

Facilitation between coastal dune shrubs: a non-nitrogen fixing shrub facilitates establishment of a nitrogen-fixer

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Understanding positive interactions among species is integral to predicting the outcomes of community-level dynamics. Few studies have explored intraspecific variation in positive associations. We investigated facilitation between two coastal dune shrubs in California, *Baccharis pilularis* and the nitrogen-fixing species, *Lupinus arboreus*. *L. arboreus* seedlings and adults were non-randomly distributed, occurring most frequently in association with *B. pilularis*. The relationship depended on the genotype of *B. pilularis*; only the prostrate architectural form of this species was positively associated with *L. arboreus*. To evaluate the nature of this association, we assessed the influence of three microhabitat types (prostrate *B. pilularis*, *Ammophila arenaria* (an introduced dune grass), and exposed sand) on four life history stages of *L. arboreus*. Prostrate *B. pilularis* benefited *L. arboreus* seedling emergence, survival, and growth but had no effect on post-dispersal seed predation or adult establishment. Enhanced resistances to granivory by rodents or to seedling predation by voles were not important mechanisms underlying facilitation. In addition, an experiment manipulating herbivory by cutworms found no effects of microhabitat on susceptibility to damage. Evidence supports the hypothesis that the prostrate form of *B. pilularis* facilitates *L. arboreus* germination, seedling growth, and establishment as compared to exposed sand and dune grass. Furthermore, in combination with prior research, the results suggest that by facilitating an important nitrogen-fixer, *B. pilularis* may have effects that cascade to other members of the coastal plant community.

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During the past decade, ecologists have begun to re-emphasize the importance of positive interactions in community dynamics (Bertness and Hacker 1994, Callaway 1995, Stachowicz 2001), reviving questions that had largely been neglected since the first half of the 20th century (Clements 1916, Allee 1949). A growing body of literature has accrued that documents the existence of positive species associations, isolates the mechanisms involved, and explores the effects of gradients in abiotic factors on these interactions (Wilson and Agnew 1992, Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997, Stachowicz 2001, Callaway et al. 2002). From this work, we know that

positive interactions occur commonly, particularly in stressful environments such as deserts, salt marshes, and alpine communities (Niering et al. 1963, McAuliffe 1988, Bertness and Shumway 1993, Hacker and Bertness 1995, Callaway et al. 2002), and that the benefits of positive associations often increase with the intensity of abiotic stress (Bertness and Hacker 1994, Hacker and Gaines 1997, Kitzberger et al. 2000, Leonard 2000, Callaway et al. 2002, but see Goldberg et al. 1999, Tielborger and Kadmon 2000).

Facilitation, whereby species positively alter the abiotic or biotic environments of their neighbors (Connell and Slatyer 1977), can occur through a variety of direct

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and indirect means. Common direct mechanisms of facilitation among plants include alteration of the local microclimate; increased soil moisture, oxygen, and nutrient availability; hyper-accumulation of heavy metals; and trapping of propagules (reviewed by Wilson and Agnew 1992, Callaway 1995, Callaway and Pugnaire 1999). Indirect effects, mediated through a third species, consist of the attraction of shared mutualists (e.g. seed dispersers, nitrogen-fixing bacteria, mycorrhizal fungi), inhibition of other plant competitors, and protection from herbivory (i.e. associational resistance), among others (reviewed by Callaway 1995). Several of these direct and indirect mechanisms may operate simultaneously.

The importance of a given mechanism may depend on the life history stages of both the benefactor species and the species receiving benefits (Wood and del Moral 1987, Morris and Wood 1989, Callaway et al. 1996, Sans et al. 1998, Bruno and Kennedy 2000). For example, large *Spartina alterniflora* patches increased the establishment of two halophytic forb species, while small patches of *S. alterniflora* did not provide ample substrate stabilization to be facilitative (Bruno and Kennedy 2000). The majority of investigations to date, however, have focused on single life history stages, usually seedlings of the beneficiary species and adults of the benefactor (Kitzberger et al. 2000, Walker et al. 2001).

In addition to life history stage, variation among individuals of the same stage may also affect the mechanisms and magnitude of benefits in positive associations. For instance, Callaway et al. (1991) found that oak trees with fine roots near the soil surface had a negative impact on grasses whereas oaks with deeper root systems conferred a benefit. Such trait-dependent facilitation has rarely been explored in the study of positive interactions among plants; yet, understanding the evolutionary consequences of facilitative interactions will hinge on studies that examine intraspecific variation, particularly when such variation has a genetic basis.

Here, we document a previously unknown positive, but facultative, association between two coastal shrubs, *Baccharis pilularis* (Asteraceae) and *Lupinus arboreus* (Fabaceae). A number of previous studies have assessed the effects of nitrogen-fixing plants on other species (Walker and Vitousek 1991, del Moral and Bliss 1993, Maron and Connors 1996). We extended this work by examining whether a non-nitrogen-fixing shrub benefits a nitrogen-fixing legume. We explored interactions in a dune system, a habitat that has been investigated infrequently in the context of facilitation among plants (only 2/145 studies reviewed in Callaway 1995 involved dunes, see also Shumway 2000). *Lupinus arboreus* can have a large impact on coastal dune communities. Mutualistic nitrogen-fixing bacteria in the nodules of this legume combined with the plant's rapid growth

create patches of nitrogen-rich soil across the coastal landscape (Alpert and Mooney 1996, Maron and Connors 1996, Maron and Jefferies 1999). In grasslands, this nitrogen enhancement affected plant communities by increasing productivity but decreasing the diversity of plant species and encouraging the establishment of non-indigenous species (Maron and Connors 1996). Thus, understanding factors that affect the distribution and abundance of *L. arboreus* provides insights into the community-level dynamics of interactions among plants in coastal California.

We also assessed whether plant genotype influences the positive association between two species; this is one of the first studies to document such an effect. *Baccharis pilularis* possesses a genetically based architectural polymorphism with both upright and prostrate forms; preliminary observations suggested that only the prostrate form was positively correlated with *L. arboreus*. We examined the natural distributions of *L. arboreus* seedlings and adults and used experiments to compare the relative benefits of three dune microhabitats, prostrate *B. pilularis*, *Ammophila arenaria* (an introduced dune grass), and exposed sand, during multiple *L. arboreus* life history stages.

Reduced herbivore damage has been a commonly implicated benefit of positive associations between plants (Tahvanainen and Root 1972, Atsatt and O'Dowd 1976, Hambäck et al. 2000); thus, we also investigated three kinds of associational resistance through which *B. pilularis* may facilitate *L. arboreus*: reduction of seed predation by rodents, reduction of granivory by voles, and reduction of insect herbivory. Specifically, we addressed the following questions; (1) Are natural distributions of seedling and adult *L. arboreus* positively associated with the prostrate form of *B. pilularis*? (2) Does the microhabitat of prostrate *B. pilularis* benefit seedling survival, growth, or adult establishment via reduced insect or vole herbivory? (3) Is seedling emergence highest in prostrate *B. pilularis* microhabitat? (4) Are post-dispersal seed predation rates lowest in the prostrate *B. pilularis* microhabitat?

Methods

Natural history of the study species

We conducted this study 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 three dominant members of the plant community in the dune habitat at this site were *Baccharis pilularis* DC., *Lupinus arboreus* L. and *Ammophila arenaria* (L.) Link.

The native perennial shrub, *Baccharis pilularis* (Asteraceae, coyote bush), grows as two distinct architectural types in coastal California, an upright form (1–4

m tall), formerly recognized as the subspecies *B. p. consanguinea*, and a prostrate form (0.1–0.2 m tall), formerly *B. p. pilularis* (Munz and Keck 1973). The architectural polymorphism in *B. pilularis* is largely genetically based. Clones of the two forms planted in a common garden at BMR retained architectural differences, and the progeny from seeds of a single form segregated into both upright and prostrate types when grown in a greenhouse (Rudgers, unpubl.). In addition, hybridization of prostrate *B. pilularis* with an upright species, *B. sarothroides* (1–3 m) resulted in F_1 progeny that segregated into prostrate and upright types (Thompson et al. 1995), consistent with the hypothesis of a heritable basis for plant architecture in *B. pilularis*.

Lupinus arboreus (Fabaceae, bush lupine) occurs commonly in dune and grassland habitats of coastal California. *L. arboreus* seedlings germinate in winter and can mature into reproductive adults in a little over one year. Seeds are produced in late July and early August. In dunes, rodent granivory and herbivory affect *L. arboreus* seed and seedling survival. Previous work has shown that mice (*Peromyscus maniculatus* and *Reithrodontomys megalotis*) consume dispersed *L. arboreus* seeds, severely depressing seedling recruitment and ultimately reducing adult plant density (Maron and Simms 1997, 2001). Vole (*Microtus californicus*) and cutworm (Noctuidae) herbivory can kill *L. arboreus* seedlings, also limiting adult plant establishment (Maron and Simms 2001, unpubl.).

Ammophila arenaria (Poaceae, European beachgrass), a perennial grass with thick rhizomes, has been planted along the California coast for dune stabilization and now dominates the dune habitat at BMR. The introduction of *A. arenaria* has modified dune topography (Wiedemann and Pickart 1996) and reduced both plant and animal species diversity (Boyd 1992, Buell et al. 1995, and references therein). The mechanisms underlying the effects of introduced *A. arenaria* on native plants, such as *L. arboreus*, have not previously been investigated experimentally.

Distributions of seedling and adult *L. arboreus*

We assessed the distribution of naturally occurring *L. arboreus* plants at the BMR. During 11–12 July 2001, we recorded the distributions of four primary microhabitat types in the dunes (*A. arenaria*, exposed sand, upright *B. pilularis*, and prostrate *B. pilularis*) along nine 30 m transects. We define these types of vegetation cover as microhabitats because they are naturally occurring; thus, we cannot separate the effects of the identity of plant species or genotypes from the microhabitats in which they occur. To obtain the natural frequencies of each microhabitat type, we recorded the length of the transect line covered by each microhabitat (to the nearest 0.1 m) and divided by the total transect

length. Along each transect, we also noted the microhabitat of all *L. arboreus* seedlings and adults within a 2 m belt on either side of the transect line. With these methods, we covered approximately 0.11 ha of the 69 ha BMR dunes. We assessed distributions of seedlings and adults with Chi-Square (χ^2) tests, using the natural distributions of the four microhabitats as the expected frequencies. When the distribution significantly differed from expected, we decomposed the effects for each microhabitat with individual tests that were Bonferroni-adjusted for multiple comparisons.

L. arboreus seedling survival, growth and adult establishment

Previous work at BMR has shown that cutworm herbivory can severely reduce *L. arboreus* seedling establishment, particularly during winter when cutworms are active in dune soils (Maron and Simms 2001). Thus associational resistance to cutworm damage is one mechanism by which prostrate *B. pilularis* may benefit *L. arboreus*. We tested this hypothesis using a 3×2 factorial design with three microhabitats (*A. arenaria*, exposed sand, and prostrate *B. pilularis*) and two insecticide treatments (insecticide and control). If insect herbivory were mediating the differences among microhabitats in the performance of *L. arboreus*, then we expect to find a significant interaction between the insecticide treatment and microhabitat.

Our initial hypothesis was that *L. arboreus* would benefit from prostrate *B. pilularis* and thus contribute to the observed positive association. We did not examine upright *B. pilularis* in this experiment. Although upright and prostrate plants occasionally grow intertwined, their distributions in the dunes do not fully overlap. Given that we used naturally occurring plants, we were interested in separating the effects of microhabitat from the effects of plant species as much as possible by employing a blocking design to remove variation due to local conditions. The unreplicated block design consisted of 50 haphazardly located blocks that were ≥ 10 m apart. This design was not compatible with examining upright *B. pilularis* due to the non-coincidental distribution of this form relative to the prostrate form within the dunes. Furthermore, preliminary observations suggested that the upright form was never positively associated with *L. arboreus*. Therefore, to confirm whether benefits accrued from association with prostrate *B. pilularis*, we felt that alternative microhabitats that had a concurrent distribution with prostrate types would be more appropriate controls in the absence of planting a common garden of both architectural forms.

To obtain seedlings, *L. arboreus* seeds collected from several plants in the local population were nicked with a knife and planted in the BMR greenhouse during

early December 1999. On 7 January 2000, we transplanted six *L. arboreus* seedlings to each block, with one seedling randomly assigned to each treatment combination. Seedlings within each microhabitat were planted ≥ 0.5 m apart. Cutworms were suppressed by regular applications of the insecticide Dursban (0.5 ml active ingredient/l of water, Dow Elanco Corporation, Midland, MI). Water was sprayed on control plants. Dursban and water were applied every 2 weeks from February through June 2000. The active ingredient in Dursban is chlorpyrifos, which has no known negative effects on nitrogen-fixing bacteria, nitrifying and denitrifying bacteria, fungal abundance, earthworms, spiders, or legume nodule size and number (ref. in Maron 1998). Dursban reduces belowground damage to *L. arboreus* by the root-boring ghost moth (*Hepialis californicus*, Lepidoptera), an important herbivore on adult plants, as well by cutworms (Noctuidae; Maron 1998, 2001, unpubl.). As substantial previous work on *L. arboreus* has documented reductions in herbivory due to Dursban, we did not examine levels of damage in this study.

Throughout the season we assessed *L. arboreus* survival and growth. We divided survival censuses into three critical life history stages: early establishment (January–June 2000), conditional survival from June to one year (June 2000–January 2001), and conditional survival from one year to the adult stage (January–July 2001). We assessed seedling growth repeatedly during the experiment (9 May, 24 May, 21 June, and 30 August 2000). For the early dates (until 21 June) we counted the number of leaves per plant and measured plant height to the nearest cm. Because plants had many more leaves during the latter two censuses, we only measured height. Both leaf number and height are highly correlated with seedling biomass (Maron, unpubl.).

We analyzed survival data using logistic regression analyses for each life history stage (Proc Genmod, SAS Institute 1999). Separate, conditional logistic regressions were used rather than survival analysis because census intervals were unequal. We included the effects of block, microhabitat, insecticide treatment, and the interaction of microhabitat \times insecticide and report likelihood ratio χ^2 values. Block was considered a random effect in all statistical analyses. When microhabitat type was statistically significant at $P < 0.05$, planned contrasts were used to compare the three types. Since logistic regression analyses were conditionally independent (by definition), we combined the P -values using Fisher's method (Sokal and Rohlf 1995).

We evaluated seedling growth data using multivariate repeated measures analysis of variance (Profile analysis, SAS Institute 1999; von Ende 2001). Plant height and number of leaves were examined separately because they were not measured for the same time intervals. To correct for multiple tests, P -values were Bonferroni-

adjusted. In both models, effects included block, microhabitat, insecticide treatment, and microhabitat \times insecticide. The number of leaves was log-transformed for normality. We reported Pillai's Trace test statistics for these analyses following Scheiner (2001).

Throughout the experiment, voles (*Microtus californicus*), which cause distinctive herbivory by removing all leaves and cotyledons, consumed 21% of the seedlings ($n = 63$). Of seedlings that were eaten by voles, 86% died by the next census. Thus, vole herbivory is one mechanism by which microhabitats might have differential effects on *L. arboreus* survival. Although we did not manipulate vole herbivory, we recorded whether or not detectable vole herbivory occurred and analyzed these data with logistic regression (SAS Institute 1999).

Seedling emergence of *L. arboreus*

To determine how dune microhabitat may alter *L. arboreus* seedling emergence, in late August 2000, when seeds are naturally dispersed from *L. arboreus*, we revisited 25 of the 50 blocks established for the 2000 seedling survival experiment. Within each block we placed a group of 10 seeds in each of the three microhabitats: *A. arenaria*, exposed sand, and prostrate *B. pilularis*. Undamaged seeds were collected from several plants in the local *L. arboreus* population, thoroughly mixed, and randomly assigned to each microhabitat. We buried seeds 3–5 cm deep and ≥ 5 cm apart, marking the location of each seed with plastic cylinders (4 cm diameter, 5 cm height) pushed most of the way into the soil surrounding each seed. Seedlings were surveyed on 25 April and 12 July 2001. We analyzed the total proportion of seedlings that had germinated by July (angular-transformed) with ANOVA including the effects of microhabitat (fixed) and block (random) (SAS Institute 1999).

Post-dispersal seed predation on *L. arboreus*

We compared seed predation in prostrate *B. pilularis* microhabitat with *A. arenaria* and exposed sand during two years, 2000 and 2001. We employed the same blocking pattern as described in the 2000 factorial seedling survival experiment. Within each block we placed seed depots in all three microhabitats, with depots approximately 1–3 m apart within each block. A seed depot consisted of 10 seeds placed on sand in a 10 cm diameter petri plate. We set out depots on 25 August 2000 and revisited them every 24 h for four consecutive days. Each day we recorded the number of seeds remaining and replenished missing seeds. We made the assumption that seeds were removed from

depots exclusively by rodents. Rodent scat was commonly found in depots with seeds removed (Rudgers, pers. obs.), BMR has no seed-harvesting ants, and granivorous birds seldom eat bush lupine seeds (Maron, pers. obs.). Since seed predation rates were low in 2000, we examined the total proportion of seeds removed during the entire 4 d period (angular transformed to meet assumptions of normality). We analyzed the effects of microhabitat and block on seed predation using ANOVA (SAS Institute 1999).

During 2001, we tested the hypothesis that seed predation differed among microhabitats on a larger spatial scale. We established two non-overlapping grids consisting of five 150 m long transects with grid markers placed approximately every 10 m (75 grid markers total). Each grid marker was randomly assigned to a single microhabitat type: *A. arenaria*, exposed sand, or prostrate *B. pilularis* ($n = 25$). At each marker we found the nearest microhabitat of the assigned type and placed there a seed depot with 10 seeds (as in 2000). Seed depots were checked for missing seeds after 48 h. We surveyed seed removal during 15–16 August along one grid and on 19–20 August at the second grid. In 2001, seed predation rates were much higher than in 2000, and most depots had either all 10 seeds or no seeds removed. This pattern of removal generated a bi-modal distribution of the proportion of seeds removed. Therefore, we used whether or not any seeds were removed as the response and analyzed these data with logistic regression including the effects of trial (15–16 or 19–20 August), transect (nested within trial), microhabitat, and trial \times microhabitat (SAS Institute 1999).

Results

Are distributions of *L. arboreus* associated with prostrate *B. pilularis*?

The distribution of both *L. arboreus* seedlings and adults significantly differed from the distribution expected under a hypothesis of no association between *L. arboreus* and particular microhabitats (Fig. 1; seedlings $\chi^2_{(3)} = 21.97$, $P < 0.001$; adults $\chi^2_{(3)} = 15.06$, $P < 0.005$). Examining each microhabitat separately, we found that seedlings and adults were more likely to occur in the prostrate form of *B. pilularis* than expected by chance, but *L. arboreus* distribution did not differ from expected for any other habitat type (Fig. 1). Thus, the prostrate form of *B. pilularis* appears to be a more favorable microhabitat for *L. arboreus* than either *A. arenaria* or exposed sand. Furthermore, both seedling and adult *L. arboreus* were positively associated only with the prostrate genotypes of *B. pilularis*. Therefore, plant genotype plays a role in the positive association between *B. pilularis* and *L. arboreus*.

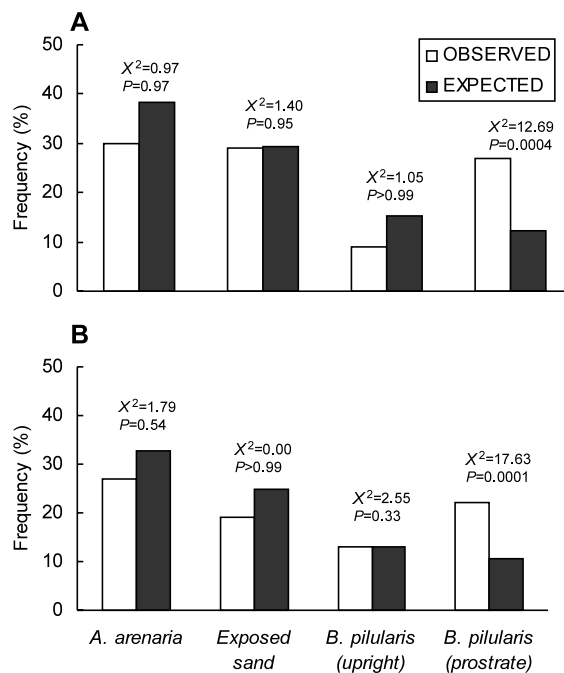


Fig. 1. Observed and expected frequencies of *L. arboreus* in four microhabitats for (A) *L. arboreus* seedlings and (B) *L. arboreus* adults. Statistical results from individual χ^2 tests are presented with P -values Bonferroni-adjusted for multiple comparisons. All tests had 1 df .

Does prostrate *B. pilularis* microhabitat benefit *L. arboreus* survival, growth, or establishment?

Seedling survival and adult establishment

The effects of microhabitat type on *L. arboreus* depended on the life history stage examined. During early seedling establishment (January–June 2000), seedlings in prostrate *B. pilularis* and exposed sand had 1.8 times higher survival than seedlings in *A. arenaria*, but prostrate *B. pilularis* and exposed sand did not strongly differ (Fig. 2A, Table 1). For the sub-set of plants that survived through June ($n = 146$), subsequent survival to the one-year old stage (June 2000–January 2001) was 1.9 times higher in prostrate *B. pilularis* than in the other two microhabitats combined; *A. arenaria* and exposed sand did not significantly differ (Fig. 2A, Table 1). If seedlings survived through January 2001 ($n = 103$), microhabitat had no additional impact on survival (January–July 2001; Fig. 2A, Table 1). While the cumulative differences among the microhabitats were most clear at the end of the experiment (Fig. 2A), the majority of the facilitative effects occurred during the first two life history stages. Combining the results from the three life history stages using Fisher's method, we found a strong effect of microhabitat (Table 1): over all life history stages, survival of *L. arboreus* seedlings was

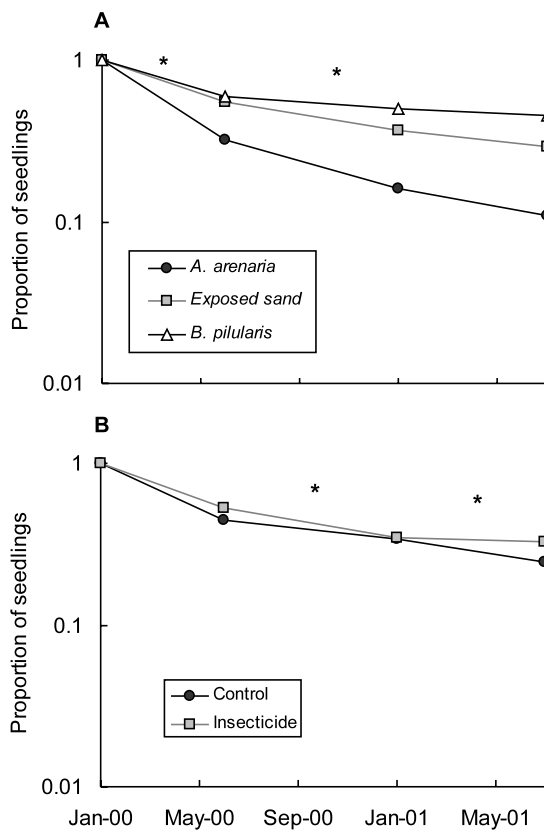


Fig. 2. Proportion of seedlings surviving (log scale) plotted for three life history stages: early seedling establishment (January–June 2000), survival through one year (June 2000–January 2001), and survival to adulthood (January–July 2001) by (A) microhabitat type and (B) insecticide treatment. Asterisks represent stages during which treatments significantly differed in conditional survival ($P < 0.05$).

highest in prostrate *B. pilularis* (45% survived), intermediate in exposed sand (29% survived), and lowest in *A. arenaria* (11% survived).

If insect herbivory were an important mechanism driving differences in seedling performance among microhabitats, then we expected a strong interaction between the insecticide and microhabitat. We did not find such interactions during any life history stage (Table 1). Therefore, the insecticide treatment had similar effects across microhabitats.

The insecticide treatment improved seedling survival, but only during the final life history stage from one year to adulthood (Fig. 2B, Table 1). In the June 2000 to January 2001 phase, insecticide actually decreased conditional survival by 15% (Table 1). However, by the final stage this effect was reversed; control plants had 23% lower conditional survival than insecticide-treated plants (Table 1). Fisher's method was not used to examine the insecticide effect because the direction of this effect depended on life history stage.

Table 1. Statistical results from logistic regression analyses examining the effects of block, habitat type, and insecticide treatment on survival of *L. arboreus* for three life history stages. χ^2 are likelihood ratio tests. Fisher is Fisher's method for combining probabilities.

Effect	Seedling establishment		Survival to one year		Survival to adulthood		Fisher	
	df	χ^2	df	χ^2	df	χ^2	df	P
Microhabitat	2	23.53	2	18.56	2	4.23	6	<0.0001
<i>A. arenaria</i> vs <i>B. pilularis</i>	1	20.26	1	16.62	1	3.44	6	<0.0001
<i>A. arenaria</i> vs exposed sand	1	14.82	1	0.93	1	0.54	6	0.0010
<i>B. pilularis</i> vs exposed sand	1	0.43	1	10.36	1	1.47	6	0.0073
Insecticide	1	2.70	1	4.81	1	4.96	6	N/A
Microhabitat × insecticide	2	0.70	2	2.66	2	0.09	6	0.7513
Block	44	52.58	29	17.57	10	5.16	6	0.6998

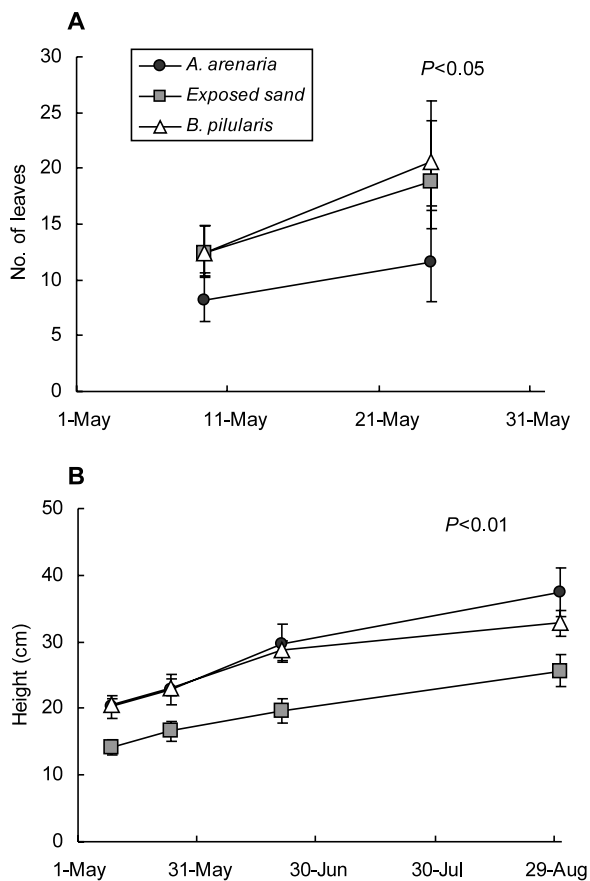


Fig. 3. Seedling growth in three microhabitats (2000) for (A) mean leaf number (back-transformed least squares (LS) means \pm 95% CI) and (B) mean height (cm) (LS means \pm SE). *P*-values given for the effect of microhabitat.

Seedling growth

Microhabitat altered seedling growth. Seedlings planted in *A. arenaria* produced 34% fewer leaves than seedlings in prostrate *B. pilularis* and 41% fewer leaves than

seedlings in exposed sand (Fig. 3A, Table 2). Leaf production by seedlings in prostrate *B. pilularis* and exposed sand did not substantially differ (Table 2). In addition, microhabitat strongly affected plant height. Seedlings in exposed sand were approximately 30% shorter than seedlings in both *A. arenaria* and prostrate *B. pilularis* combined (Fig. 3B, Table 2). Since *A. arenaria* reduced leaf production and exposed sand significantly reduced plant height, seedlings planted in prostrate *B. pilularis* had the highest combined measures of growth (Fig. 3).

No significant microhabitat by insecticide interaction occurred for seedling growth (Table 2), providing additional evidence that associational resistance was not the mechanism underlying facilitation of *L. arboreus*. The insecticide slightly reduced *L. arboreus* leaf number, but had no effect on plant height (Table 2). Back-transformed least squares (LS) mean leaf numbers with 95% confidence limits (CL) were insecticide 9.5 (8.0, 11.3) and control 12.3 (10.3, 14.7) on 9 May 2000 and insecticide 14.8 (11.7, 18.64) and control 18.5 (14.5, 23.6) on 24 May 2000. Thus, although the insecticide increased *L. arboreus* survival, it did not benefit *L. arboreus* growth. This effect is not unexpected given the type of damage that cutworms cause: seedlings attacked by cutworms are usually killed.

Seedling predation by voles

Seedling predation by voles, like insect herbivory, was not an important mechanism underlying differences in *L. arboreus* survival among the three microhabitats ($\chi^2_{(2)} = 0.65$, $P = 0.72$). The proportions of seedlings killed by voles were 0.23, 0.21, and 0.19 in *A. arenaria*, exposed sand, and *B. pilularis*, respectively. We detected no effect of insecticide ($\chi^2_{(1)} = 1.28$, $P = 0.26$), microhabitat \times insecticide interaction ($\chi^2_{(2)} = 0.24$, $P = 0.89$), or block effect on predation by voles ($\chi^2_{(37)} = 27.60$, $P = 0.87$).

Table 2. Statistical results from repeated measures analysis of variance examining the effects of microhabitat, insecticide treatment, and block ($n = 50$) on the number of leaves and plant height (cm) for *L. arboreus*. *P*-values were Bonferroni-adjusted. Pillai is Pillai's Trace statistic from the multivariate analyses.

Effect	Leaf number				Plant height (cm)			
	Pillai	<i>df</i>	<i>F</i>	<i>P</i>	Pillai	<i>df</i>	<i>F</i>	<i>P</i>
Microhabitat	0.09	2,84	4.36	0.0157	0.21	2,53	7.16	0.0018
<i>A. arenaria</i> vs <i>B. pilularis</i>	0.09	1,84	8.19	0.0053	0.004	1,53	0.23	0.6356
<i>A. arenaria</i> vs exposed sand	0.07	1,84	5.90	0.0173	0.14	1,53	8.30	0.0057
<i>B. pilularis</i> vs exposed sand	0.00	1,84	0.11	0.7465	0.19	1,53	12.33	0.0009
Insecticide	0.04	1,84	3.14	0.0802	0.01	1,53	0.44	0.5108
Microhabitat \times insecticide	0.02	2,84	0.71	0.4925	0.02	2,53	0.60	0.5499
Block	0.35	45,84	1.00	0.4821	0.39	42,53	0.80	0.7678
Time	0.50	1,84	83.99	<0.0001	0.75	3,51	51.21	<0.0001
Microhabitat \times time	0.03	2,84	1.11	0.3329	0.16	6,104	1.50	0.1853
Insecticide \times time	0.00	1,84	0.10	0.7539	0.03	3,51	0.57	0.6365
Microhabitat \times insecticide \times time	0.03	2,84	1.22	0.3009	0.14	6,104	1.30	0.2632
Block \times time	0.32	45,84	0.89	0.6549	1.24	126,159	0.89	0.7571

Is seedling emergence highest in prostrate *B. pilularis* microhabitat?

Seeds planted in the three microhabitats had marginally significantly different short-term emergence rates ($F_{(2,77)} = 2.66$, $P = 0.08$). Although overall emergence was low (< 10%), seeds planted in prostrate *B. pilularis* had approximately 65% higher emergence than seeds in *A. arenaria* and 50% higher emergence than seeds in exposed sand. Short-term seedling emergence differed little between *A. arenaria* and exposed sand. Back-transformed LS means (95% CL) were *A. arenaria* 0.03 (0.02, 0.04), exposed sand 0.04 (0.03, 0.05), and *B. pilularis* 0.08 (0.07, 0.10). Block explained variation in the model ($F_{(25,77)} = 2.60$, $P = 0.002$); thus, independent of microhabitat type, there was strong spatial variation in the emergence of *L. arboreus* seeds.

Are post-dispersal seed predation rates lowest in prostrate *B. pilularis* microhabitat?

During periods of both low (2000) and high (2001) post-dispersal seed predation, predation did not differ among microhabitats. In 2000, the proportion of seeds removed from depots was roughly equivalent among microhabitats ($F_{(2,46)} = 1.11$, $P = 0.34$). Back-transformed LS means with 95% CL were *A. arenaria* 0.009 (0.001, 0.03) exposed sand 0.03 (0.01, 0.05), and prostrate *B. pilularis* 0.01 (0.002, 0.03). Block had a strong effect ($F_{(24,46)} = 3.80$, $P < 0.0001$), suggesting a high level of spatial variation in seed predation that was independent of the type of vegetation cover. Seed removal was much greater in 2001 than in 2000 with 67% of seeds removed from depots compared to 19%. However, microhabitat did not affect rates of seed predation in 2001 ($\chi^2_{(2)} = 2.27$, $P = 0.32$). In *A. arenaria*, the fraction of seed depots with seeds removed was 0.64 in 2001, with 0.74 and 0.62 in exposed sand and prostrate *B. pilularis*, respectively. Neither the date of the 2001 trials ($\chi^2_{(1)} = 0.39$, $P = 0.53$) nor transect (nested within trial; $\chi^2_{(8)} = 5.43$, $P = 0.71$) explained additional variation in the model.

Discussion

A strong positive association between prostrate *B. pilularis* and both seedlings and adults of *L. arboreus* in the field supported the hypothesis that *B. pilularis* shrubs facilitate this legume. Unlike many other facilitative interactions, however, the association between *L. arboreus* and *B. pilularis* is highly facultative, rather than obligate, with only approximately 30% of *L. arboreus* seedlings and adults occurring in association with *B. pilularis*. Furthermore, facilitation in this system differs from previous studies that have shown nitrogen-fixing

plants to be important facilitators (Walker and Vitousek 1991, del Moral and Bliss 1993, Maron and Connors 1996) in that the microhabitat of a non-nitrogen-fixing shrub provided benefits to a nitrogen-fixing species.

The microhabitat of prostrate *B. pilularis* facilitated both the survival and growth of *L. arboreus*. Seedling survival through the first year was greater in prostrate *B. pilularis* than in *A. arenaria* or exposed sand microhabitats. Furthermore, leaf number was reduced in *A. arenaria* and plant height was lower in exposed sand relative to prostrate *B. pilularis*. As the number of leaves and plant height are correlated with seedling biomass (Maron, unpubl.), we suggest that prostrate *B. pilularis* microhabitat also benefits *L. arboreus* growth.

Associational resistance mediated neither survival nor growth effects. While the insecticide sprayed to reduce cutworm damage increased survival, the effects of the insecticide were similar across all microhabitats. Seedling predation by voles also did not vary with microhabitat. In contrast, prior studies in other systems have found important effects of associational resistance (Tahvanainen and Root 1972, Hambäck et al. 2000).

Modifications in local abiotic conditions provide one potential explanation for differential survival and growth of *L. arboreus* among microhabitats. In particular, soil moisture may be an important limiting resource in dune environments (Barbour 1984). Prostrate *B. pilularis* leaves may trap fog and condensation, increasing local water availability. Fog precipitation has been suggested as a mechanism underlying facilitation but, to our knowledge, has not been empirically demonstrated as a benefit of plant associations in any system (reviewed by Wilson and Agnew 1992, but see Ingraham and Matthews 1995, Dawson 1998).

Impacts of microhabitats on growth of *L. arboreus* suggest that this shrub may compete strongly with the introduced dune grass, *A. arenaria*, which grows in dense, monospecific stands. We suggest that leaf number may be more highly related to plant fitness than plant height because differences in height could arise from the etiolation of plants in low light environments or from increased wind pruning in the unprotected sand environment at BMR (Miller and Weis 1999). Thus, for plant growth, the evidence most strongly supports a reduction in *L. arboreus* growth (via leaf production) in *A. arenaria* microhabitats relative to other environments. This study provides the first experimental evidence that this introduced dune grass has negative impacts on the indigenous coastal flora. In California dune systems, *A. arenaria* thus has the potential to reduce the performance of a common, native shrub.

In addition to effects on survival and growth, prostrate *B. pilularis* also facilitated *L. arboreus* seedling emergence. The fraction of seedlings emerging from seeds buried in prostrate *B. pilularis* microhabitat was

65% higher than in *A. arenaria* and 50% greater than in exposed sand. Our 1-year study represented a substantial fraction of the germination period: although *L. arboreus* has a persistent seed bank, only 4% of seeds were viable after 3 years of burial at BMR (Maron and Simms 2001). The effect of prostrate *B. pilularis* on seedling emergence was probably not due to differences among microhabitats in soil surface temperatures (Seneca and Cooper 1971) as seedling emergence in exposed sand, the microhabitat expected to have the highest surface temperatures, did not differ from emergence in *A. arenaria*, a more shaded microenvironment. As with seedling survival and growth, microhabitat differences in water availability provide a possible, unexplored mechanism underlying the facilitative effect.

Unlike the benefits of prostrate *B. pilularis* that we found for *L. arboreus* survival, growth, and seedling emergence, microhabitat had no significant effects on post-dispersal seed predation. In 2000, seed removal from seed depots was similar across microhabitats on a small spatial scale (1 m), during a time of relatively low seed predation. In 2001, during a period of relatively high seed predation, we examined seed predation on a larger spatial scale (10 m) and again detected no differences in removal rates. Similarly, other studies have failed to find substantial impacts of facilitation via reduced seed predation (Dunne and Parker 1999). However, Pitts and Barbour (1979) showed that the activity of *Peromyscus maniculatus* (deer mouse) was concentrated in dense stands of *A. arenaria*, suggesting that higher rodent granivory would occur in this microhabitat, in contrast to our results.

While results suggest that *B. pilularis* facilitates *L. arboreus* during multiple life history stages, we did not examine potential facilitative interactions during the seed dispersal or reproductive phases of *L. arboreus* life history. Further facilitative effects may be revealed. For example, as has been observed for other benefactor species (Day and Wright 1989, Aguiar and Sala 1994), the low growing, prostrate form of *B. pilularis* may trap *L. arboreus* seeds, increasing the seed bank in this microhabitat. *B. pilularis* may also increase the reproductive output of *L. arboreus* and perhaps create local patches of elevated seed density, contributing to a patchwork of source-sink dynamics in this system. In contrast, interactions between *L. arboreus* and *B. pilularis* may switch from facilitative to competitive during later life stages. Nothing is currently known about the reciprocal effects of *L. arboreus* on *B. pilularis*; however, other work has demonstrated that the benefits of plant associations decrease with plant age (Walker and Vitousek 1991). In the BMR dunes, adult *L. arboreus* plants are often surrounded by “doughnuts” of prostrate form *B. pilularis*. Once *L. arboreus* seedlings establish, they grow quickly, and it appears that these large, sprawling shrubs may shade low-growing *B. pilularis*,

ultimately out-competing their benefactors for light (Flores-Martinez et al. 1994).

Both seedlings and adults of *L. arboreus* occurred more frequently in prostrate *B. pilularis* than in any of the three other microhabitats examined, including *B. pilularis* (upright form). To our knowledge, this is the first demonstration that plant genotype can mediate positive associations among plant species. In order to explore evolutionary questions concerning positive associations between plants, future studies would benefit from exploring intraspecific genetic variation in plant traits, such as height, branch architecture, and root structure, that could mediate the costs and benefits of these interactions.

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