Ecology, 90(7), 2009, pp. 1852–1862

Edaphic quality and plant–pathogen interactions: effects of soil calcium on fungal infection of a serpentine flax

Yuri P. Springer[1]

Department of Ecology and Evolutionary Biology, University of California, A316 Earth and Marine Sciences Building, Santa Cruz, California 95064 USA

Abstract. Spatial variation in the frequency and outcome of interspecific interactions is thought to play a central role in shaping geographic patterns of biodiversity. Previous empirical studies and modeling exercises suggest that negative species interactions should occur with greater frequency and intensity in high-quality/low-stress environments. I tested this hypothesis through a four-year epidemiological study of interactions between the plant *Hesperolinon californicum* and the pathogenic rust fungus *Melampsora lini*. By virtue of its association with serpentine soils, *H. californicum* is exposed to edaphic conditions that are stressful to most plants. Notable among these is the low availability of calcium, an element that plays critically important roles in the immune responses of plants to attacking pathogens. As a serpentine generalist, however, *H. californicum* grows in soils with a wide range of calcium concentrations, and this should lead to differences in the frequency and/or severity of rust infection among host populations. I investigated geographic variation in disease pressure by conducting annual surveys in 16 populations spanning the host's distribution and representing a range of soil calcium concentrations. Results indicated that plants growing in more stressful low-calcium soils experienced higher rates of rust infection, suggesting that soil calcium may modulate host susceptibility in a manner opposite to that predicted by the a priori hypothesis. Epidemiological surveys further revealed a latitudinal cline in disease prevalence, with high infection rates in northern host populations decreasing gradually toward the south. Studies of the fitness effects of disease demonstrated that rust infection caused significant reductions in host survival and fecundity, and there was evidence of a demographic feedback between infection prevalence and host density across survey years.

Key words: environmental quality; epidemiology; fitness costs; Hesperolinon californicum; Melampsora lini; *plant–pathogen interactions; serpentine soil; soil calcium.*

Introduction

Geographic variation in the frequency and outcome of species interactions is considered fundamental to the generation, maintenance, and distribution of biodiversity (Thompson 1999, 2005). While a variety of stochastic and deterministic mechanisms can contribute to this variation, spatial heterogeneity in properties of the abiotic environment may have especially important and predictable impacts. One approach to studying such effects is to examine changes in species interactions across gradients of environmental quality, often measured in terms of productivity, stability, or stress (reviewed in Bertness and Callaway 1994, Brooker et al. 2008). Results of a growing number of studies conducted in this framework, including both computer simulations (Travis et al. 2005, 2006) and field research in a variety of "harsh" or "stressful" environments including alpine, arctic, desert, and intertidal systems (Bertness and Leonard 1997, Choler et al. 2001, Callaway et al. 2002, Bruno et al. 2003) support the prediction that species interactions should become less beneficial and more antagonistic as environmental quality increases (but see Maestre and Cortina 2004).

Terrestrial plant communities have served as model systems in which to investigate deterministic impacts of environmental quality on species interactions. While a large proportion of research in this context has focused on plant–plant interactions, the potential for stressful abiotic conditions to similarly modulate a broader ranger of biotic interactions also exists. For example, there is evidence that plants in resource poor environments may invest more in anti-herbivore chemical defenses because of relatively high costs of replacing tissue lost to grazing (Grime 1977, Coley et al. 1985, Bryant et al. 1989, but see Miller and Woodrow 2008). Possible consequences of this could include a greater abundance and/or diversity of herbivores in resource-rich habitats and relatively higher frequency and intensity of grazing experienced by plants in these environments. For intimate host–symbiont interactions (parasitisms and mutualisms), Thrall el al. (2007)

Manuscript received 21 April 2008; revised 2 September 2008; accepted 2 October 2008. Corresponding Editor: J. N. Klironomos.

[1] Present address: Zoology Department, University of Otago, 340 Great King Street, Dunedin 9054 New Zealand. E-mail: yurispringer@gmail.com

proposed that elevated levels of host density in high-quality, low-stress environments should favor increased rates of horizontal transmission and the evolution of more virulent (antagonistic) symbionts. They posited that antagonistic species interactions, and associated coevolution, should occur with relatively greater frequency and intensity in these settings. Studies that collectively document this consistent, deterministic impact of abiotic stress across a broader range of biotic interactions, involving species representing a variety of taxonomic groups and functional guilds, are needed to evaluate the generality of this directional effect of environmental quality on species interactions. Further, identifying the mechanistic underpinnings of these effects would significantly improve our ability to explain and predict the spatial structure of species interactions, and resulting coevolutionary dynamics, in natural ecosystems.

Plant communities associated with serpentine soils provide a unique opportunity to assess the role of environmental quality in generating spatial variation in species interactions. Serpentine soils, generally distributed in discrete patches in areas associated with tectonic activity, are characterized by a unique suite of edaphic properties that are highly stressful to most plants. These include a coarse rocky texture, low water-holding potential, high concentrations of magnesium, iron, and various heavy metals, and extreme deficiencies in nutritive elements including calcium, nitrogen, phosphorus, and potassium (Proctor and Woodell 1975, Brooks 1987). These soils support distinct assemblages of associated or endemic plant species, and patterns of marked floristic discontinuity are usually observed at serpentine soil ecotones (Baker et al. 1991, Roberts and Proctor 1992). The inability of plant species growing on adjacent soils to invade serpentine patches is generally attributed to the extremely low nutritive value and physiologically stressful nature of serpentine soils, with low-calcium concentrations thought to be especially influential in driving this biotic partitioning (Vlamis and Jenny 1948, Kruckeberg 1954, Walker 1954, Kruckeberg 1967, Brady et al. 2005). Calcium concentrations in serpentine soils can be up to 17 times lower than levels measured in nonserpentine and agricultural soils (Mehlich and Tewari 1974). Although serpentine-associated plants are adapted to tolerate conditions of low-calcium availability, growth is often significantly more vigorous in higher calcium soils (reviewed in Brady et al. 2005). As such, natural variation in calcium concentrations within and among serpentine patches exposes associated plants to a range of conditions that likely represent an important gradient of environmental quality.

Results of a recent greenhouse study provide evidence of the potential for soil calcium concentrations to modulate interactions between serpentine plants and their pathogens. In an inoculation trial using serpentine soils with experimentally manipulated calcium levels, Springer et al. (2007) showed that rates of infection of the serpentine flax *Hesperolinon californicum* by the fungal rust *Melampsora lini* were inversely related to soil calcium concentrations. Contrary to the aforementioned findings and predictions, this result suggests that infection by pathogens might occur with greater frequency and/or severity in lower quality serpentine environments. Documenting this relationship in wild host populations would provide support for the use of soil calcium to predict spatial structure of host–pathogen interactions in natural plant communities, and would help clarify current understanding of the relationship between environmental stress and the frequency of antagonistic species interactions.

Here I present results of a field study of the effects of soil calcium on patterns of plant disease in serpentine plant communities. The study used the *H. californicum*–*M. lini* system and had three objectives. First, I conducted annual epidemiological surveys for three to four years in 16 host populations, distributed across the host's biogeographic range, to characterize spatiotemporal patterns of disease prevalence and severity. Second, I tested the prediction generated by the greenhouse inoculation experiment that infection rates should be lower among plants growing in higher quality, high calcium soils. I collected and analyzed soil samples from each study population and quantified the relative contribution of a number of demographic, edaphic, and geographic factors to observed patterns of infection. Finally, by measuring seedling survival and adult fecundity of host plants exhibiting a range of infection severity in the field, I assessed the impact of disease on host fitness and the potential for the pathogen to exert selective pressure on host evolution.

Methods

Host and pathogen

California dwarf flax, *H. californicum* Small (Linaceae), is a diminutive annual, generally 20–40 cm tall at flowering, with thin stems and leaves (McCarten 1993). The species is endemic to California, where it grows primarily in the Coast Range Mountains (Sharsmith 1961). Plants germinate in late December–early January and set seed between July and September. A seed bank appears to be present but quantitative data on its persistence are not available. While it has not been demonstrated conclusively, it is unlikely that *H. californicum* is a metal hyperaccumulator because hyperaccumulation has never been reported in the Linaceae (A. J. M. Baker, *personal communication*). Based on the lack of heterostyly, pseudocleistogamous flowers, and the observed tendency for greater phenotypic variation among vs. within populations, Sharsmith (1961) concluded that the species is primarily selfing. *H. californicum* is part of the serpentine-associated flora in California and has been classified as a "bodenvag" taxon because it grows on soils that vary widely in their degree of serpentine influence (Kruckeberg 1954, Sharsmith 1961). It therefore represents an ideal host species with

which to investigate how among-population variation in soil calcium concentrations influences the frequency and severity of infection by pathogens.

Melampsora lini Persoon is an autecious, macrocyclic rust fungus (Uredinales) that attacks stem and leaf tissue of plants in the family Linaceae (Flor 1954, Lawrence et al. 2007). It is an obligate pathogen, causing nonsystemic infections that reduce host plant vigor via destruction of photosynthetic tissue and damage to cuticle surfaces (Littlefield 1981). Given appropriate weather conditions, annual infection cycles begin in early/mid-March. *M. lini* has an asexual generation time of ~12–14 days, and wind-dispersed urediospores are produced in localized lesions on plant stems and leaves. The fungus is thought to overwinter as resting teliospores or dormant uredial infections that represent sources of primary inoculum in the subsequent growing season (Gold and Statler 1983, Burdon and Jarosz 1991). Aside from rare and relatively mild damage caused by an herbivorous Chrysomelid beetle (*personal observation*), no other natural enemies of *H. californicum* are known.

Epidemiological surveys.—To characterize spatiotemporal patterns of rust infection and host abundance I conducted annual epidemiological surveys in 16 *H. californicum* populations that collectively spanned the latitudinal extent of the host's biogeographic range (Appendix A). Populations ranged in size from ~1000 to >150 000 plants and were separated by at least 1 km. Due to fine scale patchiness of serpentine soils many of these populations consisted of spatially discrete patches of plants separated by tens to hundreds of meters. I used fixed plot surveys, conducted at the onset of flowering each season, to collect data in a subset of these populations in 2001 and at all sites in 2002, 2003, and 2004. Prior to the initial survey of each population, a rectangular survey plot was delineated around each patch of plants containing at least 300–400 individuals. These plots (one to nine per population) ranged in size from 20 to 640 m^2. Surveys utilized 0.25-m^2 quadrats placed along uniformly spaced transects within plots, with quadrat and transect spacing determined by plot size and plant abundance: (a) transects every 2 m and quadrats every 2 m along transects in plots smaller than 100 m^2 or containing fewer than 100 large adult plants, (b) transects every 4 m and quadrats every 2 m in plots between 100 and 360 m^2, and (c) transects every 4 m and quadrats every 4 m in plots larger than 360 m^2. Within quadrats I used a modified James scale (James 1971) to visually estimate the infection severity of each plant (percentage of photosynthetic tissue covered with rust pustules) and assign it to one of nine infection severity categories: 0% (uninfected), 1%, 5%, 10%, 25%, 50%, 70%, 90%, and 100%. In the process I quantified the total number of plants in each quadrat and used this value to estimate plant density. I recorded the latitude and altitude of each survey plot using a handheld GPS unit.

Factors driving infection dynamics.—To investigate the mechanistic underpinning of observed patterns of infection, I collected data on soil chemistry associated with each host population and meteorological conditions in a subset of these locations that collectively spanned the latitudinal extent of the study area. I used a statistical model to quantify the influence of these abiotic environmental parameters, as well as spatial and demographic attributes of host and pathogen populations, on epidemiological patterns characterized through the field surveys.

Depending on their spatial area I collected between six and 18 soil samples from each study population in 2002. Sampling locations were haphazardly selected to cover the entire spatial extent of each population. I used diethylene triamine pentaacetate (DTPA) extractions to quantify the concentrations of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Si, Sr, and Zn, and ammonium acetate (AA) extractions to measure the concentrations of Ca, Mg, K, and Na, associated with these samples. Specific details of the extraction protocols and analyses using ICP optical emissions spectrometry were provided by Springer et al. (2007).

In 2003 and 2004 I deployed nine HOBO meteorological dataloggers (model number H08-032, Onset, Bourne, Massachusetts, USA) to measure temperature and relative humidity (RH) at seven study populations that collectively span the latitudinal range of *H. californicum* (Appendix A). Sensors were enclosed in baffled housings positioned 23 cm above ground level and took measurements every 30 min between mid-January (shortly after plant germination but before the first observed rust infection) and mid-June (end of flowering).

Fitness consequences of infection.—I used three approaches to measure the impacts of rust infection on host fitness.

I measured the effect of infection on seedling survival using data collected in two populations (5 and 6) near the latitudinal center of the host species' range (Appendix A). The large spatial area and demographic size of these populations allowed me to compare plants presenting the full range of infection severity in relatively close proximity to one another. I measured seedling survival in 2002 using 12 permanent 0.25-m^2 quadrats (nine in population 5, three in population 6). I recorded the infection status and/or mortality of all plants in each quadrat every two weeks between 21 March, when plants were 2–4 cm tall and rust infection was first observed, and 16 May, when plants had begun to flower. I used a modified James scale (James 1971) to assign each plant to one of four infection categories based on the percentage of photosynthetic tissue covered with rust pustules: uninfected, light (<30%), moderate (30–70%), and severe (>70%).

I used data from the same two populations to quantify the effect of infection on adult plant fecundity. In 2001 I haphazardly placed a 0.25-m^2 quadrat in

survey plots where infection had previously been observed and recorded the disease status (infected vs. uninfected) of, and number of flower buds produced by, the first 10 healthy and infected plants in each quadrat. During epidemiological surveys in 2002 I tagged a haphazard subsample of plants that collectively spanned the range of infection severity. Once plants had produced seed capsules I recorded the height (soil to highest tip of branches folded into vertical orientation), longest branch length (main stem to branch tip), and number of viable seed capsules produced by these tagged individuals. Counts of viable capsules, which consistently contain 5–6 seeds, are an excellent proxy for seed production.

I estimated the potential for a feedback of infection on host demography by measuring the correlation between disease prevalence in a host patch in a given year and the change in mean host density between that year and the subsequent year.

Statistical analyses

Epidemiological surveys.—Abundance data for *H. californicum* (plant density) and *M. lini* (infection prevalence and severity) were averaged across quadrats within survey plots for each survey year to generate plot/year means (year [*n*] = 2001 [25], 2002 [40], 2003 [40], 2004 [40]). To characterize spatial patterns in the abundance of host and pathogen I averaged these plot/year means within populations across survey years and regressed them against the latitude and altitude of each host population (recorded in the survey plot closest to the geographic center of the population). To characterize temporal patterns in the abundance of host and pathogen I averaged plot/year means across populations within years and plotted them by year. All analyses were performed in JMP version 5.1.1 (SAS Institute, Cary, North Carolina, USA) unless otherwise noted.

Factors driving infection dynamics.—I used nonmetric multidimensional scaling (NMDS) to (1) determine how well host populations could be distinguished by their edaphic properties, and (2) identify the focal analytes that contributed most significantly to this differentiation. Analyses were performed using Primer version 5.2.3 (Primer-E, Plymouth, UK). Because the concentration of the analytes spanned five orders of magnitude, I standardized data so that concentrations of the different analytes converged on the same mean but retained their respective distributions. Using a Bray-Curtis dissimilarity matrix constructed with standardized data, I ran an NMDS analysis with 500 restarts to visualize the degree and nature of edaphic differentiation among samples. I performed a SIMPER analysis (Clarke 1993) to quantify the percentage contribution of each of the 17 measured analytes to this pattern. As calcium and magnesium contributed disproportionately to the NMDS solution they were the only two edaphic analytes used in subsequent analyses.

Because meteorological data were only collected in a subset of populations and survey years, I conducted separate analyses to test for correlations between weather conditions and patterns of infection. Results of previous research on the biology of *M. lini* indicate that maximal spore germination (percentage germinated, number and rate of germ tube growth) occurs at 18°C and in the presence of free water, and that the percentage and duration of spore viability is maximal under conditions of 7°C and 40–60% RH (Hart 1926, Misra 1952). Based on this information I calculated values for seven meteorological variables likely to influence patterns of rust infection. I computed "mean daily deviation from 18°C" by averaging the absolute value of the deviation of each temperature measurement from 18°C over the course of each survey (calendar) day. Values for "mean daily deviation from 7°C," and "mean daily deviation from 50% RH," were determined in an analogous way. Due to complications with survey instruments I was unable to measure free water availability directly so I used four proxy variables as estimates: for each survey day I calculated the percentage of measurements for which (1) RH $\geq$ 90%, (2) RH $\geq$ 95%, (3) RH $\geq$ 100%, and (4) temperature $<$ dew point. Regression analyses using the full weather data set (2003 and 2004) indicated that each of these percentage variables was highly correlated to the mean continuous daily duration of time over which the respective criteria were met.

I used linear regression to test for correlations between these seven meteorological variables and the prevalence of infection recorded in the survey plot where the weather sensors had been deployed. Because infection was scored only once near the end of the host's growing season it was unclear when patterns of rust prevalence were actually established. Because of this uncertainty, regressions were performed using data for the seven meteorological variables averaged across three time scales: the entire survey season, each four-week period ($n = 5$), and each two-week period ($n = 10$). Analyses were run separately for each of the two years that weather data were collected. To test for latitudinal and altitudinal trends in meteorological conditions, I regressed the latitude and altitude of each weather sensor against the mean daily temperature, mean proportion of daily RH measurements >90%, and mean proportion of daily measurements for which temperature was below dew point. All averages were calculated within, and regressions run separately for, each of the two survey years.

I quantified the contribution of different geographic, demographic, and edaphic factors to patterns of fungal infection using multiple regression. I used all survey plot/year combinations excluding data from the four populations where disease was never observed because it was unclear whether the fungus ever recruited to these sites. The nine factors included in the analyses were latitude and altitude of survey plots, host density in the

current and previous year, estimated number of host plants in the current and previous year (host abundance: product of mean host density and survey plot area), disease prevalence in the previous year, and soil calcium and magnesium concentrations. Because infection prevalence and severity were strongly correlated, only the former was used as a response variable. Prior to the analysis, data were transformed as necessary to meet the assumption of normality, and linear regression was used to test for covariance between all pairwise combinations of independent variables. When strong covariance occurred, I substituted residuals calculated from the regression for values of one of the pair of correlated parameters. Soil data were available for all but five of the 40 survey plots. These five plots were each located in one of the two largest host populations (5 and 6) where a total of 18 and 15 soil samples were collected. Given the relatively robust sampling at these two sites, population means for soil calcium and magnesium concentrations were calculated and substituted for the missing plot values in the model.

Fitness consequences of infection.—I performed Kaplan-Meyer survival analysis (Lee and Wang 2003) to examine the effect of infection severity at the initial census on the probability of a seedling surviving to flowering. Logistic regression was used to test for effects of infection severity and plant age on the probability of a seedling surviving between any two consecutive census dates. I determined the best-fit model through iterative reduction of the full model, which contained all single-factor effects and all possible interaction terms. I sequentially dropped the nonsignificant effect of the highest order and reran the reduced model until all remaining terms were significant at the $P \leq 0.05$ level (Kleinbaum 1994). For the analysis I pooled data from both populations because plants growing in the three permanent quadrats in population 6 were predominantly healthy (74%) or lightly infected (23%). Pooling data allowed for the inclusion of plants representing all levels of infection severity in a single analysis.

I used a t test to compare flower bud production of diseased and healthy plants and an ANCOVA to quantify the effects of plant height, longest branch length, and infection severity on seed capsule production. Fecundity data (flower bud and seed capsule production) were $\log_{10}(x + 1)$-transformed to meet the assumption of normality. Because t test results indicated that the two study populations in which these data were collected did not differ significantly in production of flower buds ($t = -1.12$, df = 248, $P = 0.13$) or seed capsules ($t = -0.35$, df = 220, $P = 0.36$), I pooled results across sites for analyses. For the ANCOVA, height and branch length were $\log_{10}$-transformed to meet the assumption of normality, and because these two parameters covaried (linear regression: $r^2_{adj} = 0.60$, MS = 28.56, $F_{1,221} = 332.21$, $P < 0.0001$), the residuals of the regression of $\log_{10}$ branch length vs. $\log_{10}$ height were used in place of $\log_{10}$ branch length. The best-fit model was identified using the approach described for the analysis of seedling mortality.

I used linear regression of disease prevalence in year$_t$ vs. change in mean host density between years (year$_{t+1}$ − year$_t$) to test for effects of infection on host demography. I included data from all survey plots for which there were consecutive measurement years (n = 105 plot/year combinations).

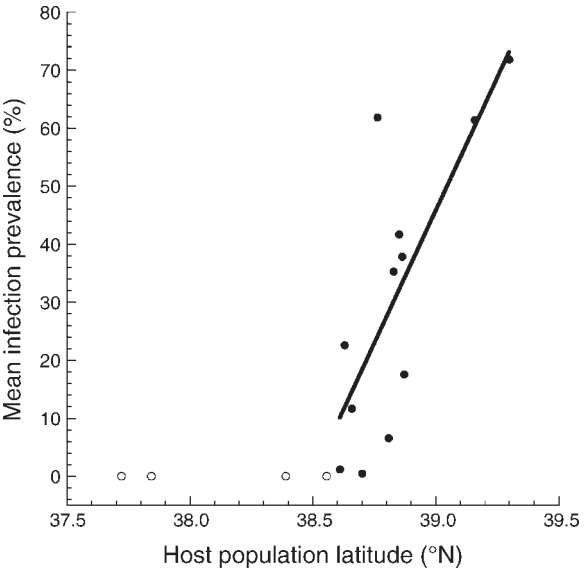

FIG. 1. Latitudinal patterns of mean pathogen prevalence calculated by averaging data for each host population across survey years. Latitude was taken from the survey plot closest to the geographic center of each population. Regression lines fit only to data collected in host populations where infection was observed at least once (solid circles); $r^2_{adj} = 0.54$, MS = 3939.71, $F_{1,11} = 13.92$, $P = 0.0039$ (mean prevalence = −3523.75 + [91.52 × latitude]).

RESULTS

Epidemiological surveys.—There was evidence of a latitudinal cline in fungal infection, with northern host populations associated with significantly higher levels of disease prevalence ($r^2_{adj} = 0.54$, MS = 3939.71, $F_{1,11} = 13.92$, $P = 0.0039$) compared to populations at lower latitudes (Fig. 1; Appendix B). Infection was never observed in the four southernmost host populations during either formal epidemiological surveys or one to two annual pre-survey visits to assess the stage of plant development. This clinal pattern was significant in all years except 2004, when infection at the five northernmost sites was lower than expected. Massive infection associated with a rust outbreak at those sites in 2002 (mean prevalence = 82%, mean severity = 52%) reduced average host density (plants per square meter) from 13.1 to 0.37 the following year. Depending on population recovery rates, very few plants were present at these sites for one or two subsequent years. A latitudinal cline in infection severity was also observed ($r^2_{adj} = 0.66$, MS = 1953.44, $F_{1,11} = 22.34$, $P = 0.0008$) and was likely

attributable to strong positive correlation between the two disease measures (severity = 5.82 + (0.31 × prevalence) + 0.0087(prevalence − 27.88)^2; $r^2_{adj} = 0.90$, MS = 27 706.9, $F_{2, 118} = 548.45$, $P < 0.0001$ based on regression using all survey plot/year combinations involving host populations where disease was documented at least once). There was no similar latitudinal trend in host density, or altitudinal trends in the abundance of either host or pathogen.

While measures of pathogen abundance showed considerable interannual variation (Fig. 2), rust infection was a regular feature of the life history of *H. californicum*. In the 12 populations where disease was observed at least once, infection prevalence and severity were 27.9% ± 3.1% and 12.9% ± 2.1%, respectively (mean ± SE, $n = 119$ survey plot/year means). When disease was actually present in a patch a given year ($n = 85$), mean prevalence and severity increased to 39.0% ± 3.8% and 18.1% ± 2.7%, respectively, and 35% of the cases had prevalence values exceeding 50%. Host density (Fig. 2) and abundance also fluctuated between years. Average host population size, estimated as the product of survey plot density and area summed across all plots within each population, was 15 959 ± 8021 (mean ± SE) in 2001, 28 521 ± 11 060 in 2002, 4326 ± 2017 in 2003, and 14 512 ± 5391 in 2004. Average host density, measured at the level of survey plot, was 7.7 ± 0.55 plants/m^2 (mean ± SE, $n = 145$ plot/year means). Density fluctuations were also high among years. For example, mean plant density in six of the study populations was reduced by >95% following a year of severe infection (minimum prevalence = 50%, mean = 81% among those sites).

Factors driving infection dynamics.—The solution of the NMDS analysis of soil data showed study populations distributed along a continuous edaphic gradient (Appendix C). Stress of the 2D plot was 0.03 in 499 of the 500 restarts. SIMPER analysis indicated that of the 17 focal analytes the two with the largest influence on this solution were calcium (mean ± SD, percentage contribution to solution = 39.42% ± 9.39%) and magnesium (magnesium = 39.76% ± 6.40%). Low-calcium and high-magnesium concentrations that are stressful to plants are universally recognized as defining features of serpentine soils (Brooks 1987).

Irrespective of the temporal period over which weather data were averaged, there was not a significant relationship between infection prevalence and any of the seven meteorological summary variables: mean daily deviation from 18°C, mean daily deviation from 7°C, mean daily deviation from 50% RH, and percentage of daily measurements for which (1) RH ≥ 90%, (2) RH ≥ 95%, (3) RH ≥ 100%, and (4) temperature < dew point. There were no significant relationships or discernable weather trends by latitude. Higher altitude sites had a significantly greater proportion of daily temperature measurements below dew point in 2004 ($r^2_{adj} = 0.58$, MS = 14.25, $F_{1,8} = 12.04$, $P = 0.01$) but this pattern was not significant in 2003 ($P = 0.14$), and while there was a trend in both years for these sites to also have lower mean temperatures, the pattern was not significant in either year ($P = 0.07$ in 2003, $P = 0.13$ in 2004).

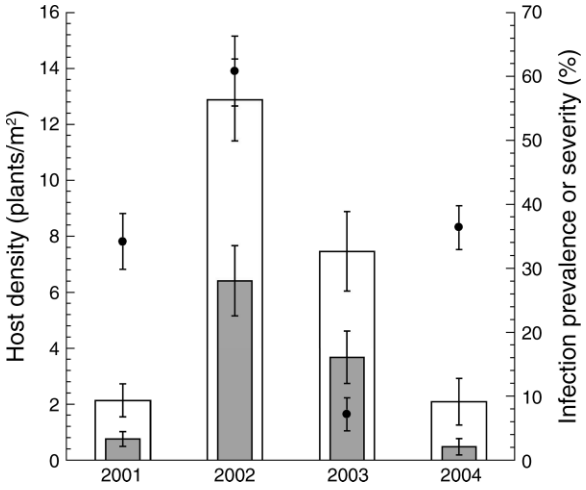

FIG. 2. Changes in host density and pathogen prevalence and severity across survey years. Annual means were calculated by averaging all plot/year means within years. Mean fungal prevalence and severity, represented by open and gray bars, respectively, was calculated using only populations where infection was observed at least once. The severity of infection is calculated as the percentage of photosynthetic tissue covered with rust pustules. Mean density of plants is plotted as solid circles. Error bars denote ±SE. The overall pattern was unchanged when annual means were calculated using only data collected in populations surveyed in 2001.

Infection prevalence was significantly greater among plants growing at higher latitudes and altitudes and in soils with lower calcium concentrations, and disease was significantly more prevalent in plots with a higher plant abundance and density in both the current and previous year (Table 1). Results were qualitatively identical when analyses were performed using infection severity as the response variable.

Fitness consequences of infection.—Relative to uninfected individuals or those with light infection, seedlings that were heavily infected early in the growing season had a greatly reduced probability of surviving to reproductive age (Kaplan Meyer log rank $\chi^2 = 186$, df = 3, $P < 0.0001$; Appendix D) and to the next census interval ($\chi^2 = 408.84$, df = 6, $P < 0.0001$, Fig. 3A). Concordance of the best-fit logistic regression model of seedling survival, which included the factors infection severity (log rank $\chi^2 = 380.81$, df = 3, $P < 0.0001$) and plant age (log rank $\chi^2 = 13.77$, df = 3, $P = 0.0032$), was 86.7% (2357 of 2720 observations correctly predicted). The severity by age interaction term was nonsignificant (log rank $\chi^2 = 14.26$, df = 9, $P = 0.11$) and was dropped from the model. The average seedling mortality rate of moderate to severely infected hosts (>30% of photosynthetic tissue covered with rust pustules) was roughly six times the rate among healthy conspecifics (6.7% vs.

TABLE 1. Results of standard least-squares analysis of infection dynamics of California dwarf flax, *Hesperolinon californicum*, by the pathogenic rust fungus *Melampsora lini* in California, USA.

Variable	Coefficient	*F*	*P*
Mean plant density per quad, previous year (square-root)	0.29	77.81	<0.0001
Mean plant density per quad (square-root)	0.23	41.26	<0.0001
Latitude	2.26	23.65	<0.0001
Soil calcium concentration ($\log_{10}$)	−1.09	15.72	0.0002
Altitude	8.4×10^{-4}	9.80	0.0025
Residuals of fourth-root mean no. plants estimated†	0.087	8.92	0.039
Residuals of fourth-root mean no. plants estimated, previous year‡	0.057	4.10	0.047
Soil magnesium concentration	7.3×10^{-5}	1.28	0.26
Prevalence, previous year (arcsine fourth-root)	−0.020	0.027	0.87

Notes: The response variable was arcsine fourth-root of infection prevalence. Analyses were based on all patch/year combinations for which data for all nine explanatory variables were available. Data transformations are indicated parenthetically. Model $r^2_{adj} = 0.57$, MS = 1.55, $F_{9,80} = 12.93$, $P < 0.0001$.

† Residuals from regression of square-root mean plant density per patch vs. fourth-root mean number of plants estimated.

‡ Residuals from regression of square-root mean plant density per patch in previous year vs. fourth-root mean number of plants estimated in previous year.

38.7%, $\chi^2 = 304.67$, df = 1, $P < 0.0001$, $n = 2063$ total observations of 852 plants).

On average, infected plants ($n = 100$) had fewer than one-half as many flower buds as uninfected plants ($n = 150$) ($t = 8.66$, df = 248, $P < 0.0001$, mean ± SE = 7.17 ± 1.08 vs. 15.09 ± 1.35), and the production of viable seed capsules declined significantly with increasing infection severity ($r^2_{adj} = 0.52$, MS = 37.54, $F_{1,221} = 239.93$, $P < 0.0001$, Fig. 3B). Relative to healthy individuals, plants with >30% of photosynthetic tissue covered with pustules produced on average almost one-eighth the number of viable seed capsules (mean ± SE, 21.0 ± 1.8 vs. 2.8 ± 0.94, $F_{1,138} = 76.29$, $P < 0.0001$).

There was a significant tendency for host density to decrease when infection prevalence in the previous year was high ($r^2_{adj} = 0.15$, MS = 1870.85, $F_{1,104} = 19.84$, $P < 0.0001$, Fig. 3C).

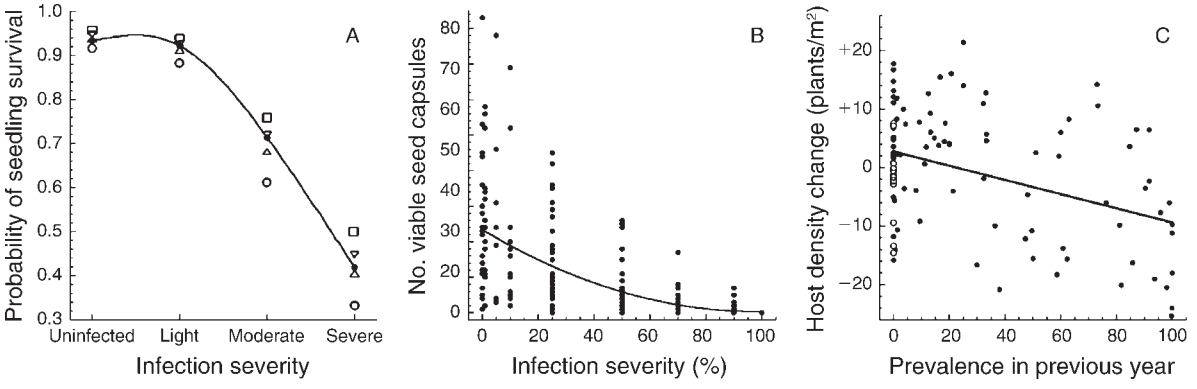

FIG. 3. Effects of rust infection on (A) host seedling survival, (B) host seed capsule production, and (C) interannual changes in host population density. (A) Predictions of best-fit logistic regression model of seedling survival to next census interval relative to infection severity and seedling age. Solid circles represent observed survival probabilities for each of the four infection severity classes averaged across all census days. Open symbols denote predicted survival probability for plants age 42 days (inverted triangle), 28 days (square), 14 days (triangle), and at initial census (circle). The smooth curve, generated from spline fit to model ($\lambda = 1$, $r^2 = 0.972$) represents the average across all census days; status at next interval = 2.40 − 0.37 × (infection severity [light–uninfected]) − 1.57 × (infection severity [moderate–light]) − 1.15 × (infection severity [severe–moderate]) + 0.31 × (age [14–0 days]) + 0.38 × (age [28–14 days]) − 0.21 × (age [42–28 days]). (B) Regression of mean number of viable seed capsules produced vs. infection severity ($r^2_{adj} = 0.31$, MS = 8064.82, $F_{2,221} = 51.27$, $P < 0.0001$; no. viable seed capsules = 19.57 − (0.28 × infection severity) + 0.0024 × [infection severity − 40.04]^2). Viable seed capsules (mean ± SD) for the nine infection categories, in order of increasing infection severity, were 21.03 ± 19.35, 27.86 ± 17.74, 23.67 ± 25.28, 16.25 ± 18.74, 13.91 ± 12.05, 5.63 ± 7.19, 1.86 ± 3.31, 0.77 ± 1.70. Data were collected in 2002 from host populations 5 and 6. (C) Linear regression of changes in host density vs. rust prevalence in the previous year. All survey plot/year combinations for which there were consecutive measurement years are shown, and populations where disease was never observed are plotted as open circles. Density change (plants/0.25 m^2) was calculated as interannual difference in mean no. plants per quadrat in each survey plot ($r^2_{adj} = 0.15$, MS = 1870.85, $F_{1,104} = 19.84$, $P < 0.0001$; host density change = 2.74 − 0.12 × [prevalence previous year]).

Discussion

Annual disease surveys revealed a latitudinal cline in infection: northern *H. californicum* populations were associated with significantly higher levels of means rust prevalence relative to southern populations, where infection occurred less frequently or was never observed. This pattern was relatively consistent across survey years despite considerable temporal variation in the abundance of both host and pathogen. Equally notable were the high rates of disease in locations where rust infection was documented at least once. When averaged across survey years, one-quarter of host patches in this geographic range had mean prevalence values exceeding 50%, and almost one-fifth had values >70%. In survey patches where the fungus was present in a given year, an average of two in five plants were infected, and sick plants lost ~30% of their photosynthetic tissue to symptom-associated damage.

The prevalence of rust infection was significantly higher among plants growing in lower calcium soils. This result substantiates findings of Springer et al. (2007), who documented this relationship experimentally in the greenhouse, and contradicts the general prediction that antagonistic species interactions should occur with lower frequency and/or intensity in harsh, low-quality environments. Interestingly, results from the annual epidemiological surveys indicated that neither host abundance nor density were higher in high-calcium soils, so the demographic mechanism underlying the predictions of Thrall and colleagues (2007) (high-quality environment → higher host abundance/density → more frequency/virulent infection by pathogens) does not appear to be involved in this edaphic effect. Instead, lower soil calcium levels may have deleterious effects on the physiology of individual host plants that make them more vulnerable to infection, perhaps by compromising immuno-defense mechanisms. Calcium is known to play a fundamental role in the signal transduction pathways involved in plant recognition of and defensive responses to attacking pathogens (reviewed in Springer et al. 2007) and the application of calcium to soils has been shown to reduce disease caused by a diverse group of plant pathogens from the genera *Aspergillus*, *Erwinia*, *Fusarium*, *Plasmodiophora*, *Pseudomonas*, *Pythium*, *Rhizoctonia*, *Sclerotium*, and *Verticillium* (reviewed in Engelhard [1989]). In their laboratory experiment Springer et al. (2007) found a significant positive correlation between calcium concentrations of *H. californicum* leaf tissue and the soil in which plants were grown. Thus, it appears that host plant biochemistry is altered in low-calcium soils in a manner that increases vulnerability to pathogen infection. Such an effect may be common when stressful environmental conditions adversely influence host physiology and could modulate the frequency and outcome of host–symbiont interactions in a direction opposite that predicted by Thrall et al. (2007).

The significant relationships between disease prevalence and the number and density of hosts are consistent with prevailing conceptual models of plant disease transmission: the probability of colonization of local host patches by wind-dispersed pathogens should be positively correlated with patch size (number of plants) (Burdon et al. 1989), and the rate and extent of subsequent spread should exhibit positive density dependence (Burdon and Chilvers 1982). Less intuitive were the significant correlations between disease and host abundance and density in the previous year but the lack of a similar association between prevalence in consecutive years. Perhaps higher numbers or densities of host plants create conditions that are favorable for rust spore overwintering, for example by modifying local microclimates. Regression analyses involving data from the meteorological sensors indicated that the significant effect of altitude could be weather related. Sensors at higher elevation sites tended to record cooler temperatures and a greater proportion of measurements for which temperature was below dew point. These results are indicative of cooler, wetter conditions that should promote fungal proliferation by increasing the germination rate, survivorship of rust spores, and the number of pathogen generations per year (Hart 1926, Misra 1952).

Findings of a second greenhouse inoculation experiment involving *H. californicum* and *M. lini* suggest that the latitudinal pattern of disease prevalence may be driven largely by the distribution of pathogen resistance genes among host populations. Genetic resistance to fungal infection was found to exhibit a latitudinal cline similar to the prevalence cline reported here, with low resistance in northern *H. californicum* populations increasing gradually toward the south (Springer 2007). Regression analyses indicated that ~60% of variation in disease prevalence was explained by host resistance structure. Examination of meteorological data collected in the present study did not reveal any large-scale latitudinal patterns in weather conditions known to affect fungal growth, and there was also no significant latitudinal gradient in soil calcium concentrations. North/south winds could have contributed to patterns of fungal prevalence through directional dispersal of fungal spores. While wind measurements recorded by a sensor in the northern part of the study area (CDEC Knoxville Creek weather station near host population 4) indicate relatively strong, regular north northeast winds with slightly weaker and less frequent southwest winds in this location between December and June of 2001–2004 (Western Regional Climate Center, data *available online*),[2] the lack of similar information from other study sites makes it difficult to speculate on the influence of wind on broadscale patterns of fungal distribution. Thus, given available information, it appears that epidemiological patterns in the *H. californicum*/*M. lini* system may be driven largely by a combination of host genetics, density, and edaphic quality. Interannual

[2] ⟨http://www.wrcc.dri.edu⟩

fluctuations in disease levels no doubt reflect sensitivity of the pathogen to variable environmental conditions, but the relative temporal consistency of the underlying prevalence cline suggests a more deterministic influence likely exerted by traits of host populations (e.g., demography, resistance structure) and the edaphic environment. Longer-term studies are needed to assess the temporal stability of this spatial pattern of rust prevalence and to quantify changes in the effects of different biotic and environmental mechanisms on it through time.

Results of the fitness surveys indicate that *M. lini* represents a potent selective force for evolution of *H. californicum*. Costs of infection were pronounced, and evidence from annual surveys suggests that disease-related reductions in host survivorship and fecundity may have rapid and measurable demographic consequences for host populations. When compared to healthy conspecifics, moderate to severely infected hosts (>30% of photosynthetic tissue covered with rust pustules) had almost a sixfold greater rate of mortality as seedlings and produced almost one-eighth the number of viable seed capsules as adults. Diseased hosts produced fewer than one-half of the number of flower buds compared to healthy individuals. High costs of infection have been documented for a variety of plant diseases (reviewed in Burdon 1987, Gilbert 2002), but reductions in fitness caused by lesion-forming pathogens such as rusts tend to be difficult to quantify because they are the result of the cumulative effects of many small lesions over extended periods of times (Burdon 1991). In a controlled outdoor garden experiment, Jarosz and Burdon (1992) found that *M. lini* infection of the flax *L. marginale* caused reductions in within-season host survivorship and fecundity comparable in magnitude to those presented here. They attributed this outcome to the timing of experimental infection that, in contrast to disease cycles in the wild, occurred earlier in the growing season and prior to host plant flowering. This timing factor may be especially important for *H. californicum* and the numerous other serpentine annuals with similar phenological traits. *H. californicum* seeds germinate in midwinter but grow very slowly, probably as a result of low temperatures and nutritive deficiencies of serpentine soils (Sharsmith 1961). During these cool, wet months conditions are highly favorable for the proliferation of fungal and bacterial pathogens, and *M. lini* infection begins to develop when host plants are only a few centimeters tall. The loss of even a small percentage of photosynthetic tissue at this ontogenetic stage is often lethal, and the host plants that survive these early infections often lack sufficient resources for reproduction when spring arrives. The annual life cycle of the host means that the consequences of these reproductive failures for host population dynamics manifest rapidly, as evidenced by the significant negative relationship between host density and disease prevalence in the previous year.

When compared to results of studies focused on biologically similar "model" plant–pathogen interactions (*Valeriana salina–Uromyces valerianae* [Ericson et al. 1999], *Filipendula ulmaria–Triphragmium ulmariae* [Smith et al. 2003], *Linum marginale–M.lini* [Burdon and Jarosz 1991, Jarosz and Burdon 1991], *Plantago lanceolata–Podosphaera plantaginis* [Laine and Hanski 2006]), a number of intriguing differences are apparent. First, while spatial patterns of prevalence are correlated to similar factors in all of the interactions (e.g., host abundance, density, disease status of nearby populations), each of the model systems is characterized by multiple disease foci with a patchy spatial distribution of disease at the regional scale. In contrast, the *H. californicum–M. lini* system presented a unidirectional, clinal pattern of infection prevalence across the host's range. While marked differences in prevalence levels among patches within larger *H. californicum* populations were often observed, population-level differences appeared more gradual, linear, and continuous. Second, infection dynamics of the model systems are often characterized as having a metapopulation signature. Dramatic bottlenecks of fungal populations at the end of each host growing season cause frequent local extinction of pathogens, and passive wind dispersal of pathogen spores during the subsequent year results in spatially random recolonization events. These factors promote temporal consistency of prevalence levels at the regional scale but large and often erratic fluctuations in prevalence at the scale of local host populations (reviewed in Burdon and Thrall 1999). The reverse appears to be true in the *H. californicum–M. lini* system. Over the four-year period prevalence levels averaged across all study sites showed large fluctuations, but the rank order of prevalence rates among *H. californicum* populations remained relatively constant. The clinal pattern of infection was significant in all years except 2004, when prevalence was lower than expected in northern host populations following one to two years of extremely high disease pressure. Examination of the circumstances surrounding this result suggests the final notable difference among these systems. In the four model systems, the hosts are perennials and local fluctuations in pathogen abundance (e.g., severe population bottlenecks, local extinctions) regularly occur in association with seasonal changes in the availability of host tissue related to plant senescence and winter dormancy. While such ontogenetic cycles should be even more pronounced in *H. californicum* because of its annual life history, disease-induced host mortality appears to represent an equally important cause of pathogen bottlenecks, pathogen local extinctions, and prevalence fluctuations in the *H. californicum–M. lini* system. Among the 40 host patches surveyed, 18 patches (45%) experienced local extinction of rust for at least one season. These extinctions occurred following a massive rust epidemic in 2002: rust prevalence averaged 62% across all 18 patches and 95% across the nine most heavily infected

patches. In the following year, average plant density in these 18 patches, and average estimated number of plants per patch, were reduced by 94% and 99%, respectively. In the nine most heavily infected patches, both measures were reduced by 99%. These dramatic reductions in *H. californicum* numbers likely constrained the ability of *M. lini* to colonize and spread in the one to two years following the epidemic, resulting in localized extinctions in these host patches and lower levels of infection at nearby sites. While it is not clear whether the patterns characterized in this relatively short study are indicative of longer-term dynamics, the documented strong reciprocal fitness effects and measurable demographic feedbacks, together with the short lifespan of the host, suggest that coevolution between *H. californicum* and *M. lini* could be much stronger, faster, and more tightly linked compared to systems with longer lived hosts.

Acknowledgments

Many thanks to P. Aigner, J. Callizo, D. Jech, C. Koehler, N. McCarten, and S. Harrison for their help in the field, and to R. Franks and colleagues at the UC Davis ANR analytical lab for assistance with soil analyses. Comments from K. Andonian, M. Carr, S. Forde, G. Gilbert, I. Parker, P. Raimondi, and four anonymous reviewers significantly improved the manuscript. I thank M. Carr and G. Gilbert for their mentoring, encouragement, and patience during the course of this research. Financial support was provided by grants from the California Native Plant Society, the National Garden Clubs of America, and Sigma Xi, an NSF Graduate Research Training Fellowship, and co-funding from the UCSC Department of Ecology and Evolutionary Biology and the Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO). Fieldwork was performed in part at the University of California Natural Reserve System's McLaughlin Reserve.

Literature Cited

Baker, A. J. M., J. Proctor, and R. D. Reeves, editors. 1991. The vegetation of ultramafic (serpentine) soils. Proceedings of the First International Conference on Serpentine Ecology (University of California, Davis, 19–22 June 1991). Intercept Limited, Andover, UK.

Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology and Evolution 9:191–193.

Bertness, M. D., and G. H. Leonard. 1997. The role of positive interactions in communities: lessons from intertidal habitats. Ecology 78:1976–1989.

Brady, K. U., A. R. Kruckeberg, and H. D. Bradshaw. 2005. Evolutionary ecology of plant adaptation to serpentine soils. Annual Review of Ecology and Systematics 36:243–266.

Brooker, R. W., et al. 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96: 18–34.

Brooks, R. R. 1987. Serpentine and its vegetation: a multidisciplinary approach. Dioscorides Press, Portland, Oregon, USA.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119–125.

Bryant, J. P., P. J. Kuropat, S. M. Cooper, K. Frisby, and N. Owensmith. 1989. Resource availability hypothesis of plant antiherbivore defense tested in a South-African savanna ecosystem. Nature 340:227–229.

Burdon, J. J. 1987. Diseases and plant population biology. Cambridge University Press, Cambridge, UK.

Burdon, J. J. 1991. Fungal pathogens as selective forces in plant populations and communities. Australian Journal of Ecology 16:423–432.

Burdon, J. J., and G. A. Chilvers. 1982. Host density as a factor in plant disease ecology. Annual Review of Phytopathology 20:143–166.

Burdon, J. J., and A. M. Jarosz. 1991. Host–pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: I. patterns of resistance and racial variation in a large host population. Evolution 45:205–217.

Burdon, J. J., A. M. Jarosz, and G. C. Kirby. 1989. Pattern and patchiness in plant–pathogen interactions—causes and consequences. Annual Review of Ecology and Systematics 20: 119–136.

Burdon, J. J., and P. H. Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. American Naturalist 153:S15–S33.

Callaway, R. M., et al. 2002. Positive interactions among alpine plants increase with stress. Nature 417:844–848.

Choler, P., R. Michalet, and R. M. Callaway. 2001. Facilitation and competition on gradients in alpine plant communities. Ecology 82:3295–3308.

Clarke, K. R. 1993. Nonparametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117–143.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895–899.

Engelhard, A. W., editor. 1989. Soilborne plant pathogens: management of diseases with macro- and microelements. APS Press, St. Paul, Minnesota, USA.

Ericson, L., J. J. Burdon, and W. J. Muller. 1999. Spatial and temporal dynamics of epidemics of the rust fungus *Uromyces valerianae* on populations of its host *Valeriana salina*. Journal of Ecology 87:649–658.

Flor, H. H. 1954. Flax rust. Pages 152–161 *in* A. G. Norman, editor. Advances in Agronomy. Academic Press, New York, New York, USA.

Gilbert, G. S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annual Review of Phytopathology 40: 13–43.

Gold, R. E., and G. D. Statler. 1983. Telium formation and teliospore germination in *Melampsora lini*. Canadian Journal of Botany 61:308–318.

Grime, J. P. 1977. Evidence for existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169–1194.

Hart, H. 1926. Factors affecting the development of flax rust, *Melampsora lini* (Pers.) Lev. Phytopathology 16:185–205.

James, C. 1971. A manual of assessment keys for plant disease. American Phytopathological Society, St. Paul, Minnesota, USA.

Jarosz, A. M., and J. J. Burdon. 1991. Host–pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: II. Local and regional variation in patterns of resistance and racial structure. Evolution 45:1618–1627.

Jarosz, A. M., and J. J. Burdon. 1992. Host–pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: III. Influence of pathogen epidemics on host survivorship and flower production. Oecologia 89:53–61.

Kleinbaum, D. 1994. Logistic regression: a self-learning text. Springer-Verlag, New York, New York, USA.

Kruckeberg, A. 1954. The ecology of serpentine soils: III. Plant species in relation to serpentine soils. Ecology 35:267–274.

Kruckeberg, A. R. 1967. Ecotypic response to ultramafic soils by some plant species of northwestern United States. Brittonia 19:133–151.

Laine, A. L., and I. Hanski. 2006. Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. Journal of Ecology 94:217–226.

Lawrence, G. J., P. N. Dodds, and J. G. Ellis. 2007. Rust of flax and linseed caused by *Melampsora lini*. Molecular Plant Pathology 8:349–364.

Lee, E. T., and J. W. Wang. 2003. Statistical methods for survival data analysis. Third edition. Wiley, New York, New York, USA.
Littlefield, L. J. 1981. Biology of the plant rusts: an introduction. Iowa State University Press, Ames, Iowa, USA.
Maestre, F. T., and J. Cortina. 2004. Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. Proceedings of the Royal Society of London B 271:S331–S333.
McCarten, N. 1993. Linaceae—flax family. Pages 738–740 *in* J. C. Hickman, editor. The Jepson manual: higher plants of California. University of California Press, Berkeley, California, USA.
Mehlich, A., and G. P. Tewari. 1974. The roles of gypsum (calcium sulfate) in agriculture. United States Gypsum Company, Chemical Division, Chicago, Illinois, USA.
Miller, R. E., and I. E. Woodrow. 2008. Resource availability and the abundance of an N-based defense in Australian tropical rain forests. Ecology 89:1503–1509.
Misra, A. P. 1952. Factors affecting variability in the development of *Melampsora lini*. Phytopathology 42.
Proctor, J., and S. Woodell. 1975. The ecology of serpentine soils. Pages 255–366 *in* A. Macfadyen, editor. Advances in Ecological Research. Academic Press, London, UK.
Roberts, B. A., and J. Proctor, editors. 1992. The ecology of areas with serpentinized rocks. Kluwer Academic, Dordrecht, The Netherlands.
Sharsmith, H. K. 1961. The genus *Hesperolinon* (Linaceae). Pages 235–314 *in* University of California Publications in Botany. University of California, Berkeley, California, USA.
Smith, D. L., L. Ericson, and J. J. Burdon. 2003. Epidemiological patterns at multiple spatial scales: an 11-year study of a *Triphragmium ulmariae–Filipendula ulmaria* metapopulation. Journal of Ecology 91:890–903.
Springer, Y. P. 2007. Clinal resistance structure and pathogen local adaptation in a serpentine flax–flax rust interaction. Evolution 61:1812–1822.
Springer, Y. P., B. A. Hardcastle, and G. S. Gilbert. 2007. Soil calcium and plant disease in serpentine ecosystems: a test of the pathogen refuge hypothesis. Oecologia 151:10–21.
Thompson, J. N. 1999. The evolution of species interactions. Science 284:2116–2118.
Thompson, J. N. 2005. The geographic mosaic of coevolution. University of Chicago Press, Chicago, Illinois, USA.
Thrall, P. H., M. E. Hochberg, J. J. Burdon, and J. D. Bever. 2007. Coevolution of symbiotic mutualists and parasites in a community context. Trends in Ecology and Evolution 22: 120–126.
Travis, J. M. J., R. W. Brooker, E. J. Clark, and C. Dytham. 2006. The distribution of positive and negative species interactions across environmental gradients on a dual-lattice model. Journal of Theoretical Biology 241:896–902.
Travis, J. M. J., R. W. Brooker, and C. Dytham. 2005. The interplay of positive and negative species interactions across an environmental gradient: insights from an individual-based simulation model. Biology Letters 1:5–8.
Vlamis, J., and H. Jenny. 1948. Calcium deficiency in serpentine soils as revealed by absorbent technique. Science 107:549–551.
Walker, R. B. 1954. The ecology of serpentine soils. II. Factors affecting plant growth on serpentine soils. Ecology 35:259–266.

APPENDIX A

Map showing locations and identification numbers of the 16 *Hesperolinon californicum* study populations in California, USA (*Ecological Archives* E090-128-A1).

APPENDIX B

Geographic and demographic data for host and pathogen by population and survey year (*Ecological Archives* E090-128-A2).

APPENDIX C

Results of NMDS analysis of all soil samples from the 16 study populations and bubble plots showing the influence of calcium and magnesium on NMDS resolution (*Ecological Archives* E090-128-A3).

APPENDIX D

Kaplan Meyer survival analysis of seedling survivorship probabilities relative to severity of infection at time of initial survey (*Ecological Archives* E090-128-A4).