

WHEN THERE IS NO ESCAPE: THE EFFECTS OF NATURAL ENEMIES ON NATIVE, INVASIVE, AND NONINVASIVE PLANTS

INGRID M. PARKER^{1,3} AND GREGORY S. GILBERT²

¹*Ecology and Evolutionary Biology, 1156 High Street, EEB/EMS, University of California, Santa Cruz, California 95064 USA*

²*Environmental Studies, 1156 High Street, 405 ISB, University of California, Santa Cruz, California 95064 USA*

Abstract. An important question in the study of biological invasions is the degree to which successful invasion can be explained by release from control by natural enemies. Natural enemies dominate explanations of two alternate phenomena: that most introduced plants fail to establish viable populations (biotic resistance hypothesis) and that some introduced plants become noxious invaders (natural enemies hypothesis). We used a suite of 18 phylogenetically related native and nonnative clovers (*Trifolium* and *Medicago*) and the foliar pathogens and invertebrate herbivores that attack them to answer two questions. Do native species suffer greater attack by natural enemies relative to introduced species at the same site? Are some introduced species excluded from native plant communities because they are susceptible to local natural enemies? We address these questions using three lines of evidence: (1) the frequency of attack and composition of fungal pathogens and herbivores for each clover species in four years of common garden experiments, as well as susceptibility to inoculation with a common pathogen; (2) the degree of leaf damage suffered by each species in common garden experiments; and (3) fitness effects estimated using correlative approaches and pathogen removal experiments. Introduced species showed no evidence of escape from pathogens, being equivalent to native species as a group in terms of infection levels, susceptibility, disease prevalence, disease severity (with more severe damage on introduced species in one year), the influence of disease on mortality, and the effect of fungicide treatment on mortality and biomass. In contrast, invertebrate herbivores caused more damage on native species in two years, although the influence of herbivore attack on mortality did not differ between native and introduced species. Within introduced species, the predictions of the biotic resistance hypothesis were not supported: the most invasive species showed greater infection, greater prevalence and severity of disease, greater prevalence of herbivory, and greater effects of fungicide on biomass and were indistinguishable from noninvasive introduced species in all other respects. Therefore, although herbivores preferred native over introduced species, escape from pest pressure cannot be used to explain why some introduced clovers are common invaders in coastal prairie while others are not.

Key words: *biotic resistance; clovers; endophyte; enemy release hypothesis; exotic weed; foliar disease; fungal plant pathogen; herbivory; Medicago; natural enemies hypothesis; Stemphylium; Trifolium.*

INTRODUCTION

When introduced into new regions, most plant species either fail to establish or persist solely in anthropogenic environments such as roadsides or agricultural fields (reviewed in Mack 1995, Williamson 1996, Colautti et al. 2004). On the other hand, a few highly successful exotics invade native communities in the new range, reach high densities, and displace native species. These few weedy invaders represent one of our most challenging environmental problems (Vitousek et al. 1996, Parker et al. 1999, Mack et al. 2000). What distinguishes the vast majority of failed invasions from the few successful ones has been a central question in invasion ecology since its beginning (Elton 1958, Baker and

Stebbins 1965). One line of inquiry focuses on distinguishing the successful invaders based on life history traits, genome size, or other features (Rejmánek and Richardson 1996, Reichard and Hamilton 1997). These approaches emphasize invaders as a definable group and support the view that, to some degree, invasions are generalizable and predictable based on inherent traits of the invading organism. A second line of inquiry focuses instead on ecological interactions as drivers of invasion success. Perhaps most prominent in this line is the idea that escape from natural enemies plays a pervasive role in invasion by introduced species (Darwin 1859, Gillett 1962, Crawley 1987, Maron and Vilà 2001, Keane and Crawley 2002). This idea forms the justification for classical biological control, in which natural enemies are brought from the native range to control weedy invaders (Huffaker and Messenger 1976, DeBach and Rosen 1991). Despite its central importance, until very recently there have been few rigorous empirical tests of this idea

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³ E-mail: parker@biology.ucsc.edu

(Maron and Vilà 2001). Not only is it unresolved whether invasive introductions differ from noninvasive introductions, it remains unclear how often native plant populations themselves are controlled by natural enemies and whether introduced species can be distinguished as a group by the nature of their interactions with consumers (Colautti et al. 2004).

Cross-continental comparisons of plants in their introduced vs. native ranges have tended to show a reduction in the diversity of herbivores or pathogens or the amount of damage they cause in the new range (Waloff 1966, Wolfe 2002, Mitchell and Power 2003, Genton et al. 2005, Vilà et al. 2005) or in the demographic impact of enemies (Reinhart et al. 2003, Callaway et al. 2004, DeWalt et al. 2004; but see Beckstead and Parker 2003). Such biogeographic comparisons are necessary to explain a difference in behavior of the species (increased plant vigor, population growth, or competitive ability) when it is in its native context vs. when it is an invader (Hierro et al. 2005). However, insofar as plant communities are structured by competition, the ecological effect of natural enemies is driven by the relative effect of those enemies on native vs. introduced hosts (Keane and Crawley 2002). Some studies with herbivores have shown greater negative effects on introduced hosts, whereas other studies have found greater effects on natives (reviewed in Keane and Crawley 2002, Colautti et al. 2004, Parker and Hay 2005). Surprisingly few such comparative studies have been conducted looking at pathogen effects on native and introduced hosts (Blaney and Kotanen 2001, Goergen and Daehler 2001, Agrawal et al. 2005), also showing mixed results. Rather than experiencing release from natural enemies, introduced species may be more susceptible to local natural enemies because they have not evolved resistance mechanisms or because they arrive with a smaller complement of genetic diversity at resistance loci (Colautti et al. 2004, Parker and Gilbert 2004).

Instead of treating introduced species as a homogeneous group, one might focus instead on the fact that the great majority of plant introductions do not result in successful invasions or high-impact weeds (Lockwood et al. 2001). Studies using subjective designations of impact suggest that noxious species may suffer less herbivory or disease than more innocuous species (Mitchell and Power 2003, Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005). Post hoc statistical models designed to differentiate between failed and successful introductions using life history characteristics more readily predict which species failed than which species were successful invaders (Reichard and Hamilton 1997). One possible reason that some introductions fail when they "should" succeed is that native herbivores or pathogens colonize exotic plants and eliminate them before they can establish a viable population (the biotic resistance hypothesis; Elton 1958, Simberloff 1986, Mack 1996, Maron and Vilà 2001).

Surprisingly few studies have directly tested this hypothesis that local pathogens and herbivores may prevent invasions into particular habitats (Gilbert and Parker 2005). Native pathogens have had large impacts on introduced plants in examples from horticulture, forestry, and agriculture (Mack 1996, Wingfield et al. 2001), but we know very little about where and when species introductions fail in natural systems. While we may never be able to track the initial failures of one-time introductions, we can approach the problem in another way by focusing on explaining local rather than global patterns. For introduced species present at a local site, does susceptibility to natural enemies explain distribution patterns? That is, do local pathogens and herbivores determine which introduced species invade natural habitats and which are constrained to disturbed, anthropogenic habitats?

In this study we use a suite of phylogenetically related native and nonnative species and the large assemblage of foliar fungal pathogens and invertebrate herbivores that attack them to answer two questions. Do introduced species escape the effects of natural enemies relative to native species in the same site? Is there greater pest pressure on noninvasive than invasive introduced species, contributing to the exclusion of noninvasive species from a native community? We divide the predictions of the theories of natural enemy escape and biotic resistance into three parts: the diversity and incidence of enemies, the damage they cause, and their effects on plant fitness.

METHODS

Study site and focal species

Field studies were done at the University of California's Bodega Marine Reserve (BMR), a 147-ha protected area in Sonoma County, California, USA (38°19' N, 123°04' W). Conditions at the Reserve are wet and cool in winter (January mean rainfall is 103 mm and mean daily temperature is 9°C), and dry but foggy and cool in summer (August mean rainfall is 1.5 mm and mean daily temperature is 11.7°C; Bodega Ocean Observing Node, University of California, Davis, California, USA). All experiments were performed across an expanse of coastal prairie bordered on one side by a cliff 40 m above sea level overlooking the Pacific Ocean. The site contains no major anthropogenic disturbances, is ~300 m from the nearest road, and has not been grazed since 1961 (Barbour et al. 1973). The site is directly exposed to wind and moist air coming off the ocean. Because of coastal fog during the growing season, plants at this site experience many hours per day of free water on leaf surfaces, leading to high rates of fungal infection (Bradley et al. 2003).

The BMR supports sympatric populations of 10 native species and eight exotic species of clover (*Trifolium* spp., Fabaceae) and burclover (*Medicago* spp., Fabaceae), hereafter collectively referred to as "clovers." All but two species are annuals, and they

TABLE 1. Characteristics of clover species (*Trifolium* and *Medicago*) at Bodega Marine Reserve, California, USA.

Species	Origin	Annual/ perennial	Earliest reported date†	Frequency (% of plots)		Annual mean dry biomass (g)
				In prairie	Along roads	
<i>M. arabica</i> (L.) Huds.	introduced	A	1831 ²	14.9	27.5	0.104–1.882
<i>M. lupulina</i> L.	introduced	A	1890 ²	0	0	0.011–0.069
<i>M. polymorpha</i> L.	introduced	A	<1790 ¹	23.4	58.8	0.137–0.659
<i>T. campestre</i> Schreb.	introduced	A	1894 ²	0	25.5	0.012–0.095
<i>T. dubium</i> Sibth.	introduced	A	1896 ²	0.7	70.6	0.011–0.174
<i>T. glomeratum</i> L.	introduced	A	1927 ²	0	47.1	0.007–0.169
<i>T. repens</i> L.	introduced	P	1888 ²	0	0	0.006–0.062
<i>T. subterraneum</i> L.	introduced	A	1921 ²	0	51.0	0.058–0.498
<i>T. barbigerum</i> Torr.	native	A	1866 ²	66.0	23.5	0.113–0.161
<i>T. bifidum</i> A. Gray	native	A	1862 ²	22.7	0	0.124–0.250
<i>T. fucatum</i> Lindl.	native	A	1862 ²	8.5	0	0.301–1.325
<i>T. gracilentum</i> Torr. & A. Gray	native	A	1862 ²	46.1	23.5	0.125–0.348
<i>T. macraei</i> Hook. & Arn.	native	A	1878 ²	44.7	0	0.123–0.402
<i>T. microcephalum</i> Pursh	native	A	1880 ²	2.1	0	0.072–0.346
<i>T. microdon</i> Hook. & Arn.	native	A	1862 ²	77.3	5.9	0.146–0.370
<i>T. variegatum</i> Nutt.	native	A	1863 ²	0	0	0.202
<i>T. willdenovii</i> Spreng.	native	A	1862 ²	6.4	9.0	0.218–0.373
<i>T. wormsjkoldii</i> Lehm.	native	P	1863 ²	0	0	0.054–0.098

Notes: The two most invasive species appear in boldface type. The earliest California records for each species are the earliest dates found in herbarium collections or referenced in early California floras 1880–1936 (first records of native species are provided for comparison). Frequencies were generated from 141 prairie and 51 roadside plots that supported at least one clover species (of a 2003 survey of 355 prairie plots and 60 roadside plots total). Biomass data (dry mass) are the smallest and largest annual means (averaged across all individuals within a year) across four years of common garden experiments.

† Earliest reported date in California. Sources: 1, Mabberley (1997); 2, UC Berkeley Jepson Herbarium.

range in size (dry mass) over two orders of magnitude (Table 1). Some exotic clovers were first reported in California as much as 200 years ago and others as recently as the 1920s. In the BMR, two of the exotic species (*M. polymorpha* and *M. arabica*) are invasive and have become common components of the coastal prairie, whereas the other species are either confined to anthropogenic habitats or found only occasionally in natural habitats. From 2001 to 2003 we did systematic surveys of the distribution and abundance of all clover species at BMR, across 355 4-m² plots in the prairie habitat and 60 plots along roadsides. The size and local density of clovers has varied from year to year, but their distribution has been fairly constant at this scale (I. M. Parker and G. S. Gilbert, unpublished data). In 2003, *M. polymorpha* was found in 23% and *M. arabica* in 15% of all prairie plots with clovers; they were the only introduced species with a substantial presence in the native prairie habitat and were more abundant there than half of the native species (Table 1).

We attempted to include as many as possible of the system's potentially important and discriminating natural enemies. We studied all foliar fungi that could be cultured from living leaves. Extensive studies of damping-off pathogens and preliminary surveys for viruses suggested that these interactions are not important drivers in the system (data not shown). We included all invertebrate herbivores and distinguished three principal types of herbivore damage: thrips damage caused by the introduced *Apterothrips apteris*, scraping and shredding caused by unidentified lepidopteran and/or coleopteran larvae, and snail herbivory caused by the native *Helminthoglypta arrosa*. Although the introduced snail

Helix aspersa is also present at BMR, extensive diurnal and nocturnal surveys showed that only *Helminthoglypta* was present at our experimental site. Gopher damage by burial and digging was frequent, but there was no evidence that gopher impacts differed among different clover species. Comparison between whole experiments that were either exposed to deer or protected from them suggested that deer did not exert substantial herbivore pressure on clovers. Florivores and seed predators could not be studied because of the risk of releasing nonnative seeds into the site. However, observations during the collection and handling of thousands of seeds of each species suggested negligible levels of seed predation except in the case of one native species in one out of five years.

Overview of common garden experiments

In pilot surveys of fungal infection and leaf damage on naturally occurring clovers at BMR, we noted significantly greater infection and disease development on native than on introduced host species (data not shown). However, because most native species were found in wetter prairie areas and most nonnative species were found in drier, disturbed sites where disease pressure is lower (e.g., Bradley et al. 2003), observational survey data could not be used to evaluate differences in susceptibility. Therefore, common garden experiments were undertaken to equalize environmental conditions and exposure to pathogens and herbivores for all the host species.

We conducted common garden experiments each growing season over four years (November–May of 1997–1998 to 2000–2001) to assess differences among

native and exotic clovers in levels of fungal infection, damage caused by pathogens and herbivores, and fitness effects of pathogens and herbivores. The experiments included all available clover species in each year (up to 18 spp.), but due to seed limitation, species varied slightly from year to year (Appendix A). Seeds of each species were collected from wild populations at BMR, from within the experimental site whenever possible, bulking collections from many individuals. Seeds were germinated on filter paper, then planted into non-sterilized field soil in Conetainers (2.5 × 16.5 cm; Stuewe and Sons, Corvallis, Oregon, USA). Soil was collected from <15 cm depth at many points across the experimental site and homogenized. Seedlings were grown in the BMR greenhouse, with overhead misting but without artificial heat or light, and were transplanted to the field when they had at least one true leaf (at 7–9 weeks). Sample sizes were 38, 34, 80, and 12 individuals per species in years 1–4, respectively. In different years, the experiments were blocked or completely randomized, and plants were spaced from 1 to 5 m apart; details of each experimental layout are provided in Appendix A. Most of the native clover species were common within the experimental site, and the addition of our individuals had a minimum effect on the local density of host plants. Plants grew until the start of flowering but were harvested before setting seed to ensure that no introduced species were inadvertently released into the coastal prairie. We used plant biomass as a fitness correlate. Within similar coastal prairie habitat in 1998, biomass was highly correlated with seed number ($r = 0.75–0.87$) and total seed mass ($r = 0.75–0.81$) for three species chosen to span the range of morphologies (*M. polymorpha*, *T. dubium*, and *T. macrei*).

Weather conditions varied substantially among the years (Bodega Ocean Observing Node, University of California, Davis, California, USA). Year 1 was an extremely wet, El Niño year (November–June rainfall = 1730 mm); year 2 was wet (1150 mm), year 3 was close to the annual mean (680 mm; mean, 787 mm), and year 4 was very dry (400 mm).

Diversity of natural enemies and levels of infection

In each year of the common garden experiment, we recorded herbivores and their damage both in the field and after harvest by inspecting every leaf under a stereoscope. In addition, close to the time of harvest, we isolated fungi from the leaves of every plant. Leaves were surface-sterilized and placed on growth media (see Appendix B for details); fungi were grown to sporulation and identified when possible. A total of 1178 fungal collections were identified to genus, species, or morpho-species.

Identifications were done using morphological characteristics combined with nuclear rDNA sequences (internal transcribed spacers [ITS] 1 and 2) of representative strains to identify fungal morphotypes to species (Appendix B). To determine whether our common

fungal species are native to North America, historically cosmopolitan, or probably native to Europe, we combined phylogenetic information with records in the USDA fungus host distributions database and the USDA plant fungus database on published studies (Farr et al. 2006). We also did widespread surveys of foliar fungi from clovers in southern Europe over two years to characterize the suite of common pathogens from the native range of our introduced species (Appendix B).

We assessed mean levels of total fungal infection for each host species. In addition to overall fungal infection, we were able to reliably detect the five most common fungal genera (*Stemphylium*, *Cladosporium*, *Alternaria*, *Colletotrichum*, and *Leptosphaerulina*) by visual identification. We compared the proportion of plants infected by fungi overall and by each focal fungal taxon for native vs. introduced species and for invasive vs. noninvasive introduced species. Transformations were not done because they did not alter the variance structure or the results. For all analyses, we used unpaired *t* tests assuming unequal variances with species as the replicate.

Susceptibility to experimental inoculation

We used the most common clover pathogen (*Stemphylium* sp.) in a controlled inoculation experiment to measure both the ability to infect and the tendency to cause disease on native and introduced clover hosts. Ten individuals per species (eight native species and eight introduced) were grown in a growth chamber on a 12-h day/night cycle at 18°C for ~1 mo (1–6 true leaves, with 67% of plants having 2–4 leaves). Five plants per species were dip-inoculated using a solution of mitospores from a strain of *Stemphylium* collected from BMR (with five water controls). After inoculation, plants were incubated for two days inside plastic tents to keep the humidity high, and then tents were opened. After 11–13 d, all leaves were assessed for senescence, chlorosis, necrotic spots, and necrotic flecking, and 1–5 leaves (both symptomatic and asymptomatic) per plant were surface-sterilized and plated on malt extract agar. Fungal growth was assessed every several days until no further growth was seen.

Disease symptoms and herbivore attack

For the common garden experiments, we did two types of damage assessments: a prevalence census and a severity census. In the prevalence census we recorded presence/absence of disease symptoms and herbivory on all plants, closely inspecting each leaf on every plant using a hand lens, taking steps not to transmit fungal spores between plants. In years 1 and 2, prevalence censuses were done every two weeks throughout the growing season (seven times in year 1 and nine in year 2); blocks were assessed in a different random order every time. The severity census recorded the percentage of leaf tissue affected by damage symptoms, estimated visually using categories of percentage of area (0%, 1%,

5%, 30%, 50%, 70%, 90%, 100%). Because of the time-consuming nature of this census, only a subset of plants (7–14 per species) was used to collect severity data. Five severity censuses were done in year 1 and three in year 2.

At harvest, symptom assessments were done for each plant in the laboratory using a stereoscope, allowing careful confirmation of the disease symptoms and types of herbivory assigned in the field. Isolations from symptomatic leaves and experimental inoculations revealed that the same symptom (mostly different patterns of necrosis, chlorosis, and discoloration) may be associated with several different fungi, and the same fungal species may be associated with a range of symptoms both among host species and within the same host. Therefore we focused on damage type rather than on particular pathogens. We also combined forms of herbivory to simplify the results, although separate analyses on thrips, snail, and lepidopteran/coleopteran herbivory revealed remarkable congruity with the patterns presented here (data not shown). We found no relationship between the percentage of leaf damage per plant for disease and for herbivory (e.g., year 1 data, $R^2 = 0.008$, $N = 221$, $P = 0.19$), suggesting that herbivores do not drive disease dynamics.

For years 1 and 2, prevalence census data (proportion of individuals showing symptoms) were aggregated over time using area under the disease or damage progress curve (AUDPC; Campbell and Madden 1990). The advantage of AUDPC over a single prevalence measure is that it more accurately reflects the total effect of disease or damage throughout the season. An AUDPC score was calculated for each species for disease and herbivory. For years 3 and 4, disease and herbivory assessments were done only at the final harvest date. Each species was treated as a replicate in an unpaired t test assuming unequal variances to compare either mean AUDPC score or final prevalence for native vs. introduced species and invasive vs. noninvasive species. Severity census data for disease and herbivory were analyzed using blocked, repeated-measures ANOVA, with species nested within origin (native vs. introduced) or invasiveness (invasive vs. noninvasive).

Effects of natural enemies on host fitness: correlative and experimental tests

The effects of pathogens and herbivores on host fitness were assessed in a number of ways. For the two years in which we followed plant mortality over multiple censuses (years 1 and 2), we used logistic regression models to test whether individuals showing disease or herbivory symptoms were more likely to suffer mortality in any two-week period. A separate nominal logistic regression was performed (JMP 5.1.2; SAS Institute, Cary, North Carolina, USA) for each plant species in each year, including only the main effects of herbivory, disease, and plant size (number of leaves). The independent variables herbivory and disease were nominal values of yes or no at time t , number of leaves

at time t was a continuous variable, and the dependent nominal value was alive or dead at census $t + 1$. Variables for which the estimate of the coefficient was unstable were excluded from further analysis (JMP 5.1.2; SAS Institute). Within each year, we then used a t test assuming unequal variances to compare the mean coefficients of introduced and native species and invasive and noninvasive introduced species considering each species the statistical unit.

Finally, we experimentally assessed the fitness effects of fungal infection using fungal removal experiments in two years. We reduced fungal infection using Daconil (Syngenta, Greensboro, North Carolina, USA) in year 3 and Dithane F45 (Dow AgroSciences, Indianapolis, Indiana, USA) in year 4. Fungicides were mixed according to manufacturer's directions with a nonionic surfactant (Tween 80 [Fisher Chemicals, Pittsburgh, Pennsylvania, USA] or Rainguard), with distilled water and surfactant as a control. Plants were sprayed weekly with a handheld or backpack sprayer until each leaf was fully wet. Leaves were sprayed precisely to avoid the surrounding vegetation. Separate greenhouse experiments demonstrated no phytotoxic effect of either Daconil or Dithane F45 on clover growth (Appendix C). We also tested for a toxic effect of the foliar fungicide on mycorrhizal infection levels and found no difference between fungicide and control plants (Appendix C).

At harvest, we noted all disease symptoms and herbivory damage and for six pairs of plants per species, we assessed the percentage of leaf damage per symptom as above. We isolated fungi from one leaflet from each of 20–29 plants per treatment per species in year 3 (802 total isolations) and from three leaflets from each of 5–6 plants per treatment per species in year 4 (520 total isolations). Shoots and roots were separated; roots were washed and used to measure biomass in year 3 and mycorrhizal colonization in year 4. Samples were dried in a 60°C oven and weighed. We compared pre-flowering mortality for fungicide and control treatments paired within species. We tested for differences in the effect of fungicide on the proportion of dead (control – fungicide) between native and introduced species and between invasive and noninvasive species using an unpaired t test assuming unequal variances (transformation was not needed). We compared fungal infection, disease symptoms, herbivory, and final biomass for surviving fungicide and control plants. For year 3, control and fungicide plants were paired, and the difference between log-transformed biomass ($\ln(\text{fungicide}) - \ln(\text{control})$) for each pair was used in a nested ANOVA with species nested within origin. For year 4, plants were not paired, and overall means of the (log-transformed) biomass of fungicide and control plants were used to generate a difference value per species; we then tested for effects of origin or invasiveness using t tests assuming unequal variances. For year 3, analyses of aboveground biomass, belowground biomass, and total

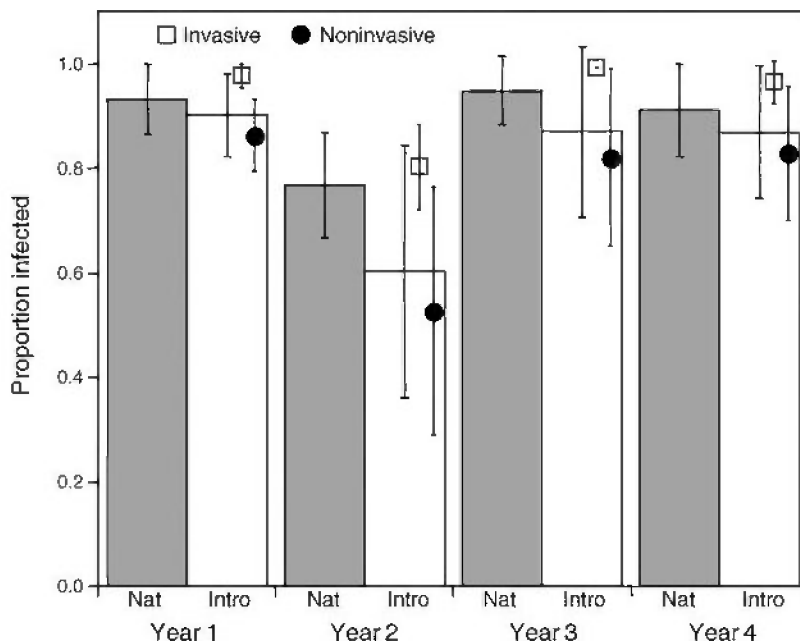


FIG. 1. Proportion of plants infected with foliar fungi (mean \pm SD) in four common garden experiments using sympatric native and nonnative clovers (species are replicates) at Bodega Marine Reserve, California, USA. Native species (Nat) are not significantly different from introduced species (Intro) in any year. Invasive introduced species (open squares) are not significantly different from noninvasive introduced species (solid circles) in any year. For statistics, see Appendix D.

biomass produced similar results; only aboveground biomass results are presented here.

RESULTS

Diversity of natural enemies and levels of infection

Fungal infection was common and involved more than a dozen fungal morphospecies; however, five genera (*Stemphylium* [64%], *Cladosporium* [16%], *Alternaria* [12%], *Colletotrichum* [2.8%], and *Leptosphaerulina* [1.3%]) made up >95% of all infections identified. Comparing sequence data to our collections from Europe, USDA databases, and published phylogenies, we found that these foliar pathogens represent a mixture of cosmopolitan species and California native species absent from Europe (see Appendix B for details). Although it is extremely difficult to separate historically cosmopolitan species from recent introductions, none of our common species groups fall clearly into the latter category. The *Stemphylium* group is made up of two species, *S. solani* and a species allied to the Câmara et al. (2002) complex Group E; one is clearly a native California species and the other is either native or cryptogenically cosmopolitan. *Cladosporium cladosporioides* is cosmopolitan and known from a wide range of hosts in North America and Europe. The *Alternaria* group comprises two species, *A. alternata* and *A. infectoria*, both of which are known from many hosts on both continents. Although never reported from European clovers in the literature, we commonly found *A. alternata* in our own European collections (data not

shown). *Colletotrichum trifolii* is widespread in the United States but rare in Europe. *Leptosphaerulina trifolii* is widespread on numerous host species in the United States and apparently absent from Europe. All five common fungal taxa were associated with all clover host species. Any fungal taxa at our site that may have shown a narrow host range were rare.

In four years of common garden experiments, we found no significant differences between native and nonnative species for the degree to which leaves were infected by fungi (Fig. 1; Appendix D). For the introduced species, there were no significant differences between invasive and noninvasive species (all $P > 0.07$; Appendix D). The trend was for the two most invasive species to show the highest levels of infection (Fig. 1). Patterns were consistent and overall infection levels were high (60–95%) in all years despite large differences in weather patterns.

We assessed relative infection for the five common fungal taxa for each year with sufficient sample size (Table 2). Of 17 possible comparisons between native and nonnative clover species, one showed significantly more infection on the native species (*Stemphylium* year 2) and one showed significantly more infection on the introduced species (*Colletotrichum* year 1). Of 16 possible comparisons between invasive and noninvasive introduced species, three showed significantly more infection on the most invasive species (*Stemphylium* year 4 and *Alternaria* years 2 and 3). Taken together, patterns of infection by all fungi as a group and by five separate taxa do not suggest either that natives are

TABLE 2. Proportion (mean with SD in parentheses) of plants infected by each of four fungal genera, for native vs. introduced species and for invasive introduced vs. noninvasive introduced species, in four years of common garden experiments.

Species and sample year	Native (SD)	Introduced (SD)	<i>t</i>	df	<i>P</i>	Invasive (SD)	Noninvasive (SD)	<i>t</i>	df	<i>P</i>
A) <i>Stemphylium</i> spp.										
Year 1	0.86 (0.16)	0.80 (0.21)	0.61	14	0.55	0.94 (0.09)	0.74 (0.23)	1.11	5	0.32
Year 2	0.69 (0.12)	0.44 (0.22)	2.91	15	0.01	0.68 (0.11)	0.40 (0.20)	1.78	5	0.14
Year 3	0.84 (0.15)	0.76 (0.23)	0.70	13	0.50	1.00 (0.00)	0.68 (0.21)	2.06	6	0.08
Year 4	0.80 (0.12)	0.70 (0.16)	1.58	15	0.14	0.88 (0.08)	0.64 (0.13)	2.50	6	0.047
B) <i>Cladosporium cladosporioides</i>										
Year 1	0.10 (0.13)	0.06 (0.10)	0.76	14	0.46	0.00 (0.0)	0.08 (0.11)	0.98	5	0.37
Year 2	0.13 (0.10)	0.11 (0.10)	0.41	15	0.69	0.18 (0.12)	0.11 (0.08)	1.01	5	0.36
Year 3	0.22 (0.10)	0.12 (0.09)	2.07	13	0.06	0.21 (0.07)	0.09 (0.08)	1.70	6	0.14
Year 4	0.17 (0.16)	0.24 (0.18)	0.91	15	0.38	0.21 (0.12)	0.25 (0.20)	0.26	6	0.80
C) <i>Alternaria</i> spp.										
Year 1	0.05 (0.08)	0.08 (0.11)	0.64	14	0.53	0.10 (0.14)	0.08 (0.11)	0.21	5	0.84
Year 2	0.07 (0.08)	0.05 (0.07)	0.56	15	0.58	0.15 (0.07)	0.01 (0.03)	4.08	5	0.01
Year 3	0.17 (0.02)	0.10 (0.10)	1.67	13	0.12	0.22 (0.15)	0.06 (0.05)	2.68	6	0.04
Year 4	0.25 (0.14)	0.24 (0.19)	0.04	15	0.97	0.30 (0.24)	0.23 (0.19)	0.44	6	0.68
D) <i>Colletotrichum trifolii</i>										
Year 1	0.02 (0.06)	0.16 (0.14)	2.32	12	0.04	0.16 (0.05)	0.16 (0.17)	0.03	5	0.98
Year 3	0.04 (0.04)	0.07 (0.06)	1.08	13	0.30	0.06 (0.03)	0.08 (0.07)	0.40	6	0.70
Year 4	0.02 (0.03)	0.02 (0.03)	0.01	15	0.99	0.00 (0.0)	0.02 (0.03)	0.87	6	0.42
E) <i>Leptosphaerulina trifolii</i>										
Year 1	0.124 (0.131)	0.049 (0.085)	1.26	14	0.23	0.100 (0.141)	0.028 (0.064)	0.69	1.2	0.60
Year 2	0.011 (0.020)	0 (0)	1.52	7	0.17	0 (0)	0 (0)			

Note: Data were insufficient for analysis for *Colletotrichum trifolii* in year 2 and for *Leptosphaerulina trifolii* in years 3 and 4.

preferentially infected or that the most invasive species escape pathogen attack.

All three types of herbivore damage, associated with snails, thrips, and lepidopteran/coleopteran larvae, were seen on every species. Pea aphids (*Acyrtosiphon pisum*) were also observed occasionally on every species. In summary, there was no evidence in this system of a group of host-specific natural enemies that preferentially attack native species.

Susceptibility to experimental inoculation

We used controlled inoculations in the laboratory to measure susceptibility to *Stemphylium*, the most common foliar pathogen, which appears to be a native California species complex (see Appendix B). Of 79 plants inoculated with *Stemphylium*, 75 yielded at least one infected leaf; only one control plant out of 80 was infected. The proportion of leaves per plant yielding cultures is a measure of the ability of the pathogen to enter the leaf. The mean percentage of leaves infected varied from 38% to 93% among native hosts and from 43% to 100% among introduced hosts. Native and introduced hosts were not significantly different in terms of infection levels, although there was a trend for greater infection in introduced hosts ($59.8\% \pm 17.1\%$ vs. $77.3\% \pm 21.6\%$ [mean \pm SD]; $t = 1.80$, $df = 13$, $P = 0.096$). Invasive and noninvasive introduced hosts did not have significantly different levels of infection ($79.2\% \pm 17.7\%$ vs. $76.7\% \pm 24.2\%$; $t = 0.16$, $df = 2$, $P = 0.89$).

Although asymptomatic leaves were as likely as symptomatic leaves to yield *Stemphylium* cultures

(82.6% vs. 82.3%), inoculated leaves were much more likely to show disease symptoms than were uninoculated leaves (42.0% vs. 2.1%), and necrotic spots and necrotic flecking only appeared on inoculated leaves (26.1% and 4.3%, respectively). The mean percentage of leaves showing disease symptoms varied from 26.7% to 55.0% among native hosts and 13.3% to 93.3% among introduced hosts. Native and introduced hosts were not significantly different for disease symptom expression ($37.0\% \pm 10.1\%$ vs. $52.3\% \pm 22.8\%$; $t = 1.73$, $df = 10$, $P = 0.11$). Similarly, invasive and noninvasive hosts were not significantly different ($56.7\% \pm 14.1\%$ vs. $50.8\% \pm 26.0\%$; $t = 0.40$, $df = 4$, $P = 0.71$).

Disease symptoms and herbivore attack

We measured the impact of disease and herbivory by prevalence measures (i.e., proportion of individuals showing symptoms or damage) and also by severity measures (percentage of leaf tissue damaged). Considerable variation in disease pressure was seen among host species. In contrast, overall means were consistent from year to year despite highly variable weather patterns (Figs. 2 and 3).

Native and introduced species did not differ for the prevalence of disease symptoms in the population measured at the end of the season (years 3 and 4) or for cumulative effect of the disease through time (years 1 and 2; Fig. 2, all $P > 0.25$; Appendix D). For severity of disease damage per individual, introduced species showed significantly higher damage levels than native species in year 1 ($P = 0.0001$) but not in any other year

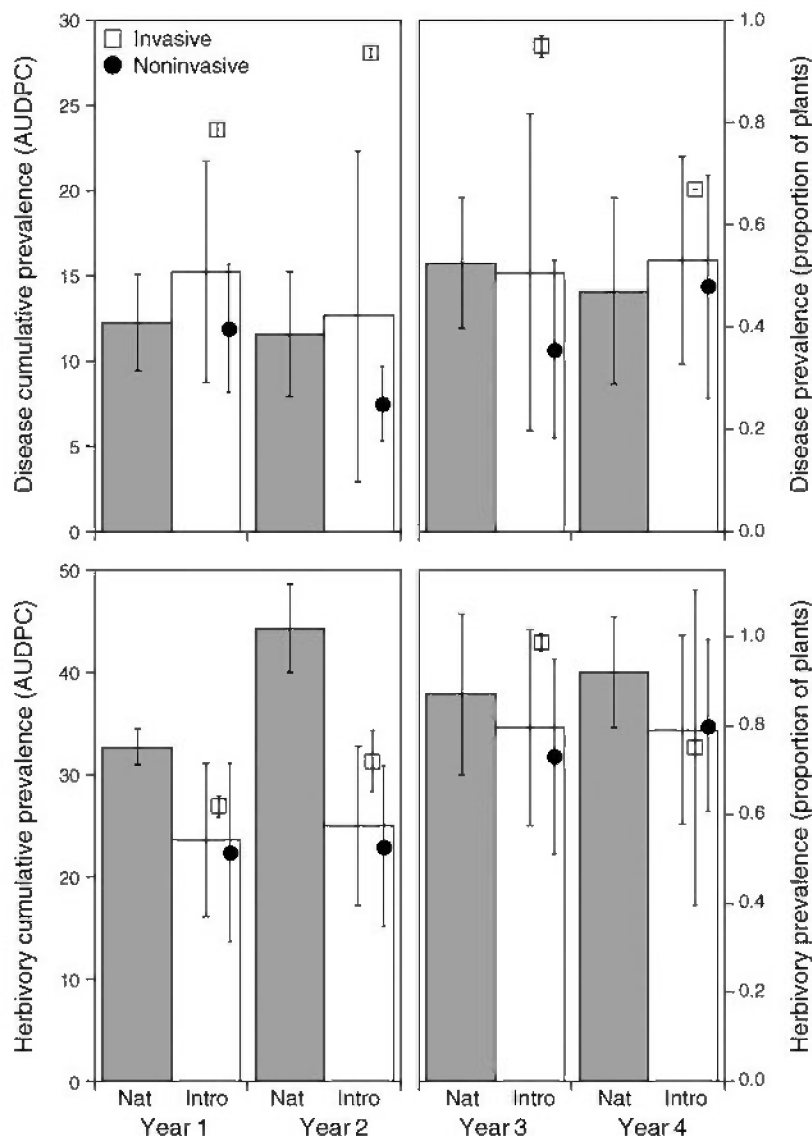


FIG. 2. Prevalence of disease and herbivory (mean \pm SD) for native species (Nat) vs. introduced species (Intro) and for invasive introduced species (open squares) vs. noninvasive introduced species (solid circles). Years 1 and 2 show an integrated measure of cumulative incidence (area under the disease [damage] progress curve, AUDPC), and years 3 and 4 show prevalence at harvest only. For statistics, see Appendix D. Replication is at the level of the species.

(Fig. 3, Appendix D). In addition, significant variation among species was found within native and introduced groups in year 1 ($P = 0.0001$) and year 2 ($P = 0.008$). There was a significant effect of sampling date in year 2 ($P = 0.017$).

Leaf damage from herbivores was greater on native than introduced species in two years. Prevalence of damage in the population was significantly higher for native species (Fig. 2) when measured cumulatively in years 1 ($P < 0.0001$) and 2 ($P = 0.016$), but not when measured only at harvest in years 3 and 4 ($P > 0.14$; Appendix D). For severity of herbivory per individual, native species showed higher damage levels than introduced species (Fig. 3) in year 1 ($P = 0.0001$) and

year 2 ($P = 0.0006$), but not at harvest in years 3 or 4 ($P > 0.20$). In addition, there was a significant effect of species within origin in year 1 ($P = 0.0001$) and year 3 ($P = 0.0036$) but not years 2 or 4 ($P > 0.26$). There was no significant effect of sampling date or interaction between date and origin.

The most invasive introduced species (*M. polymorpha* and *M. arabica*) never showed less damage from pathogens than did the noninvasive introduced species. In fact, all patterns went the opposite direction (Figs. 2 and 3). Disease symptoms were significantly or marginally significantly more prevalent on the invaders in all years ($P < 0.00001$ to $P = 0.094$; Appendix D). Severity of disease was also significantly higher on the invaders in

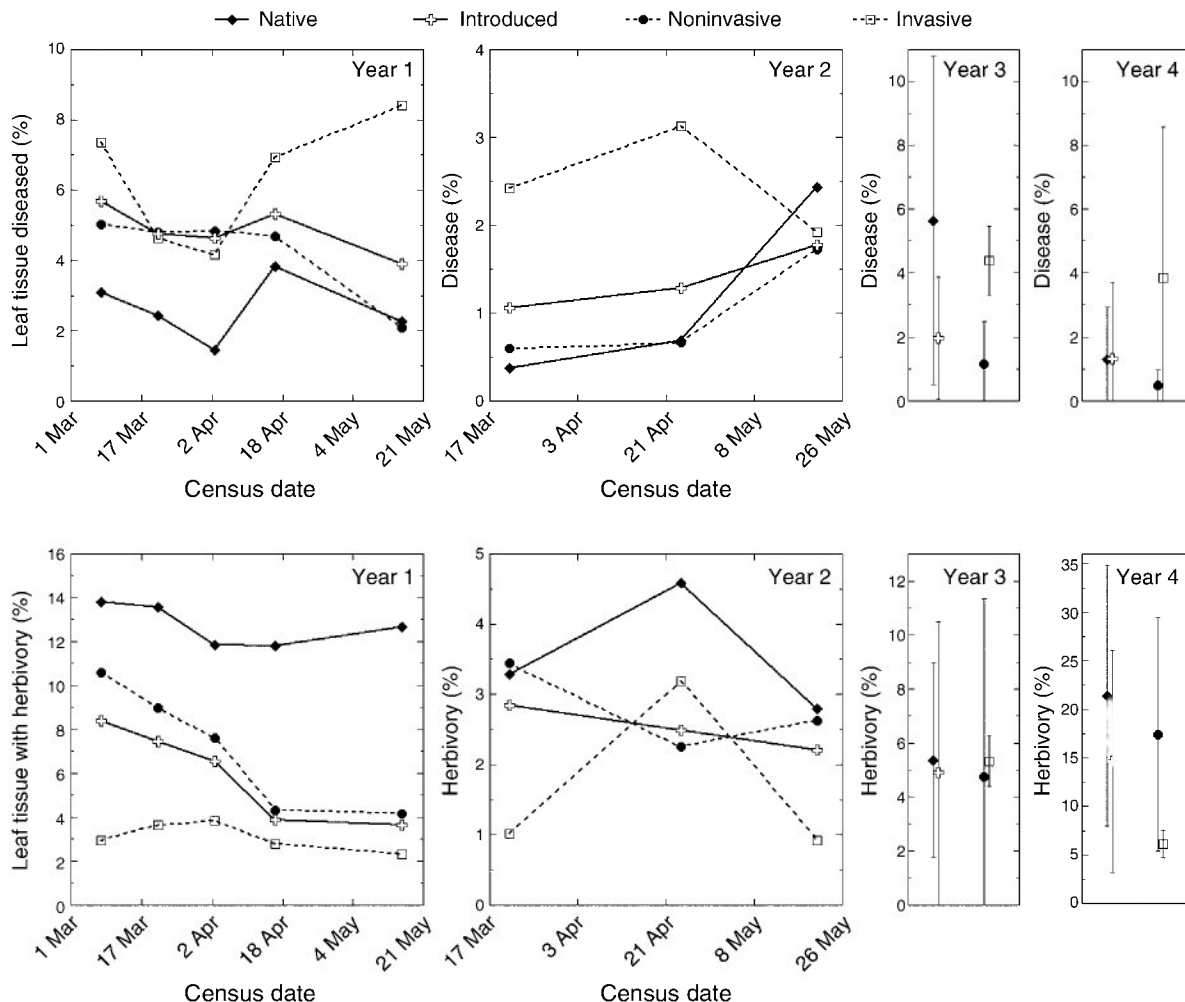


FIG. 3. Severity of damage (percentage of leaf tissue) over time for disease symptoms and herbivory in native vs. introduced species and in invasive vs. noninvasive introduced species. Values indicate the means of the mean damage per species. Years 3 and 4 show damage at time of harvest (mean \pm SD). Introduced species had significantly more disease than natives in year 1 and significantly less herbivory in years 1 and 2. Invasive species had significantly more disease than noninvasive species in all years, and herbivory was never significantly different. For statistics and the error terms for years 1 and 2, see Appendix D.

all years ($P < 0.0001$ to $P = 0.036$; Appendix D). A significant effect of species nested within origin was found only in year 1 ($P = 0.0009$). There was no significant effect of sampling date.

Similarly, damage from herbivores tended to be more prevalent on the most invasive introduced species (Fig. 2), although this was only significant in year 3 ($P = 0.037$) and marginally significant in year 2 ($P = 0.083$; Appendix D). Severity of damage was not significantly different between invasive and noninvasive species in any year ($P > 0.08$; Appendix D). There was a significant effect of species nested within invasiveness for year 1 ($P = 0.0001$) and year 3 ($P = 0.0069$), no significant effect of sampling date, and a marginally significant interaction between sampling date and invasiveness in year 2 ($P = 0.051$).

As a whole, patterns of damage suggest that native and introduced clovers are equally susceptible to

pathogens and equally likely to express disease symptoms, but that herbivores at this site appear to prefer native species, at least in some years. However, escape from herbivory cannot be used as an explanation for invasion in this system because herbivores showed no preference for those introduced species that were not invasive over species that were invasive. In fact, both herbivory and disease damage were more prevalent in the most aggressive invaders.

Effects of natural enemies on fitness: correlative and experimental tests

For years 1 and 2, we used logistic regression models to test for effects of disease and herbivory on the semi-weekly probability of mortality. These effects were generally weak, although the comparisons that were significant or marginally significant always showed a positive association between mortality and damage

TABLE 3. Coefficients (mean \pm SD) from logistic regression models of the effects of disease, herbivory, and leaf number on the probability of mortality of clover plants in two-week census intervals.

A) Origin					
Independent variable	Native	Introduced	<i>t</i>	df	<i>P</i>
Year 1					
Disease	0.149 \pm 0.319	0.119 \pm 0.427	0.14	6.6	0.89
Herbivory	-0.048 \pm 0.261	0.014 \pm 0.387	0.32	6.1	0.76
Leaves	-0.411 \pm 0.347	-0.515 \pm 0.372	0.51	7.9	0.62
Year 2					
Disease	0.285 \pm 0.272	0.195 \pm 0.330	0.57	11.7	0.57
Herbivory	0.236 \pm 0.297	0.139 \pm 0.358	0.56	11.8	0.58
Leaves	-0.063 \pm 0.036	-0.223 \pm 0.246	1.71	6.2	0.14
B) Invasiveness, year 2					
Independent variable	Invasive	Noninvasive	<i>t</i>	df	<i>P</i>
Disease	-0.066 \pm 0.340	0.299 \pm 0.295	1.33	1.65	0.34
Herbivory	0.178 \pm 0.564	0.124 \pm 0.334	0.13	1.29	0.91
Leaves	-0.113 \pm 0.048	-0.268 \pm 0.286	1.17	4.49	0.30

Notes: Shown are the results of *t* tests comparing model coefficients from separate models run on each clover species (see Appendix D). The general form of the logistic model was $\text{Pr}(\text{mortality}) = \text{herbivory} + \text{disease} + \text{number of leaves} + \text{intercept}$. Species excluded from the analysis because of unstable model parameter estimates were: year 1, *Medicago arabica* and *Trifolium subterraneum*; year 2, *T. glomeratum*, *T. willdenovii*, and *T. wormskjoldii*. Invasiveness could not be tested for year 1 because there was only one invasive species with stable model coefficients. Sample sizes are: origin, year 1, native, $n=9$; origin, year 1, introduced, $n=5$; origin, year 2, native, $n=8$; origin, year 2, introduced, $n=7$; invasiveness, year 2, invasive, $n=2$; invasiveness, year 2, noninvasive, $n=5$.

(three species/year combinations for disease and three for herbivory; Appendix D). There were no significant differences between introduced and native species in the logistic regression coefficients for the effects of disease or herbivory on plant mortality ($P > 0.5$; Table 3). There were also no significant differences between invasive and noninvasive introduced species ($P > 0.3$). In other words, the tendency of disease symptoms or herbivory damage to predict mortality throughout the growing season did not depend either on origin or on invasiveness.

Fungicides were successful in reducing, but not eliminating, fungal infection as well as symptom expression. In year 3, infection was reduced by 7% and symptoms by about two-thirds; in year 4, infection was reduced by 21% and symptoms by about half (Appendix C). Fungicide treatment did not reduce mycorrhizal colonization or show any phytotoxic effects (Appendix C).

In year 3, fungicide treatment reduced mean plant mortality from 21% to 15%, representing a significant, 27% decrease in mortality (paired $t = 2.76$, $df = 15$, $P = 0.014$; Fig. 4). Aboveground biomass at harvest showed only weak effects of fungicide, with no significant treatment effect with all species combined (fungicide 0.199 ± 0.474 g, control 0.193 ± 0.356 g; paired $t = 0.39$, $df = 457$, $P = 0.69$), and a significant effect for only one individual species (Appendix C).

The reduction in mortality due to the fungicide treatment was not significantly different between native and introduced species (mean difference \pm SD: native = 0.059 ± 0.077 , introduced = 0.053 ± 0.091 ; $t = 0.15$, $df =$

13.6, $P = 0.88$) nor between invasive and noninvasive species (mean difference \pm SD: invasive = 0.000 ± 0.071 , noninvasive = 0.071 ± 0.095 ; $t = 1.12$, $df = 2.4$, $P = 0.36$). The difference in biomass between paired fungicide and control plants showed no significant effect of origin (fungicide minus control [F-C] = 0.004 ± 0.26 g vs. 0.008 ± 0.40 g for native and introduced, respectively; $F_{1,14} = 0.11$, $P = 0.74$). However, invaders showed a greater increase in biomass with fungicide treatment than did noninvaders (F-C = 0.030 ± 0.053 g vs. -0.002 ± 0.013 g; $F_{1,6} = 4.10$, $P = 0.044$). Patterns were similar for belowground biomass (data not shown).

In year 4, overall mortality was $<8\%$. Mortality was not lower in fungicide plants than in control plants; in fact it was slightly higher ($t = 2.06$, $df = 16$, $P = 0.056$; Fig. 4). Fungicide did not have a significant effect on biomass at harvest for all species combined (fungicide, 0.167 ± 0.227 g; control, 0.168 ± 0.161 g; $t = 0.92$, $df = 183$, $P = 0.36$) or in any individual species (Appendix C).

The response of mortality to fungicide did not differ between native and introduced species (0.062 ± 0.124 vs. 0.037 ± 0.073 , respectively; $t = 0.51$, $df = 11.1$, $P = 0.62$). Nor did invasive species differ from noninvasive species (0.083 ± 0.118 vs. 0.056 ± 0.136 , respectively; $t = 0.28$, $df = 2.0$, $P = 0.81$). Using the difference between mean biomass of fungicide and control plants at the species level as replicates, we found no significant difference between the response of native and introduced plants to fungicide (F-C = -0.014 ± 0.026 g vs. 0.010 ± 0.095 g, for native and introduced plants, respectively; $t = 0.29$, $df = 13.3$, $P = 0.78$). Among the introduced taxa, we did not find an effect of invasiveness (F-C = -0.006 ± 0.009

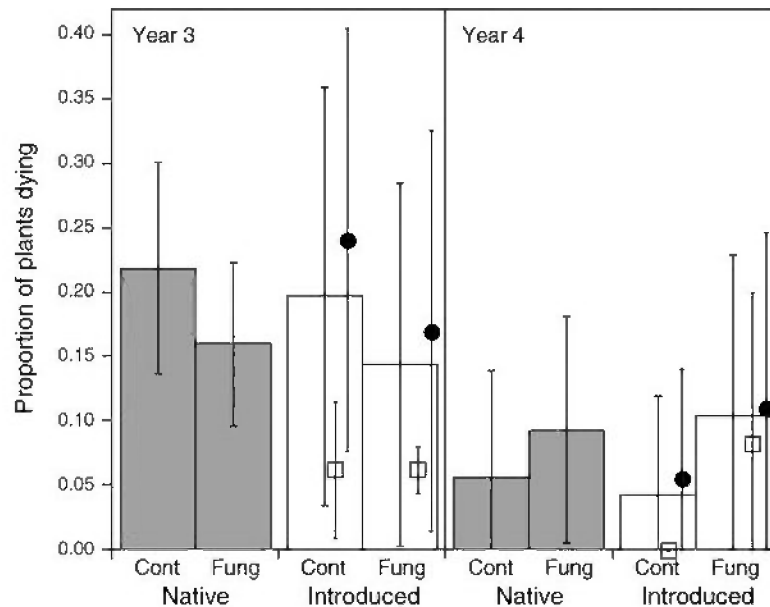


FIG. 4. Effect of fungicide on the proportion of plants dying before reproduction (mean \pm SD) for native and introduced species (Fung, fungicide treatment; Cont, control). Open squares represent invasive introduced species; solid circles are noninvasive introduced species. Replication is at the level of the species.

g vs. -0.017 ± 0.029 g for invasive and noninvasive, respectively; $t = 0.84$, $df = 5.9$, $P = 0.43$).

In summary, reduction in fungal infection and disease development following fungicide treatment led to a large and significant reduction in plant mortality in year 3. The treatment had little detectable effect in year 4 when overall plant mortality was low, but the trend was for higher mortality in fungicide plants. In all comparisons, native and nonnative species responded similarly to the removal of fungi; that is, there was no indication that fungal pathogens have larger fitness consequences for native species. The most invasive species benefited significantly more from reduced fungal infection than did the noninvasive introduced species, for both survival and final size (year 3).

DISCUSSION

Escape from enemies

Comparing a broad suite of sympatric native and introduced plant species, we found no evidence that the introduced species had escaped the effects of pathogens. Our preliminary surveys had suggested that introduced species suffered lower levels of disease, but these species were also generally associated with drier roadside habitats, confounding origin with habitat differences in disease pressure. This underscores the necessity of common garden studies in any native/introduced comparison. In the common garden, native and introduced species were nearly indistinguishable in terms of foliar infection levels in the field, prevalence and severity of disease symptoms, the relationship between

disease symptoms and mortality, and fitness effects of release from pathogens in removal experiments.

These findings are consistent with the generalization that comparisons of natural enemy attack within a community have tended not to find differences between native and introduced species (reviewed in Colautti et al. 2004). Comparisons between native and nonnative congeners may be less likely to show differences because of the tendency of close relatives to share pathogens and herbivores. That is, species that are introduced into areas with closely related native species are least likely to demonstrate an escape from natural enemies, which might explain why there tends to be a negative relationship between invasiveness and phylogenetic relatedness to local native species (Mack 1996, Strauss and Webb 2006; but see Duncan and Williams 2002). Interestingly, the idea that host-switching shows a strong phylogenetic signal has seldom been explicitly tested (Parker and Gilbert 2004). Abiotic requirements as well as shared mutualisms should also show a phylogenetic signal (Mitchell et al. 2006, Webb et al. 2006), making it hard to disentangle the various factors influencing broad-scale patterns of invasion success.

In contrast to the pathogens, herbivores inflicted more damage on native species than on introduced species, although herbivore attack was not predictive of mortality, and we cannot fully evaluate whether more herbivory on natives translated into a greater fitness cost. Unlike predictions based on "escape" or "release" from natural enemies, this pattern was due not to a greater number of herbivore species on native hosts, but rather to a consistent preference for natives by generalist herbivores, both native and introduced. This is the

opposite of the preference for introduced species found in a number of herbivore taxa by Parker and Hay (2005, but see Lankau et al. 2004). One hypothesis for our results is that the introduced species might share a novel chemical defense not present in the native species. The unusual cyanogenesis defense of *T. repens* (Dirzo and Harper 1982) suggests one possibility, and genotypes of *T. repens* are cyanogenic at our site (Hayden and Parker 2002); however, this chemical defense is not utilized by any of our other clover species, introduced or native (K. Hayden, I. M. Parker, and G. S. Gilbert, *unpublished data*). A second explanation is that herbivores are more likely to locate and feed on species with which they are familiar (Feeny 1975, Bernays and Chapman 1994, Lankau et al. 2004). Most of the introduced clovers in this study were rarely if ever found in the coastal prairie away from roads. Herbivores might be expected to increase their use of the introduced species as food plants if they did become more common in that habitat, a suggestion that is supported by the observation that the two introduced species most commonly found in the prairie suffered higher herbivore attack.

The natural enemies in this system are dominated by species that are generalists within the phylogenetic context of this study. All herbivores observed were common on all the clover species. For pathogens, we were able to identify roughly 95% of the fungi infecting plant leaves; these fungi appear to be either native or cosmopolitan in origin, and all species were found to infect all hosts. We did find some fungi that appeared to be associated with only one or two hosts (for example, two rusts [Uredinales]), but these were always rare. In a separate study, we identified and tabulated clover host use for many fungal morphospecies from different experiments and different sites; we found no evidence that rare morphotypes were more commonly associated with native species or that native species yielded more morphospecies on average (I. M. Parker and G. S. Gilbert, *unpublished data*). For pathogens, we still know very little about whether generalists or specialists dominate communities in terms of overall disease effects on plants (Hawksworth 2001). For herbivores as well, the difficulty of assigning blame for particular types of leaf damage hinders assessment of whether generalists or specialists are most important in terms of their effects at the community level. Even a detailed inventory of suitable hosts for all herbivores in a community does not necessarily predict whether specialists or generalists dominate impacts on plants (Novotny and Basset 2005).

Fungal removal experiments reduced mortality in one of two years, but did not significantly increase final plant biomass overall or in most individual species. This was no doubt partly due to the inability of fungicide application to completely control fungal infection. In addition, fungal exclusion experiments may exclude mutualists along with pathogens, masking the effects of escape from enemies (Colautti et al. 2004). The marginally significant increase in mortality for fungicide

plants in year 4 is consistent with the possibility that fungi may sometimes act as mutualists in this system. Mycorrhizal fungi were not negatively affected by our fungicide treatments. However, foliar fungi themselves can sometimes act as mutualistic symbionts. *Stemphylium* was the most common fungus in clovers at BMR and is definitely pathogenic: in experimental inoculations it produced leaf necrosis and other disease symptoms on all clover species tested. However, *Stemphylium* was also common in asymptomatic leaves, and it is possible that it and/or other fungi play a role as facultative mutualists in some hosts, in some years, or in some parts of the growing season. Foliar fungi may improve plant water relations or nutrition (e.g., Scharldt et al. 2004) or may act as mutualists indirectly by competing with more aggressive pathogens or inducing defenses that mitigate the effects of more aggressive pathogens (Herre et al. 2007). In our isolations, we did not see evidence of a shift to a different suite of (more virulent) fungi in the fungicide-treated plants, nor did we find significantly lower incidence or severity of herbivory in these plants (data not shown). More experiments are needed to discover the underlying mechanism for the dual pathogenic/mutualistic nature of foliar fungi in this system. The different responses we found in two years of fungicide experiments underscore the importance of replicating experiments over multiple years.

Biotic resistance

While it is instructive to compare groups of native and introduced species, in order to test whether pest pressure can be used as an explanation of invasiveness we need to look at local patterns of invasion. We want to know whether resident communities of pathogens and herbivores might eliminate incipient invasions and therefore help determine who invades and who does not. In contrast to expectations, the most invasive clovers showed higher infection rates, more frequent and more severe disease symptoms, and greater disease effects on fitness than those introduced clovers that are not generally found in the coastal prairie despite being common along roadsides. In addition, the prevalence and severity of herbivory, and its effect on mortality, was either higher on the invasives or indistinguishable between the two groups. Therefore, although herbivores preferred native over introduced species, escape from pest pressure cannot be used to explain why some introduced species are common invaders in coastal prairie while others are not.

These results are not consistent with Mitchell's and Power's (2003) analysis suggesting that escape from pathogens predicts plant invasiveness. They compared the number of pathogen records from plant species on noxious weed lists vs. those not on such lists, using plant pathogen databases at the continental scale. Mitchell and Power focused exclusively on obligate, biotrophic pathogens, which would be expected to show the strongest signal of specialization and loss during the

introduction process (Parker and Gilbert 2004). In contrast, the pathogens at our study site were dominated by generalist necrotrophs, which are likely to be common in many systems.

Our study did not use phylogenetically constrained comparisons. Recent phylogenetic studies suggest that California *Trifolium* cluster as a monophyletic group within the rest of *Trifolium* (Steele and Wojciechowski 2003, Ellison et al. 2006). Our European *Trifolium* taxa come from several different sections of the genus, and *Medicago* and *Trifolium* are well-supported as separate genera within the tribe Trifolieae. Therefore, the European taxa represent a wider phylogenetic sample, and the California taxa should have a tendency towards similarity based solely on phylogenetic history. The one significant difference we found between native and introduced taxa was in herbivore damage, and phylogenetic relatedness might contribute to that difference. In general, however, we found that both European and California clovers showed great variability for every factor examined, including size and leaf morphology, susceptibility to disease, and response to fungicide. This variability among species swamped any differences due to origin. Interestingly, the two most invasive introduced species, *Medicago polymorpha* and *M. arabica*, are fairly closely related to each other (Bena et al. 1998), and similarities between them (e.g., in disease prevalence) may be due in part to shared phylogenetic ancestry. However, *Medicago lupulina* was not invasive and may be as closely related to these species as they are to one another (Bena et al. 1998).

The pattern of host–pathogen interactions in this system is broadly characterized by great variability among hosts, with little of that variability associated with the origin of the host. In a separate study (Bradley et al. 2003), we identified leaf wetness as a key trait influencing susceptibility of clovers to disease. We found that because of the close correlation between leaf size and time to drying, variability in disease susceptibility was largely driven by leaf area. *Medicago polymorpha* and *M. arabica* have relatively large leaves, which may entirely explain why these two invasive clovers show high infection rates, symptom levels, and disease effects on fitness. Of all the introduced species in our study, these species were also among the first to be introduced to western North America (Table 1). One might expect that these species would have had the opportunity to accumulate more pathogens; however, patterns of host use and infection did not suggest that the accumulation of more native pathogens drives disease pressure in this system.

CONCLUSIONS

What explains invasiveness among introduced clovers, if not escape from natural enemies? The two most invasive species have fruits with recurved spines that apparently aid in long-distance dispersal, while the other species have no obvious adaptations for dispersal. It is possible that the noninvaders were primarily dispersal-

limited. However, the invaders were also able to attain much larger size than any of the noninvasive introduced species, which were, as a group, smaller than native species when planted within the native community (Table 1). The invaders also flowered earlier than the other introduced species, more in line with the phenology of native species (data not shown). In general, the success of these clovers in invading California coastal prairie may be explained by physiological and life history similarities between them and native clover species, rather than differences.

Although release from natural enemies has recently received much attention as an organizing theme in invasions research, we believe our findings may be quite general: introduced and native species often will not be distinguishable by their interactions with pathogens and herbivores. If broad host ranges are the rule for pathogens and herbivores and if most introduced plants either have relatives in their introduced range, bring pests with them, or encounter many cosmopolitan enemies, as in our system, rapid equalization of pest pressure would be expected. That is not to say that all species will be the same in their susceptibility to natural enemies, just that this susceptibility will not be driven by their origin per se. The strength of comparisons that utilize many species within native and introduced groups is that they allow the critical evaluation of how origin, as well as variation in factors unrelated to origin or invasiveness, may influence plant–consumer interactions.

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LITERATURE CITED

- Agrawal, A. A., P. M. Kotanen, C. E. Mitchell, A. G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology* 86:2979–2989.
- Baker, H. G., and G. L. Stebbins. 1965. *The genetics of colonizing species*. Academic Press, New York, New York, USA.
- Barbour, M. G., R. B. Craig, R. R. Drysdale, and M. T. Ghislen. 1973. *Coastal ecology of Bodega Head*. University of California Press, Berkeley, California, USA.
- Beckstead, J., and I. M. Parker. 2003. Invasiveness of *Ammophila arenaria*: Release from soil-borne pathogens? *Ecology* 84:2824–2831.
- Bena, G., B. Lejeune, J. M. Prosperi, and I. Olivieri. 1998. Molecular phylogenetic approach for studying life-history evolution: the ambiguous example of the genus *Medicago* L.

- Proceedings of the Royal Society of London B 265:1141–1151.
- Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York, New York, USA.
- Blaney, C. S., and P. M. Kotanen. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology* 38:1104–1113.
- Bradley, D. J., G. S. Gilbert, and I. M. Parker. 2003. Susceptibility of clover species to fungal infection: the interaction of leaf surface traits and environment. *American Journal of Botany* 90:857–864.
- Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. *Nature* 427:731–733.
- Campbell, C. L., and L. V. Madden. 1990. Introduction to plant disease epidemiology. John Wiley and Sons, New York, New York, USA.
- Cappuccino, N., and D. Carpenter. 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. *Biology Letters* 1:435–438.
- Carpenter, D., and N. Cappuccino. 2005. Herbivory, time since introduction and the invasiveness of exotic plants. *Journal of Ecology* 93:315–321.
- Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7:721–733.
- Crawley, M. J. 1987. What makes a community invisable? Pages 429–454 in A. J. Gray, M. J. Crawley, and P. J. Edwards, editors. British Ecological Society Symposium. Volume 26. Colonization, succession and stability. Blackwell Scientific, Oxford, UK.
- Darwin, C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. J. Murray, London, UK.
- DeBach, P., and D. Rosen. 1991. Biological control by natural enemies. Cambridge University Press, Cambridge, UK.
- DeWalt, S., J. S. Denslow, and K. Ickes. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology* 85:471–483.
- Dirzo, R., and J. L. Harper. 1982. Experimental studies on slug-plant interactions. III. Differences in the acceptability of individual plants of *Trifolium repens* to slugs and snails. *Journal of Ecology* 70:101–117.
- Duncan, R. P., and P. A. Williams. 2002. Darwin's naturalization hypothesis challenged. *Nature* 417:608–609.
- Ellison, N. W., A. Liston, J. J. Steiner, W. M. Williams, and N. L. Taylor. 2006. Molecular phylogenetics of the clover genus (*Trifolium*-Leguminosae). *Molecular Phylogenetics and Evolution* 39:688–705.
- Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen, London, UK.
- Farr, D. F., A. Y. Rossman, M. E. Palm, and E. B. McCray. 2006. Fungal databases. USDA Systematic Botany and Mycology Laboratory. (<http://nt.ars-grin.gov/fungal/databases/>)
- Feeny, P. 1975. Biochemical coevolution between plants and their insect herbivores. Pages 3–19 in L. E. Gilbert and P. H. Raven, editors. Coevolution of animals and plants. University of Texas Press, Austin, Texas, USA.
- Genton, B. J., P. M. Kotanen, P. O. Cheptou, C. Adolphe, and J. A. Shykoff. 2005. Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment. *Oecologia* 146:404–414.
- Gilbert, G. S., and I. M. Parker. 2005. Invasions and the regulation of plant populations by pathogens. Pages 299–315 in M. W. Cadotte, S. M. McMahon, and T. Fukami, editors. Conceptual ecology and invasions biology: reciprocal approaches to nature. Kluwer Academic, New York, New York, USA.
- Gillett, J. B. 1962. Pest pressure, an underestimated factor in evolution. *Systematics Association Publication* 4:37–46.
- Goergen, E., and C. Daehler. 2001. Inflorescence damage by insects and fungi in native pili grass (*Heteropogon contortus*) versus alien fountain grass (*Pennisetum setaceum*) in Hawai'i. *Pacific Science* 55:129–136.
- Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* 105:1422–1432.
- Hayden, K. J., and I. M. Parker. 2002. Plasticity in cyanogenesis of *Trifolium repens* L.: inducibility, fitness costs and variable expression. *Evolutionary Ecology Research* 4: 155–168.
- Herre, E. A., L. C. Mejia, D. A. Kyllo, E. Rojas, Z. Maynard, A. Butler, and S. A. Van Bael. 2007. Anti-pathogen effects of fungal endophytes in roots and leaves of *Theobroma cacao*: implications for tropical host plants. *Ecology* 88, *in press*.
- Hierro, J. L., J. L. Maron, and R. M. Callaway. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93:5–15.
- Huffaker, C. B., and P. S. Messenger, editors. 1976. Theory and practice of biological control. Academic Press, New York, New York, USA.
- Jepson, W. L. 1936. A flora of California. California School Book Depository, San Francisco, California, USA.
- Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17:164–170.
- Lankau, R. A., W. E. Rogers, and E. Siemann. 2004. Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. *Ecological Entomology* 29:66–75.
- Lockwood, J., D. Simberloff, M. McKinney, and B. Von Holle. 2001. How many, and which, plants will invade natural areas? *Biological Invasions* 3:1–8.
- Mabberley, D. J. 1997. The plant-book. Cambridge University Press, Cambridge, UK.
- Mack, R. N. 1995. Understanding the processes of weed invasions: the influence of environmental stochasticity. Pages 65–74 in C. H. Sturton, editor. Weeds in a changing world. British Crop Protection Council, Brighton, UK.
- Mack, R. N. 1996. Biotic barriers to plant naturalization. Pages 39–46 in V. C. Moran and J. H. Hoffmann, editors. Proceedings of the IX International Symposium on Biological Control of Weeds. University of Cape Town, Stellenbosch, South Africa.
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10:689–710.
- Maron, J. L., and M. Vilà. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95:361–373.
- Mitchell, C. E., and A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:625–627.
- Mitchell, C. E., et al. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9:726–740.
- Novotny, V., and Y. Basset. 2005. Review: host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society of London B* 272:1083–1090.
- Parker, I. M., and G. S. Gilbert. 2004. The evolutionary ecology of novel plant-pathogen interactions. *Annual Review of Ecology, Evolution and Systematics* 35:675–700.
- Parker, I. M., et al. 1999. Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1:3–19.
- Parker, J. D., and M. E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters* 8:959–967.

- Reichard, S. H., and K. Hamilton. 1997. Predicting invasions of woody plants introduced into North America. *Conservation Biology* 11:193–203.
- Reinhart, K. O., A. Packer, W. H. Van der Putten, and K. Clay. 2003. Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters* 6:1046–1050.
- Rejmánek, M., and D. M. Richardson. 1996. What attributes make some plant species more invasive? *Ecology* 77:1655–1661.
- Schardl, C. L., A. Leuchtman, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology* 55:315–340.
- Simberloff, D. 1986. Introduced insects: a biogeographic and systematic perspective. Pages 3–26 in H. A. Mooney and J. A. Drake, editors. *Ecology of biological invasions of North American and Hawaii*. Springer-Verlag, New York, New York, USA.
- Steele, K. P., and M. F. Wojciechowski. 2003. Phylogenetic analyses of tribes Trifolieae and Viciae, based on sequences of the plastid gene *matK* (Papilionoideae, Leguminosae). Pages 355–370 in B. Klitgaard and A. Bruneau, editors. *Advances in legume systematics*. Part 10. Higher level systematics. Royal Botanic Garden, Kew, UK.
- Strauss, S. Y., and C. O. Webb. 2006. Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences (USA)* 103:5841–5845.
- Vilà, M., J. L. Maron, and L. Marco. 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum*. *Oecologia* 142:474–479.
- Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* 84:468–478.
- Waloff, N. 1966. Scotch broom (*Sarothamnus scoparius* (L.) Wimmer) and its insect fauna introduced into the Pacific Northwest of America. *Journal of Applied Ecology* 3:293–311.
- Webb, C. O., G. S. Gilbert, and M. J. Donoghue. 2006. Phylodiversity dependent seedling mortality, size structure, and disease in a Bornean rain forest. *Ecology* 87:S123–S131.
- Williamson, M. H. 1996. *Biological invasions*. Chapman Hall, New York, New York, USA.
- Wingfield, M. J., B. Slippers, J. Roux, and B. D. Wingfield. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the southern hemisphere. *BioScience* 51:134–140.
- Wolfe, L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. *American Naturalist* 160:705–711.

APPENDIX A

Methodological details of common garden experiments for testing effects of pathogens and herbivores on native and introduced clover species (*Ecological Archives* E088-076-A1).

APPENDIX B

Isolation, identification, and geographical origin of fungi from clovers (*Ecological Archives* E088-076-A2).

APPENDIX C

Detailed description of fungicide experiments (*Ecological Archives* E088-076-A3).

APPENDIX D

Results of hypothesis testing: tables of *F* statistics, *P* values, and additional analyses described in the text (*Ecological Archives* E088-076-A4).

APPENDIX E

A photo of a common garden site in the coastal prairie at Bodega Marine Reserve (*Ecological Archives* E088-076-A5).