58} = 3.83$, $P < 0.03$). Among the plants that survived to reproduce, insecticide-treated individuals produced more and heavier seeds than controls (Seed number, randomization test contrast $P < 0.03$, Seed mass, randomization test contrast: $P = 0.02$) and denodulated plants (Seed number contrast: $P < 0.05$, Seed mass contrast $P = 0.05$) (Figures 1b–1c). Controls and denodulated plants did not significantly differ (Figures 1b–1c, Seed number contrast: $P = 0.21$, Seed mass contrast $P = 0.12$). By multiplying the number of plants per treatment that survived to set seed by the number of seeds per plant (only for those plants that survived to reproduce), we obtained a population level assessment of the treatment effects, which confirmed our statistical results that denodulation reduced fitness estimates, while insecticide spray enhanced fitness estimates: Denodulation 632.2 seeds, Control 833.3 seeds, Insecticide 1236.6 seeds, with an original sample size of 37 plants per treatment.

What are the combined effects of above- and belowground herbivory on L. nanus survival and reproduction?

Insecticide treatments in 1998 effectively reduced nodule and leaf herbivory and targeted only above or belowground damage. Aboveground, Sevin reduced leaf damage by 40% compared to controls, while Dursban had no impact on folivory and did not interact with Sevin (Figure 2a, Table 1). The combined analysis on the number of nodules and nodule damage demonstrated a strong effect of Dursban only (Sevin $F_{1,94} = 0.01$, $P = 0.99$; Dursban $F_{1,94} = 22.78$, $P < 0.0001$; Sevin \times Dursban $F_{1,94} = 0.44$, $P = 0.65$; Pre-treatment leaf number $F_{1,94} = 10.77$, $P < 0.0001$). Dursban reduced belowground damage from *Sitona* sp. by 98% compared to controls, and Sevin did not alter belowground herbivory or interact with the Dursban treatment (Figure 2b, Table 1). Neither insecticide altered the number of nodules per plant (Table 1).

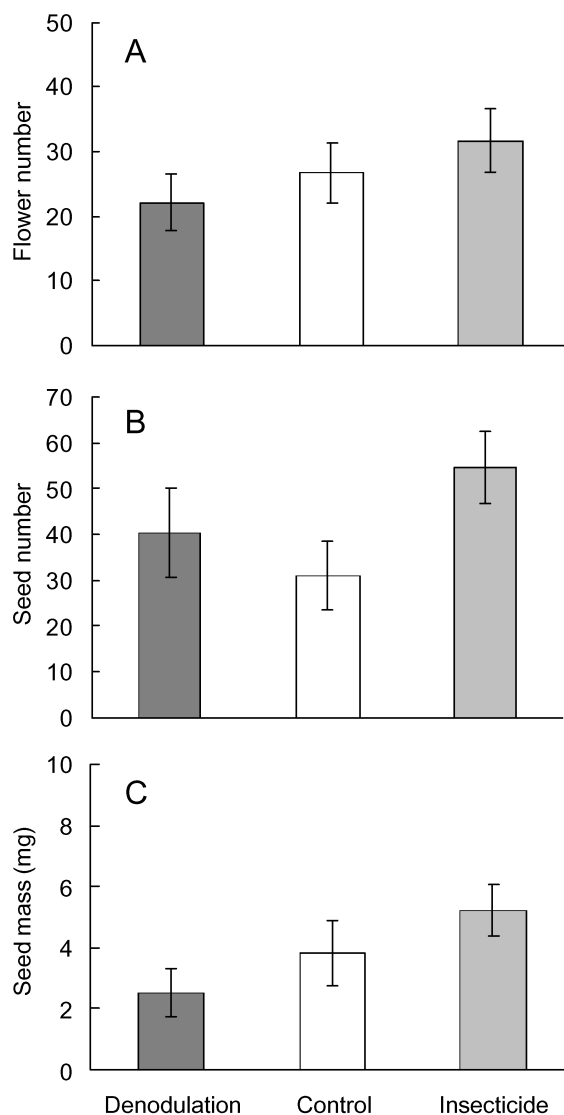


Figure 1. Effect of belowground herbivory treatments in 1997 on the number of flowers/plant (A) the number of seeds/plant (B) and seed mass/seed/plant (mg) (C). Means presented \pm SE.

Only aboveground damage, i.e., primarily leaf herbivory, had a strong effect on *L. nanus* survival. Survival analysis demonstrated that aboveground herbivory significantly reduced the amount of time that plants survived (Wald $X^2 = 3.92$, $P = 0.048$). Plants treated with Sevin lived for an average of 77.3 ± 4.9 SE days, while control plants lived only 67.0 ± 4.2 SE days, Dursban plants 62.0 ± 4.7 SE days, and plants treated with both insecticides, 66.3 ± 4.5 SE days. Belowground herbivory reduction did not affect survival (Wald $X^2 = 0.01$, $P = 0.91$), nor was there a

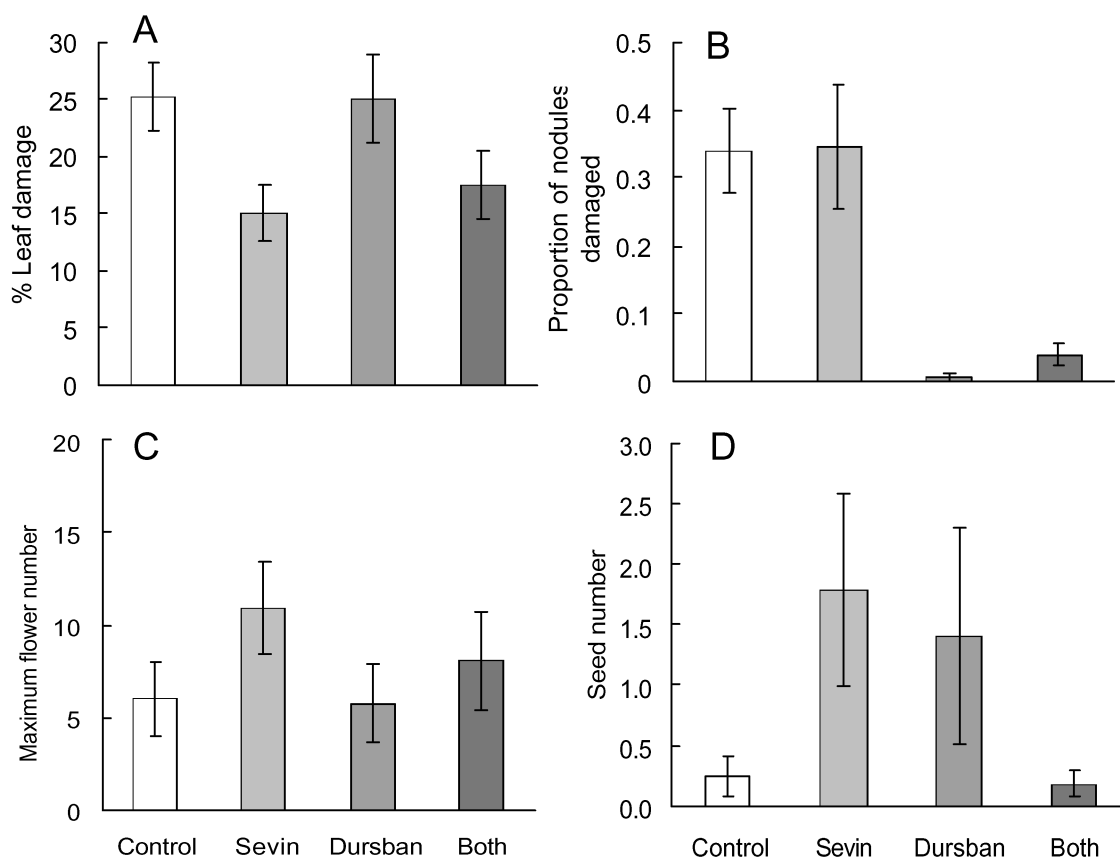


Figure 2. Mean \pm SE responses of *L. nanus* to insecticide treatments in the 1998 field experiment for percentage leaf damage/plant (A), proportion of nodules damaged/plant (B), maximum number of flowers/plant (C), and number of seeds/plant (D), where Sevin = above-ground herbivory reduction and Dursban = belowground reduction.

Table 1. Analysis of the effects of two insecticides, Sevin (aboveground) and Dursban (belowground) on percentage leaf damage, proportion of nodules damaged, and nodule number for *L. nanus* in 1998. Pre-treatment percentage leaf damage or leaf number was used as a covariate.

Effect	Response Variable					
	Percentage leaf damage		Proportion of nodules damaged		Number of nodules	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sevin	11.04	0.0012	0.01	0.9007	0.02	0.8816
Dursban	0.15	0.7006	37.96	< 0.0001	0.29	0.5910
Sevin \times Dursban	1.44	0.2328	0.03	0.9219	0.85	0.3599
Block	4.52	0.0355	3.61	0.0875	0.73	0.4164
Transect (Block)	1.09	0.3729	0.30	0.5855	1.28	0.2632
Initial leaf damage	12.01	0.0007	N/A	N/A	N/A	N/A
Initial leaf number	N/A	N/A	10.47	< 0.0001	18.09	< 0.0001

significant interaction between the insecticide treatments (Wald $X^2 = 2.13$, $P = 0.14$). These differences in survival may not have been large enough to affect female reproductive output, but pollen production and potential siring success could be significantly affected

by such differences in longevity. The block effect, i.e., transect location, explained substantial variation in the model (Wald $X^2 = 5.85$, $P = 0.02$), but the covariate (pre-treatment leaf number) was not important (Wald $X^2 = 1.71$, $P = 0.19$).

Table 2. Statistical results for the effects of two insecticides, Sevin (aboveground) and Dursban (belowground) on *L. nanus* in the 1998 field experiment. MANCOVA (flower number and seed number) and ANCOVA (seed number only).

Effect	Response Variable			
	MANCOVA		Seed Number	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sevin	1.74	0.1792	0.61	0.4358
Dursban	0.73	0.4821	1.01	0.3160
Sevin × Dursban	3.31	0.0387	6.58	0.0111
Block	1.08	0.3411	1.96	0.1635
Transect (Block)	1.57	0.0954	1.62	0.1432
Initial leaf number	1.67	0.1905	3.33	0.0696

In 1998, many plants in the experiment were killed by factors not affected by the insecticides. Of the plants for which we could assign a proximate cause of death ($n = 65$), 11% were eaten by gophers and 28% were submerged under water and died.

In spite of this mortality, the insecticide treatments had some effects on *L. nanus* reproduction. Although we found no main effects of Sevin or Dursban in a MANCOVA model with maximum flower number and total seed production, there was a significant interaction between the two insecticide treatments (Table 2) that did not affect flower number (Figure 2c) or seed mass (see below), but did influence seed production (Figure 2d). Planned contrasts for seed number demonstrated that Sevin-treated plants had more seeds than control plants ($F_{1,287} = 6.24$, $P = 0.01$) and more seeds than plants treated with both insecticides ($F_{1,287} = 6.79$, $P = 0.01$); however, Dursban-treated plants did not produce significantly more seeds than plants treated with both insecticides ($F_{1,287} = 1.46$, $P = 0.23$). We found no effects of treatments on seed mass; however, the sample sizes were low (ANOVA Model $F_{10,10} = 0.75$, $P = 0.67$). Plants treated with Sevin had more flowers than plants in other treatments (Figure 2c), but the ANCOVA model for flower production was non-significant ($F_{11,188} = 1.16$, $P = 0.32$). Combining results for the number of plants that survived to reproduce and mean seed number per plant, we obtained population level estimates of fitness: Control 12.9 seeds, Sevin 89.0 seeds, Dursban 58.0 seeds, Both 24.0 seeds (original $n = 25$ plants per treatment).

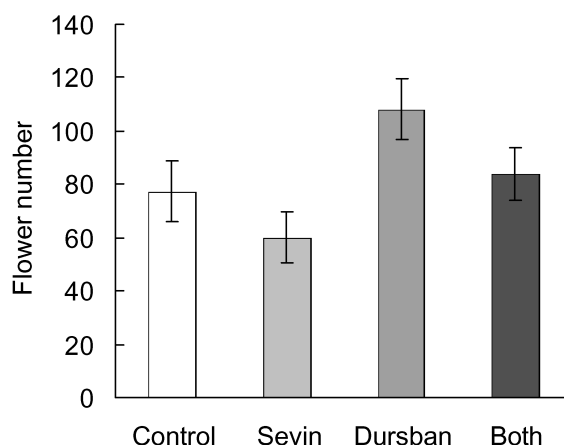


Figure 3. Non-target effects of the insecticides Sevin and Dursban on *L. nanus*. Mean (\pm SE) number of flowers produced per plant in the absence of herbivores (growth chamber).

Do the insecticides have non-target effects?

Insecticides had slight effects on *L. nanus* flower number in the absence of herbivores. The growth chamber experiment revealed no differences among treatments in survival to seed set (Sevin $X^2 = 0.26$, $P = 0.61$; Dursban $X^2 = 0.26$, $P = 0.61$; Sevin × Dursban $X^2 = 0.00$, $P = 0.97$). However, both insecticides altered flower and seed production combined (MANOVA Sevin $F_{1,60} = 4.23$, $P = 0.02$; Dursban $F_{1,60} = 3.68$, $P = 0.03$; Sevin × Dursban $F_{1,60} = 1.02$, $P = 0.37$). Although the individual ANOVA model for flower number was not significant at $P < 0.05$ (Model $F_{23,60} = 1.56$, $P = 0.09$), Dursban increased flower production (Figure 3, $F_{1,60} = 4.12$, $P = 0.05$), Sevin decreased flower production ($F_{1,60} = 7.03$, $P = 0.01$), and there was no significant interaction between the treatments ($F_{1,60} = 0.12$, $P = 0.73$) nor a strong effect of family ($F_{20,60} = 1.23$, $P = 0.27$). In an individual ANOVA model, neither insecticide altered seed production (Model $F_{23,60} = 0.94$, $P = 0.55$). For plants that produced seeds, treatment did not affect seed mass (Model $F_{23,40} = 1.41$, $P = 0.17$). These data suggest that in the field increases in flower production with Dursban and reductions in flower production with Sevin may not be completely independent of the direct effects of the insecticides in the absence of herbivores.

*How does temporal variation in the impact of herbivores on *L. nanus* relate to variation in abiotic conditions?*

During the winter of 1997–1998, coastal California was strongly affected by an El Niño event. As a result, cumulative rainfall during the peak rainfall months (November–February) of 1997–1998 was approximately 2.4 times the 33-year average at BMR (Figure 4a). In contrast, during the other two winters included in our study (1996–1997 and 1999–2000), rainfall was within one standard deviation of the 33-year average (Figure 4a). Minimum temperatures varied much less across years than did rainfall (Figure 4b). The lowest temperatures occurred in January 1999, and these lows did not correspond to the El Niño event of 1997–1998.

The three years differed strikingly in patterns of both aboveground leaf herbivory and in *L. nanus* survival (Figure 5). First, percentage leaf damage was 3.4-fold higher in 1998 than in 2000 (Figure 5a; $F_{1,59} = 17.87$, $P < 0.0004$). Unlike aboveground herbivory, nodule damage did not differ significantly among years (Figure 5b, $F_{2,64} = 2.20$, $P = 0.48$). Percentage survival of *L. nanus* depended significantly on the year (Figure 5c; $X^2 = 15.52$, $P < 0.002$). Plants had the lowest survival in 1998, the El Niño year, which differed from both 1997 (1998 v. 1997 contrast $X^2 = 14.14$, $P = 0.008$) and from 2000 (1998 v. 2000 contrast $X^2 = 10.27$, $P < 0.006$). Survival was similar in 1997 and 2000 (1997 v. 2000 contrast $X^2 = 3.84$, $P = 0.20$). Seed number (for plants surviving to reproduce) was not statistically significantly different among years (Figure 5d; $F_{2,34} = 3.22$, $P = 0.21$), although the average seed production per plant in 2000 was greater than in 1998. The lack of statistical significance in this case is due to the small number of control plants that produced seeds in 1998 ($n = 3$).

Discussion

Belowground herbivory

Although other work has shown that belowground herbivory can have important effects on the reproductive success of native plants (e.g., Gange et al. (1991) and Müller-Schärer and Brown (1995), Maron (1998)), to our knowledge, this study provides the first experimental evidence demonstrating that subterranean damage to nodules (a plant-bacteria mutual-

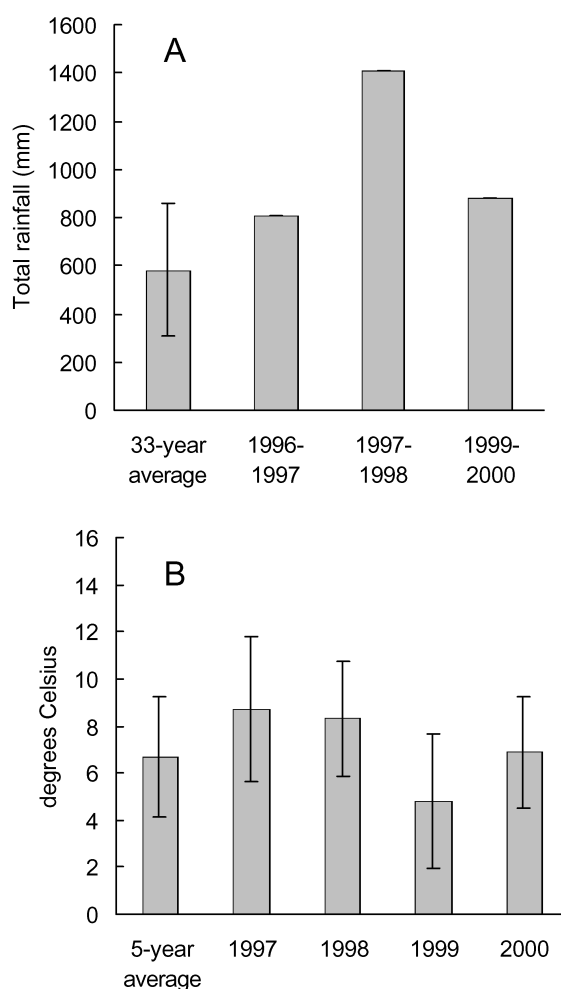


Figure 4. Temporal variation at BMR in total rainfall (mm) during the peak rainfall months of November through February (1996–1997, 1997–1998, and 1999–2000) plus mean \pm SD for the years 1968–2001 (33-year average) (A), mean \pm SD minimum daily temperatures ($^{\circ}$ C) during the month of January for 1997–2000 and for the 5-year average (1997–2001) (B).

ism) reduces the lifetime reproduction of a non-agricultural plant. Nodule damage reduced *L. nanus* survival and reproduction in one year of the study (1997) in which denodulation decreased the probability that a plant survived to produce seeds. While the insecticide treatment and the control did not differ with respect to survival, and none of the treatments altered *L. nanus* flower production, insecticide-treated plants produced more and heavier seeds than control or denodulated plants. Thus, assuming that the main effect of the insecticide, Dursban, was to reduce nodule damage, we conclude that high levels of simulated damage reduced *L. nanus* survival, whereas moderate

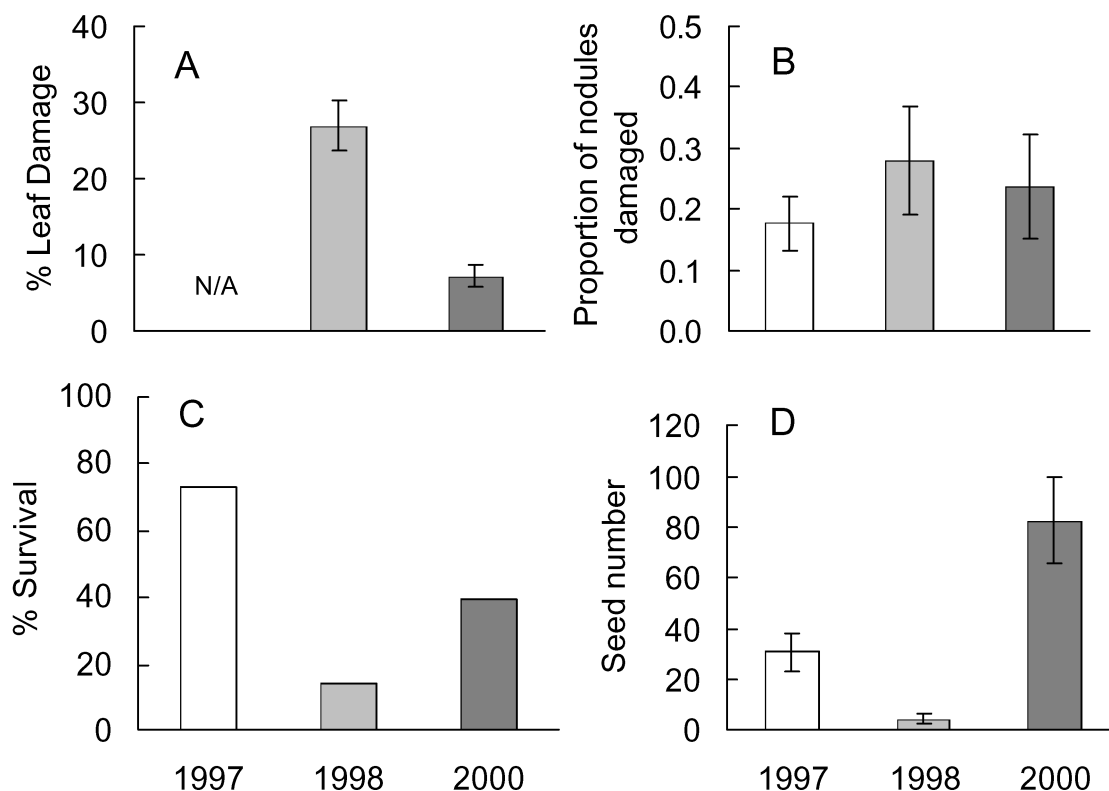


Figure 5. Temporal variation in herbivory, survival, and seed production for *L. nanus* control plants in 1997, 1998, and 2000. Mean \pm SE percentage leaf damage in late March (A), mean \pm SE proportion of nodules damaged in late March (B), percentage survival from late March to seed production (C), and mean \pm SE number of seeds/plants that set seed (D).

levels of natural damage decreased reproduction compared to the least damaged, insecticide-treated plants. Therefore, levels of belowground damage had effects that were contingent on the life history stage of the plant.

Results from 1997 must be qualified with limitations of the experimental design: there was no direct evidence that the insecticide, Dursban, reduced damage to nodules in 1997, and evidence from the growth chamber experiment suggested that Dursban increased flower production. We assert that because Dursban successfully reduced nodule damage in 1998 that it likely had the same effect in 1997. Because Dursban was only applied once in 1997, and the locations of experimental plants were different between 1997 and 1998, the effect on nodule damage in 1998 was not due to Dursban persistence in the soil from 1997. Additionally, because flower number did not differ significantly between Dursban-treated and control plants in the field, the non-target effects of this insecticide may not have been as important in the

field as they were in the growth chamber environment.

Although we found strong effects of nodule damage on seed production, whether these impacts translate into reduced population growth of *L. nanus* depends on levels of seed limitation in this species (e.g., Juenger and Bergelson (2000) and Greiling and Kichanan (2002)). In the related species *L. arboreus*, which co-occurs with *L. nanus* at BMR, seedling recruitment can be limited by rodent granivory and possibly by cutworm herbivory (Maron and Simms 1997, 2001). Further work in the *L. nanus* system would benefit from a better understanding of seed limitation, which could link the effects of herbivores on seed production to plant population dynamics.

Relative importance of above- versus belowground herbivory

In 1998, insecticide treatments strongly reduced herbivory: Sevin targeted aboveground folivory, while Dursban decreased nodule damage. During this year,

L. nanus plants had high levels of mortality due to factors other than insect damage; 11% of plants died from gopher predation, and 28% died from flooding. Overall, only 6% of control plants survived to produce seeds, although survival was greater in all other treatments.

Reductions in herbivory had no effect on flower production, but the aboveground insecticide (Sevin) increased plant survival and seed number relative to controls. This result indicates that damage to leaves, particularly in years of low survival, can reduce the lifetime fitness of *L. nanus*, a conclusion not uncommon for other annual plant species (reviewed in Marquis (1992)). In the case of Sevin, the effects of herbivory on reproduction may be even greater than reported, because in the absence of herbivores Sevin may directly reduce flower production, as shown by the growth chamber experiment on non-target effects reported here.

For subterranean herbivory, Dursban-treated plants in the field did not produce significantly more seeds or flowers than controls in 1998. Thus, belowground damage appeared to be less important to *L. nanus* in 1998 than it was in 1997. Moreover, belowground herbivory had less of an impact on plants than did aboveground herbivory in 1998. Previous work has suggested that increased belowground damage can lead to enhanced performance of aboveground herbivores (Gange and Brown 1989; Masters 1995a, 1995b; Masters and Brown 1992; Masters et al. 2001), due to a plant stress response and accompanying increased leaf nutrient concentrations. One prediction from this hypothesis might be that levels of leaf damage would decrease after application of belowground insecticide, because folivores need to consume less tissue to obtain equivalent resources. This effect did not appear to occur in *L. nanus* as changes in leaf damage were not observed when plants were sprayed with the belowground insecticide (Table 1). Another prediction of this hypothesis might be that belowground herbivory has a stronger detrimental effect on plants when aboveground herbivores are present than when they are absent. In our experiment to examine above- and belowground herbivory simultaneously, this was not found to be true, as belowground herbivory was found to be unimportant regardless of the presence of the aboveground insecticide. One possible reason for the lack of support of our results for the hypothesis that belowground herbivores have a positive effect on aboveground herbivores is that we focused on folivores,

mainly leaf-chewing insects, for both our manipulation of aboveground herbivory and our estimation of damage levels. Direct examination of seed predators could yield a very different result and would be an important component of future work in this system. Secondly, excess soil moisture, such as was present for much of the 1998 growing season, has been shown to lessen the effects of root herbivory (see Masters et al. (1993) and works cited therein), which could explain why we observed no response of plants or aboveground herbivores to the belowground insecticide in a very wet year, 1998 (Figure 4a).

Finally, in concert, Sevin and Dursban resulted in lower survival and seed production than Sevin alone. Because the combination of Sevin and Dursban had no effect in the absence of herbivores, it is unlikely that these insecticides had synergistic and negative direct effects on *L. nanus* in the field. However, non-target effects of the insecticides on community members that we did not monitor, such as pollinators of *L. nanus* or enemies of herbivores (including belowground enemies; e.g., van Tol et al. (2001)), might explain this unexpected result. As in other studies that have altered above- and belowground damage jointly (e.g., Müller-Schärer and Brown (1995) and Maron (1998)), our work did not suggest that reducing both types of herbivory had non-additive *benefits* for plants, but rather that a more complicated type of non-additivity can occur. Non-additivity between different types of interactions has been shown to be quite important in other systems. For example, the effect of herbivory on a plant can be strongly contingent on the presence of neighboring, competitor plants (e.g., Müller-Schärer (1991) and Burger and Louda (1994, 1995), Reader and Bonser (1998)). Thus, simultaneously assessing the effects of multiple processes will be necessary to understand their importance in the field.

The importance of assessing non-target effects of insecticides

The insecticides used to reduce herbivory in the field had moderate effects on plant reproduction in the absence of herbivores: Dursban increased flower number and Sevin decreased flower number in the growth chamber. Neither insecticide affected seed number, however. For the most part, we did not find similar effects of these insecticides on flower number in the field, where Dursban did not significantly increase flower production relative to controls. However, be-

cause Sevin reduced flower number in the absence of herbivores, this direct effect of Sevin may have prevented us from detecting larger impacts of aboveground herbivory in the field. Results from the 1998 experiment are, therefore, conservative with respect to the impact of Sevin on aboveground herbivory. Findings from the growth chamber experiment suggest that Sevin and Dursban should be used with caution in the future and that more research into their non-target effects is warranted, particularly as these insecticides are commonly sprayed to manipulate herbivory in ecological experiments (e.g., Müller-Schärer and Brown (1995) and Maron (1998), Masters et al. (2001), but see Brown and Gange (1989a)).

Inter-annual variation in plant-herbivore interactions

During the three years that we examined this system, both aboveground herbivory (in the form of leaf damage) and *L. nanus* survival exhibited dramatic temporal variation. In the El Nino year of significantly higher average rainfall, 1998, *L. nanus* plants at the BMR received the highest levels of leaf damage, whereas in 2000, a year of only slightly above-average rainfall, leaf damage was 27% that of 1998. Because percentage leaf damage was so low in 2000 (only 7%), folivory was probably not an important ecological factor influencing *L. nanus* during that season. A low abundance of aboveground herbivores at BMR in 2000 has been suggested by other data: *Philaenus spumarius* (spittlebug) densities in 2000 were the lowest of 14 years at BMR (R. Karban, University of California-Davis, personal communication). Insect abundances may have been depressed in 2000 due to the below average minimum temperatures in January 1999. High levels of variation in aboveground damage among years may also be related to variation in rainfall from El Nino and La Nina events. For example, the increase in precipitation associated with El Nino substantially modified the insect assemblage on islands in the Gulf of California (Polis et al. 1997); however, the consequence of this modification for plant-herbivore dynamics is unknown.

As mentioned briefly above, water availability in the soil can have a substantial impact on the importance of belowground herbivores. For example, Gange and Brown (1989) found that damage by beetle larvae to roots of an annual herb had stronger impacts when plants experienced greater water stress.

Similarly, we found that the impact of belowground herbivores on plant performance was significant in 1997, a year of normal rainfall, but not in 1998, a year of excess rainfall due to El Nino. It is possible that in general, the impact of subterranean herbivory might be stronger in years when the availability of plant resources is low. Because *Sitona* weevils consume the nitrogen-fixing nodules of plants, damage when nitrogen is limited might have more severe consequences than when plants are not constrained by nitrogen availability. Continuing work to determine the conditions under which herbivory is important to *L. nanus* would benefit from experiments that alter resource availability.

Finally, *L. nanus* survival to reproduction varied greatly among years. In 1998, when rainfall was more than double the 33-year average at BMR, survival was very low; many plants died after being flooded by the heavy rains. Thus, although the aboveground insecticide treatment increased reproduction in 1998 relative to controls, flooding had a much greater impact on plant success. This result suggests that in some years, abiotic conditions may be more important to selection on *L. nanus* than biotic factors such as insect herbivory. Indeed, in order to understand the selective importance of abiotic factors, herbivory and other interactions for plants, an expanded temporal perspective is necessary (van der Putten et al. 2001). For *L. nanus*, selection for defense against aboveground herbivores, defense against belowground herbivores, and tolerance of extreme abiotic conditions may vary strongly from year to year.

In conclusion, plant-herbivore dynamics in the *L. nanus* system are characterized by substantial temporal variation. In one year, belowground damage had large impacts on plant survival and reproduction. In another year, belowground damage had no impacts on correlates of plant fitness and was less important relative to aboveground damage and flooding. In a third season, aboveground damage was quite low, and presumably less critical to *L. nanus* success. In environments where large-scale weather patterns cause dramatic shifts in rainfall, temporal variation may be a key factor to consider in both the ecology and evolution of plant-herbivore interactions.

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