LETTER

Ecological resistance, seed density and their interactions determine patterns of invasion in a California coastal grassland

Meredith A. Thomsen,[1]* Carla M. D'Antonio,[2] Kenwyn B. Suttle[1] and Wayne P. Sousa[1]

[1]Department of Integrative Biology, University of California-Berkeley, Berkeley, CA 94720-3140, USA

[2]Department of Ecology, Evolution and Marine Biology, University of California-Santa Barbara, Santa Barbara, CA 93106-9610, USA

*Correspondence: E-mail: mthomsen@berkeley.edu

Abstract

Relatively little experimental evidence is available regarding how ecological resistance and propagule density interact in their effects on the establishment of invasive exotic species. We examined the independent and interactive effects of neighbour cover (biotic resistance), winter vs. spring water addition (abiotic resistance) and seed density on the invasion of the European perennial grass *Holcus lanatus* into a California coastal grassland dominated by exotic annual grasses. We found that decreased competition from resident exotic grasses had no effect. In contrast, increased late-season water availability eroded the abiotic resistance offered by naturally dry conditions, facilitating invasion. Finally, watering treatment and seed density interacted strongly in determining seedling survival: while seedling mortality was close to 100% in ambient and winter water addition plots, survivor numbers increased with seed density in spring-watered plots. Thus, decreased abiotic resistance can amplify the effect of increased propagule density on seedling establishment, thereby increasing the likelihood of invasion.

INTRODUCTION

The factors determining whether a plant species establishes in a new site can be divided into three broad categories: (i) the biotic interactions individuals experience; (ii) abiotic conditions; and (iii) the supply of available propagules (density, timing, quality, etc.) (Harper 1977; Lonsdale 1999; Tilman 2004). The relative importance of these factors is central to debate in plant ecology on topics such as neutral vs. tradeoff models of community assembly (Tilman 2004), seed- vs. safe site-limitation of plant communities (Andersen 1989) and mechanisms of successional change (Connell & Slatyer 1977). The interplay among biotic interactions, abiotic conditions and propagule arrival in their effects on site colonization is also central to the topic of exotic plant invasion, the specific case of the establishment and spread of a species newly introduced to a given geographical area (Mack *et al.* 2000).

The net effect on an exotic plant species of the interactions and conditions in a site determines site invasibility (Lonsdale 1999; Alpert *et al.* 2000; Brown & Fridley 2003) or conversely, site resistance to invasion (Elton 1958; Levine *et al.* 2004; Von Holle & Simberloff 2004). Resident competitors, high resident diversity and herbivores often function as effective biotic resistance (D'Antonio *et al.* 2001; Levine *et al.* 2004). High competitive resistance is not predicted to be limited to native-dominated habitats. For example, although resource complementarity (one proposed mechanism of competitive resistance) could arise through the coevolution of native species, it is also predicted through community assembly in ecological time, as species with resource requirements different from residents are more likely to establish (Tilman 2004). High availability of bare ground, eutrophication and frequent or severe disturbance are abiotic factors frequently correlated with high invasibility (Burke & Grime 1996; Mack *et al.* 2000). Stochastic events (Tilman 2004), fluctuating resource availability (Davis & Pelsor 2001) and alterations associated with global change (Kriticos *et al.* 2003) that facilitate invasion represent disruptions of abiotic resistance (D'Antonio *et al.* 2001).

The role of propagule supply in influencing invasion has received less experimental attention than have the mechanisms of biotic and abiotic resistance, a troubling omission as differences in site invasibility can only be assessed once differences in propagule supply are accounted for (Rouget & Richardson 2003). If two habitats exert equally negative effects on new species but one receives greater propagule input, the habitat receiving more propagules is more likely to become invaded, resulting in an apparent but no actual difference in invasibility. Seed-sowing experiments illustrate the potential for propagule supply to drive patterns of exotic plant spread: adding more seeds of species already present can increase recruitment, and adding seeds of new species commonly increases species richness (Turnbull *et al.* 2000).

The applicability of such experiments to the question of how propagule supply affects patterns of exotic plant invasion is limited by the fact that few studies have sown plants outside their native range, perhaps due to conservation concerns (Turnbull *et al.* 2000). Furthermore, although we know that the abundance of invasive plant species increases with proximity to propagule sources, such as planted sites (Williamson & Harrison 2002) and trails or roads (Tyser & Worley 1992), and with factors increasing the likelihood of propagule delivery (Jesson *et al.* 2000; Levine 2000), we lack detailed information on how the likelihood of invasion increases with propagule density (Turnbull *et al.* 2000; Brown & Fridley 2003). Invasion may be unlikely when propagule density is below a threshold value, because of the sensitivity of small populations to stochastic events (Tilman 2004) and Allee effects (Leung *et al.* 2004). There are also likely to be propagule densities above which safe sites are saturated, so that increasing propagule density has no effect on the number of recruits (although the per capita establishment rate declines) (Andersen 1989; Turnbull *et al.* 2000; Fig. 1). There is a clear need for manipulative experiments using a wide range of seed densities to elucidate the relationship between the propagule density of individual exotic species and the likelihood of invasion (D'Antonio *et al.* 2001; Rouget & Richardson 2003).

Our understanding of resistance and propagule supply in determining patterns of invasive plant spread would also benefit from the evaluation of their interactions. The effect of increasing seed density on the seedling establishment of native species has been shown to vary across habitats in both natural (Crawley & Long 1995) and manipulative (Keddy 1981) experiments. Similarly, D'Antonio (1993) estimated that the seed density required to invade several different habitats varied by an order of magnitude, and Rouget & Richardson (2003) showed that propagule density and environmental factors interacted strongly in determining the distribution of several invasive tree species. However, more experimental evidence is needed to clarify
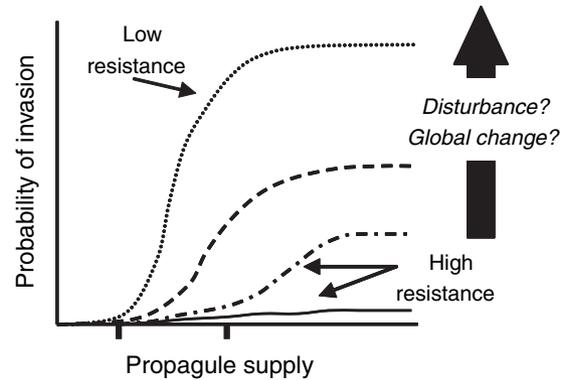

Figure 1 Interaction of ecological resistance to invasion (different lines within graph) and propagule supply (*x*-axis). Hatch marks on the *x*-axis represents threshold values for the top resistance curve, below or above which the likelihood of plant establishment is unlikely to change with changing propagule supply. The interaction between resistance and propagule supply may depend on the strength or type of resistance functioning in a system: while weak resistance may allow for a high probability of invasion at relatively low levels of propagule supply, strong resistance may be relatively unaffected by increasing propagule supply. In systems without a frequent or severe natural disturbance regime, disturbance may cause a relaxation of resistance, thereby changing the system's response to a given level of propagule supply. In habitats in which current climatic conditions function as abiotic resistance to invasion, global climate change may similarly erode resistance. Redrawn from D'Antonio *et al.* (2001).

the interaction of propagule density and resistance, for example, whether strong resistance can be overcome by high enough propagule densities, or if some forms of abiotic resistance are relatively unaffected by increasing propagule inputs (Robinson *et al.* 1995; D'Antonio *et al.* 2001; Rouget & Richardson 2003; Fig. 1).

We tested hypotheses about the interaction of ecological resistance and propagule density in a northern California coastal prairie experimentally invaded by the exotic perennial grass *Holcus lanatus* L. (hereafter *Holcus*). Our site lacks *Holcus*, although it is present in an adjacent, wetter meadow, suggesting that *Holcus* is unlikely to spread outside the experimental plots. We examined the independent and interactive effects of neighbour cover (biotic resistance), winter (January to March) vs. spring (April to June) water addition (abiotic resistance) and seed density on *Holcus* invasion. We predicted that clipping, by reducing resident cover (primarily exotic annual grasses), would decrease resistance to *Holcus* invasion. Competition from annual grasses frequently decreases the growth and survival of California native perennial grass seedlings (Dyer & Rice 1999; Hamilton *et al.* 1999; Kolb *et al.* 2002; Corbin & D'Antonio 2004; but see Seabloom *et al.* 2003); it may similarly limit the establishment of an exotic perennial grass.

We hypothesized that water addition would also decrease resistance to *Holcus* invasion. In California, *Holcus* is most common in wetter areas such as in the coastal fog zone, slope bottoms and seeps (M. Thomsen, personal observation). Similarly, Kolb *et al.* (2002) found a trend for higher *Holcus* cover on deeper, wetter soils vs. shallow, drier patches in coastal prairie. Both patterns suggest that California's Mediterranean climate (i.e. dry summers) may put *Holcus* near the boundary of its physiological tolerance for drought, so increased water availability could increase its ability to invade (Alpert *et al.* 2000). We expected winter water addition to benefit *Holcus* less than spring watering because water is less limiting to plant growth during the rainy winter (Pitt & Heady 1978). Furthermore, our water addition treatments tested the contrasting effects of two global change scenarios. Several major climate models have predicted increased precipitation in northern coastal California, occurring during either the winter (increased amount of rainfall only) or in the spring and summer (increased amount and duration of rainfall) (Field *et al.* 1999; National Assessment Synthesis Team 2000). The winter water addition treatment also mimics an El Niňo event, which could become more frequent as a result of global climate change (Field *et al.* 1999).

Finally, we intended to determine the effect of a wide range of seed densities on the likelihood of *Holcus* establishment, and to identify any threshold densities above or below which establishment did not respond to changing seed inputs (Fig. 1). We hypothesized that seed density and manipulations of resistance (clipping and watering) would interact in determining *Holcus* seedling establishment and survival (D'Antonio *et al.* 2001). We expected higher seed density to decrease the effectiveness of resistance, so that increased seed input would increase *Holcus* establishment even in 'high resistance' control plots (Fig. 1).

This study represents a novel test of explicit hypotheses about the interaction between ecological resistance and propagule density in determining the invasion of an exotic species. Working in a site from which the exotic species is naturally absent provides the opportunity to examine mechanisms of resistance and their interactions with propagule supply in the earliest stages of invasion.

METHODS

Study system

The study was conducted in South Meadow at the University of California Angelo Coast Range Reserve, Mendocino County, California (39°43′45″ N, 123°38′40″ W). Coastal prairie at the Angelo occurs in meadow openings on abandoned river terraces; soils derive from rocky mudflows on terrace surfaces and contain at least 50% clay throughout the profile (Collins 1979). Average annual rainfall is 215 cm, primarily between October and April (Herring 1997); local topography blocks coastal fog, making summers hotter and drier than on the immediate coast (Johnson 1979). Poor soils and harsh summer drought conditions reduce productivity in our experimental plots relative to many coastal prairie sites; peak standing crop (155 and 136 g m^{-2} in 2002 and 2003; K. Suttle and M. Thomsen, unpublished data) is comparable with that of annual grasslands in the San Joaquin Valley (Bartolome *et al.* 1980).

In general, California coastal prairies are less invaded by annual grasses than grasslands elsewhere in the state; higher water availability and lower historic grazing pressures may contribute to this pattern (Heady *et al.* 1977). Similar to other coastal prairie sites, native perennial bunchgrasses, especially *Danthonia californica* Bolander (hereafter *Danthonia*), are abundant in the Angelo meadows, but exotic annual species dominate some areas (Kotanen 1997). This may result from a history of soil disturbance by feral pigs (Kotanen 1997) and cultivation (Johnson 1979), which decrease perennial grass cover in coastal prairie (Stromberg & Griffin 1996; Kotanen 1997). Both disturbance types, and thus the likely prominence of exotic annuals, date to homesteading in the mid- to late 19th century (Johnson 1979). In South Meadow, peak biomass is on average 50% exotic annual grasses, 20% each nitrogen-fixers and winter-flowering forbs (both primarily natives), and 10% native and exotic perennial forbs; native perennial grasses are rare throughout most of this meadow but dominate at one end (K. Suttle and M. Thomsen, unpublished data).

The native species that coexist with exotic annual grasses in the Angelo meadows are threatened by recent increases in *Holcus* cover (Kotanen 2004). *Holcus* was introduced outside its native range as a seed contaminant, and has spread throughout coastal North America, temperate South America, eastern Asia, South Africa, Australia, New Zealand and Hawaii (Thompson & Turkington 1988). In California, land managers consider *Holcus* a conservation threat in coastal prairie (Anderson *et al.* 1999) and target it for control (Pitcher & Russo 1988). *Holcus* is well-suited to research on the role of propagule density in invasion, because its prolific seed production is cited as an explanation for its invasiveness (Thompson & Turkington 1988). Estimates of *Holcus* seed production range from 19 000 seeds per m^2 in England (Mortimer 1976) to 82 000 m^{-2} in coastal California (Peart 1982); Watt (1978) reported 177 000 seeds per plant under agricultural conditions.

Field methods

In November 2000, we established eighteen 75 m^2 circular watering treatment plots in South Meadow. Treatments

Figure 2 Schematic diagram of experimental design. Blocks are based on the three main water lines (thick solid lines). Two replicate plots of each watering treatment (circles) are dispersed within each block in a stratified random design, one on each of the two arms of each watering line (doubled lines). Within each plot, eight subplots (squares) were established in an arc along the perimeter, beginning at a randomly determined point. Clipping (shaded vs. unshaded squares) and seed density (numbers within each square) treatments were randomly assigned to each subplot. Another set of subplots was established for the second year of seed additions (not shown).
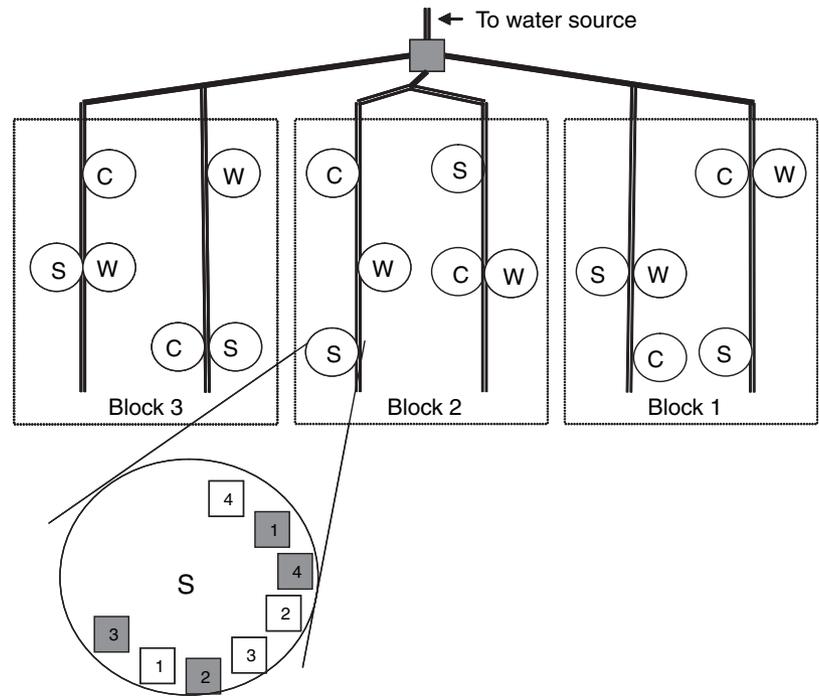
included ambient rainfall, winter water addition (January to March) or spring water addition (April to June); $n = 6$ for each treatment. The experiment was divided into three blocks based on the plot irrigation lines, each block containing two replicate plots per treatment, assigned in a stratified random design (Fig. 2). Because of constraints on the water supply, blocks were watered sequentially over 3 days.

Watered plots were irrigated by Rain Bird® Rain Curtain™ sprinklers (Rainbird, Azusa, CA, USA). Water was collected from a nearby seasonal creek; ammonium and nitrate concentrations in added water were indistinguishable from those of natural rainfall during the watering period (K. Suttle, unpublished data). Watered plots received a total of 42 cm of supplementary water per year, as 1.4 cm of water every 3 days either in the winter or spring (Fig. 3). The amount of supplementary water represents a 20% increase over the long-term mean annual rainfall (215 cm), conservative relative to the 50–100% increase predicted by global climate change models (National Assessment Synthesis Team 2000) and to the increased winter rainfall associated with El Niños (Fig. 3).

In November 2000 and 2001, we established eight 900 cm^2 subplots per plot. Clipping and seed addition treatments were randomly applied to subplots in a fully crossed design (two clipping treatments × four seed levels = eight subplots per plot; Fig. 2); combinations were not replicated within plots. Subplots were separated by > 50 cm. In blocks 2 and 3, all subplots had < 5% cover by perennial grass. Because of the patchy distribution of
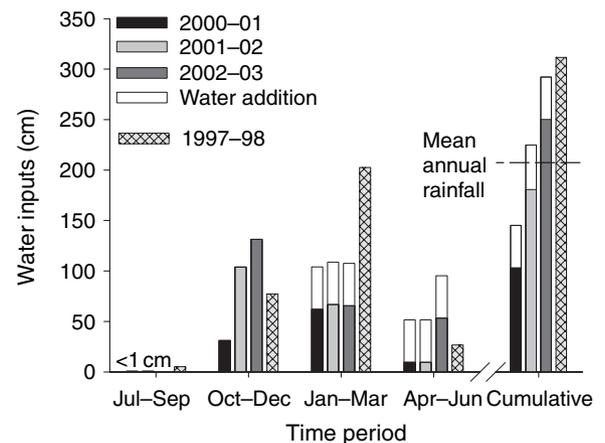

Figure 3 Quarterly and cumulative water inputs (cm) to experimental plots for each rain year of the experiment (July to June), and for the 1997–98 El Niño (presented for comparison). The winter and spring water addition treatments increased total water inputs by 42 cm (clear bars); winter water addition increased water inputs during the period of ambient rainfall (January to March), while spring water addition extended the period of water inputs (April to June). Dashed line at right indicates mean annual cumulative rainfall (215 cm).

perennial grasses across the site, it was impossible to avoid the inclusion of three plots in block 1 in which all subplots had > 50% *Danthonia* cover. We hand-clipped vegetation (other than *Holcus* seedlings) in half the subplots per plot in November and March of the first growing season after each

seed addition. The clipping treatment killed annual individuals, while perennials had some regrowth. In March 2001 and 2002, we evaluated the clipping effect by counting the number of times an inserted pin hit a resident individual at three points per subplot. The clipping treatment significantly decreased resident cover by 46% in 2001 and 16% in 2002; biomass reductions were much greater (M. Thomsen, personal observation).

We collected *Holcus* seeds in August 2000 and 2001 from a population 50 m from our site. We germinated subsamples to assess the number of viable seeds per mg and calculate the mass needed to obtain a given number. In November 2000 and 2001, we added *Holcus* seeds to all subplots by sprinkling them inside a high-sided frame; seeds naturally germinate at this time, minimizing the possibility of seed movement. In 2000, we added 0, 50, 134 or 363 viable seeds per subplot (0, 555, 1488 and 4033 seeds per m^2). In 2001, we replaced the zero-seed level with a higher one (984 seeds per subplot, or 10 933 seeds per m^2). Because we collected an inadequate amount of seed in 2001, we omitted three randomly selected replicates of the highest density (stratified to one per watering treatment). We included the means of the other five replicates as dummy values in our analyses, to balance the statistical design. Using a density function for *Holcus* seed production and dispersal (Peart 1982), we estimate that our seed additions in 2000 and 2001 approximated *Holcus* seed rain 1.8–10 and 1–10 m away, respectively, from a 1 m^2 source patch.

In early June 2001 and 2002, we sampled *Holcus* seedlings using a point frame (established seedlings). We examined the area within 1 cm of each of 25 evenly spaced points per subplot in 2001; in 2002 we examined 48 points to accommodate the greater number of seedlings at the highest seed density. Seedlings rooted within a sampling area were counted and marked with a toothpick; if more than one seedling was present, we marked the centre one. We recensused marked seedlings in late July 2001 and 2002 to evaluate survival into the summer drought (surviving seedlings). In the second year after seeding (June 2002 and 2003), we recorded the small number of new germinants, but those data are not presented. We also counted the total number of flowering and second-year individuals (i.e. plants that had survived the entire summer drought and the following growing season). To minimize the risk of causing an invasion, flowering individuals were removed. A final census the third year after seeding (June 2003 and 2004) revealed no *Holcus* seedlings, suggesting that all added seed had germinated, but we continue to monitor all subplots for *Holcus*.

Analysis

We evaluated our data using a modified randomized block design, with two replicates of each watering treatment per block and plot nested within block and watering treatment; clipping and seed density treatments were fully crossed within each plot (Fig. 2). Block and plot were random factors, while watering, clipping and seed density treatments were fixed. The number of established and surviving seedlings sampled each year was multiplied by the ratio of total plot area (900 cm^2) to the area examined in the point frame census (78.5 cm^2 in 2001, 150.2 cm^2 in 2002) to estimate the total number of established and surviving seedlings per subplot. We used this estimate to calculate the proportion of added seeds that established and survived each year; when this value was greater than one (26 of 576 cases across both variables and years), we replaced the calculated value with one in analyses and figures. Seedling numbers and masses were square root $(x + 0.5)$ and proportional data were arcsine-square root transformed to improve normality and equality of variances.

We used the GLM module in SYSTAT$^®$ 11 (SYSAT Software, Inc., Richmond, CA, USA) to fit our complete models for established and surviving seedlings each year; the significance of each factor was then tested using the appropriate error term (see Table 1). We evaluated the total number of second-year and flowering individuals per plot as a function of block, plot, watering treatment and their interactions. For all significant main effects and interactions, we compared group mean values using Tukey tests with $\alpha = 0.05$ (Zar 1999). Finally, we used a *t*-test to compare the mean mass of *Holcus* seedlings blooming in winter vs. spring water addition plots.

RESULTS

Holcus seedling establishment and survival

In 2001, the estimated number of *Holcus* seedlings establishing per subplot increased with seed density (Table 1), varying from none in the zero-seed addition plots to an average of 106 in the highest seed density treatment; all densities differed significantly (Fig. 4a). Clipping increased the number of established *Holcus* seedlings in block 1 by 76%, but had no effect in blocks 2 and 3 (Table 1; clipping × block interaction). In spring water addition plots, the proportional establishment of added seed in the 50-seed subplots was nearly twice that of the higher densities (Table 1, Fig. 5a). Proportional establishment also differed significantly with watering treatment in the 50-seed subplots, with spring (80%) > winter (55%) > ambient (30%). Finally, the interaction of clipping and block had an effect on proportional establishment similar to that on seedling numbers (Table 1).

Watering treatment and seed density interacted in their effects on seedling survival in 2001 (Table 1). At the two highest seed addition rates, an average of 31 and 62

Table 1 Results of significance testing for effects on the estimated number of *Holcus* seedlings establishing and surviving, and on the estimated proportion of added seed establishing (PAE) and surviving (PAS), per subplot

Factor	MSE	d.f.	2000 seed addition (sampled 2001)								2001 seed addition (sampled 2002)							
			Estab-lished		Surviving		PAE		PAS		Estab-lished		Surviving		PAE		PAS	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between plots																		
BK	2	2.9	0.3	0.760	0.6	0.589	0.2	0.850	1.0	0.396	0.1	0.915	2.3	0.157	0.7	0.507	3.0	0.099
WT	1	2.4	5.5	0.072	15.0	*0.014*	7.6	*0.043*	9.9	*0.028*	25.9	*0.005*	13.0	*0.018*	17.1	*0.011*	14.0	*0.016*
BK*WT[1]	2	4.9	0.3	0.880	0.6	0.696	0.3	0.848	1.0	0.444	2.0	0.184	4.8	*0.024*	1.8	0.205	5.8	*0.014*
PL (BK, WT)[2]		9	Not tested															
Within plots																		
SD	3	3.27	68.3	*0.001*	3.1	*0.042*	45.0	*0.001*	2.6	0.070	19.2	*0.001*	5.3	*0.006*	26.9	*0.001*	10.0	*0.001*
SD*WT	3	6.27	1.8	0.138	3.1	*0.019*	3.0	*0.022*	2.6	*0.038*	1.3	0.305	0.8	0.579	1.7	0.151	11.9	*0.001*
SD*BK	3	6.27	1.2	0.198	0.4	0.896	1.3	0.285	1.1	0.362	2.1	0.087	0.9	0.516	1.5	0.218	1.5	0.208
SD*BK*WT	3	12.27	0.7	0.717	0.4	0.966	0.7	0.750	1.1	0.365	0.9	0.538	0.8	0.648	1.2	0.360	2.3	*0.033*
SD*PL (BK, WT)[3]		27	Not tested															
CL	4	1.9	0.2	0.649	1.4	0.270	0.3	0.593	2.5	0.149	5.0	0.052	2.1	0.183	2.8	0.131	0.5	0.502
CL*WT	4	2.9	1.0	0.407	1.4	0.300	2.0	0.189	2.5	0.138	3.7	0.066	0.0	0.991	3.8	0.063	0.3	0.756
CL*BK	4	2.9	5.1	*0.033*	0.1	0.906	8.7	*0.008*	0.1	0.940	0.3	0.766	0.9	0.437	0.5	0.630	2.8	0.115
CL*BK*WT	4	4.9	0.4	0.796	0.1	0.980	0.8	0.579	0.1	0.992	0.8	0.577	1.5	0.288	0.3	0.878	2.8	0.094
CL*PL (BK, WT)[4]		9	Not tested															
SD*CL	5	3.27	1.5	0.248	0.3	0.824	2.0	0.131	0.6	0.643	2.3	0.097	0.3	0.803	0.7	0.552	0.3	0.795
SD*CL*WT	5	6.27	0.8	0.544	0.3	0.931	0.6	0.722	0.6	0.754	0.8	0.594	0.1	0.995	2.1	0.089	0.2	0.974
SD*CL*BK	5	6.27	1.1	0.386	0.3	0.936	1.4	0.254	0.3	0.911	2.2	0.073	0.6	0.722	1.1	0.365	2.5	*0.046*
SD*CL*BK*WT	5	12.27	0.4	0.933	0.6	0.832	0.4	0.968	0.3	0.974	3.4	*0.004*	0.3	0.992	1.2	0.342	1.5	0.190
SD*CL*PL (BK, WT)[5]		27	Not tested															

BK, block; WT, watering treatment; PL, plot; SD, seed density; CL, clipping treatment. Superscripts in the factor column identify MSE (mean squared error) terms. Data were analysed separately for all variables each year. Significant factors are italicized ($P < 0.05$).

seedlings survived per subplot in the spring watering treatment, while no seedlings survived in the winter and ambient treatments (Fig. 4c). Furthermore, within the spring watering treatment, the highest seed density caused a 980% increase in the number of surviving seedlings compared with the two lowest densities. Spring water addition increased the proportional survival of added seeds to *c.* 20% across all seed densities, compared with zero in the winter and ambient treatment plots (Table 1, Fig. 5c).

In 2002, spring water addition increased the estimated number of established *Holcus* seedlings by 41% compared with the winter water addition or ambient rainfall plots (Table 1; Fig. 4b). The two highest seed densities had approximately twice as many established *Holcus* seedlings as the two lowest. Watering, clipping, seed density and block interacted significantly in determining seedling numbers in 2002 (Table 1). We did not test for significant pairwise differences among the group mean values for this interaction, or account for it in our Tukey comparisons of the main effects of the other treatments. Spring water addition more than doubled the proportional establishment of added seed relative to winter and ambient plots (Table 1, Fig. 5b). Across watering treatments, proportional survival was highest at the lowest seed density (56%) and decreased with increasing seed density.

The estimated number of surviving seedlings in 2002 in the spring rain treatment ranged from 30 to 100 across seed density treatments, while remaining near zero in the winter addition and ambient treatments (Fig. 4d). Within the spring watering treatment, the two highest seed addition densities resulted in 155% more surviving *Holcus* seedlings than did the two lowest. Spring water addition did not increase the number of surviving seedlings in block 1, as it did in blocks 2 and 3 (Table 1; watering × block interaction). Spring water addition increased the proportional survival of added seeds relative to winter and ambient treatments, where it was close to zero (Table 1; Fig. 5d). Within spring-watered plots, proportional survival was greatest at the lowest seed addition density (56%), intermediate for the middle two (28%) and lowest at the highest density of added seed (10%). The interaction of block and water treatment (Table 1) mirrored that for estimated seedling numbers.
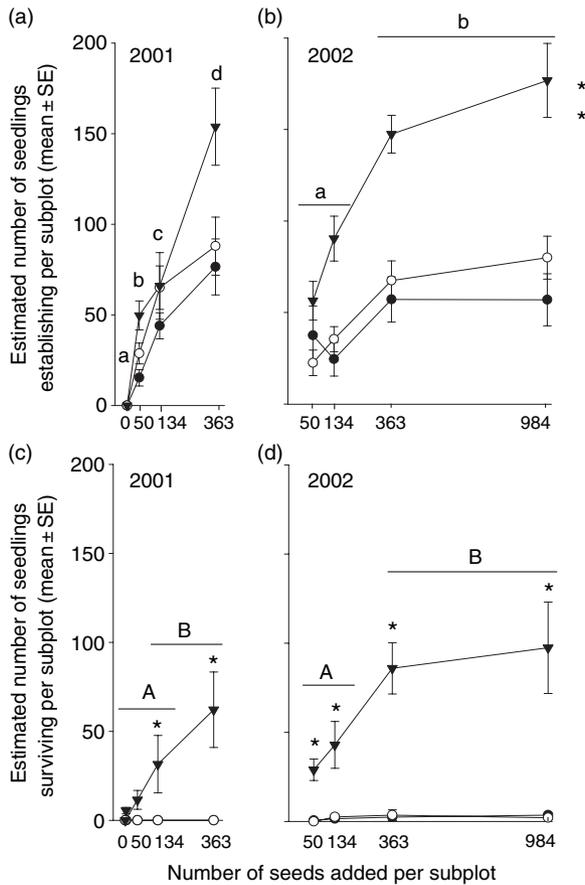
Figure 4 Interaction plot showing the estimated number of *Holcus* seedlings establishing and surviving until July of their first year per subplot (mean ± SE), as a function of seed density and watering treatment (●, spring; ○, winter; ▼, ambient watering treatment). In (a) and (b), lowercase letters indicate overall significant differences among seed densities; the two vertical stars indicate a significant difference between the spring treatment and both winter and ambient treatments in 2002 (main effects). In (c) and (d), uppercase letters indicate significant differences among seed densities within the spring rain treatment, and stars indicate significant differences between the spring vs. winter and ambient water treatments, within each seed density. Panels (a) and (c) show results for the November 2000 and (b) and (d) the November 2001 seed additions. See Table 1 for *F*-values and significance of treatments.

Figure 5 Interaction plot showing the estimated proportion of added *Holcus* seeds establishing and surviving until July of their first year per subplot (mean ± SE), as a function of seed density and watering treatment (●, spring; ○, winter; ▼, ambient watering treatment). In (a), (c) and (d), uppercase letters indicate significant differences among seed densities within the spring rain treatment, and stars indicate significant differences between the spring vs. winter and ambient water treatments, within each seed density. In (b), lowercase letters indicate overall significant differences among seed densities; the two vertical stars indicate a significant difference between the spring treatment and both winter and ambient treatments (main effects). Panels (a) and (c) show results for the November 2000 and (b) and (d) the November 2001 seed additions. See Table 1 for *F*-values and significance of treatments.

Second-year survival and flowering

None of the *Holcus* seedlings from the November 2000 seed addition survived until their second year. Twenty-eight seedlings from the November 2001 addition survived the 2002–2003 summer drought and growing season. There was one second-year seedling in an ambient plot, four in winter water addition plots and 23 in spring water treatment p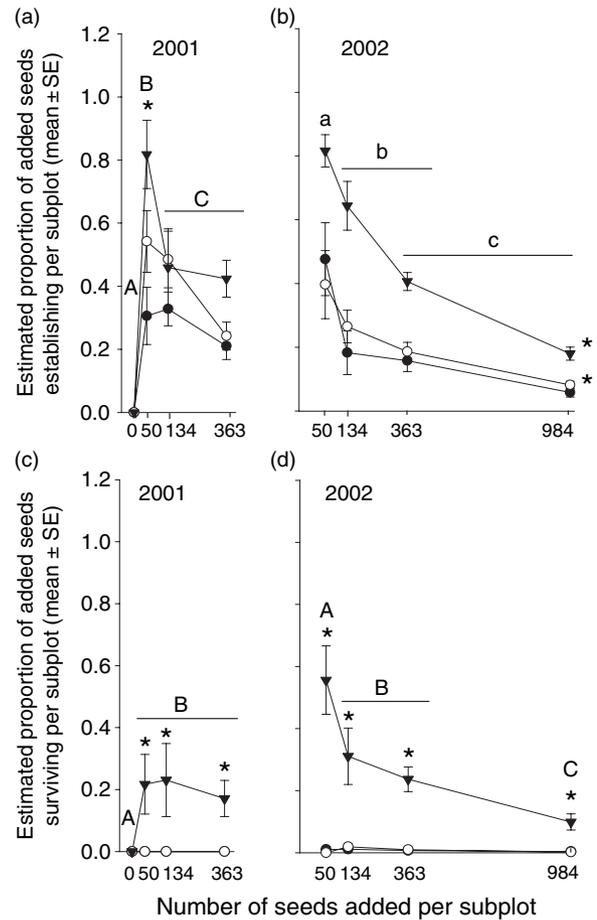lots. Thus, spring watering appeared to enhance survival, although within-treatment variability and many zero values precluded statistical confirmation ($F_{2,4} = 2.2$; $P = 0.230$). Furthermore, the five second-year individuals in winter and ambient plots died by late July 2003, while 17 of the 23 in spring rain plots flowered during the summer of 2003, at which point they were removed. These individuals were large (mean biomass = 1.68 g) and robust at the time of their removal.

In the first year of the experiment no *Holcus* individuals flowered. In 2002, 27 individuals produced inflorescences: 10 in winter and 17 in spring water addition plots. In 2003, 17 second-year individuals in spring-watered plots flowered. High variability within treatments made it impossible to detect a watering effect on flowering ($F_{2,4} = 4.3$; $P = 0.102$). However, *Holcus* plants flowering in the spring watering treatment plots were significantly larger than those in winter-watered plots (1.1 g compared with 0.17 g; $t_{42} = 2.474$, $P < 0.02$).

DISCUSSION

Weak competitive resistance

Competition from resident vegetation has frequently been shown to decrease the establishment of invasive species (Burke & Grime 1996; Davis & Pelsor 2001; Levine *et al.* 2004). However, in our study a clipping treatment that decreased the density of resident exotic annual grasses in a California grassland had no independent effect on the establishment or survival of seedlings of *Holcus*, an invasive European perennial grass. The failure of European annual grasses to negatively affect *Holcus* seedlings contrasts with their competitive suppression of native perennial grass seedlings in many studies (e.g. Dyer & Rice 1999; Hamilton *et al.* 1999; Kolb *et al.* 2002; Corbin & D'Antonio 2004; although see Seabloom *et al.* 2003). The competitive dominance of annual grasses may derive from their higher relative growth rate (Dyer & Rice 1999; Kolb *et al.* 2002); *Holcus* may be unaffected by annual grass neighbours because it also has a high growth rate (Thompson & Turkington 1988). Alternatively, exotic annual grasses may be relatively poor competitors against perennial species in our site. If this is the case, the failure of native grasses to spread into the annual-dominated area of the meadow must result from some factor other than competitive suppression, most likely propagule limitation (Seabloom *et al.* 2003) or intermittent feral pig disturbance (Kotanen 1997).

Furthermore, although our experiment was not designed to compare the resistance of resident annual exotic and native perennial grasses to *Holcus* invasion, the inclusion in block 1 of three plots with high *Danthonia* cover provides circumstantial evidence that competitive resistance to *Holcus* invasion is greater in perennial-dominated sites. In that block, clipping of residents increased 2001 seedling numbers, and the number of surviving seedlings in July 2002 was not increased by spring water addition: strong competition may have limited their response. We attribute the apparently greater competitive resistance of *Danthonia* to *Holcus* invasion to its perennial life history rather than its geographical origin, as exotic perennial grasses have been shown to limit *Holcus* establishment in California coastal prairie more than exotic annuals (Peart 1989). Future work comparing the resistance of native and exotic perennial grass-dominated patches could reveal whether coevolution plays any role in determining the invasibility of native vegetation once life history is accounted for.

Effective abiotic resistance

The stress associated with a mismatch between an individual's physiology and its abiotic environment can limit the spread of invasive species (Alpert *et al.* 2000; D'Antonio *et al.* 2001). Our results suggest that low spring and summer water availability may be a key element of resistance to *Holcus* invasion in coastal California. Increasing springtime water availability increased the establishment and survival of *Holcus* seedlings, compared with winter addition and ambient conditions. Without added late-season water, resistance to *Holcus* invasion was extremely high: across 2 years of seed additions, virtually all seedlings in winter addition and ambient rainfall plots died by the end of July of their first year. Conditions that increase germination and early survival do not always allow the establishment of adult individuals (Turnbull *et al.* 2000), and in this study, many individuals in spring water addition plots that survived until July died later that summer. However, the increase in surviving seedlings in spring-watered plots suggests an increase in the ultimate likelihood of *Holcus*'s successful invasion.

Furthermore, although high within-treatment variability prevented us from detecting statistical differences in the number of second-year and flowering individuals among watering treatments, the pattern suggests *Holcus* could invade our site if propagules were present and late-season water availability increased. At the start of each successive growing season, established perennials benefit from their incumbency when competing against germinating annual grasses (Seabloom *et al.* 2003; Corbin & D'Antonio 2004). The 17 second-year plants that flowered in spring water addition plots appeared to be fully established individuals capable of surviving another summer. Similarly, first- and second-year reproductive *Holcus* individuals were more common and attained significantly greater biomass in spring water addition plots than winter ones. Thus, when late-season water availability is increased *Holcus* is more likely to establish a reproductive population, and thus make the transition from the establishment to the spread phase of invasion (Mack *et al.* 2000).

The pattern of *Holcus* performance relative to annual rainfall variation during the experimental period underscores the effect of water availability. Fewer *Holcus* seedlings flowered in their first year and survived until their second year in 2001 than in 2002, when greater total rainfall may

have improved pre-drought seedling condition and delayed soil drying (Fig. 3). Similarly, in 2003 April-June rainfall was greater, and many individuals bloomed in winter and spring water addition plots. Thus, the results of this study highlight the influence of stochastic factors such as climate on invasion (D'Antonio *et al.* 2001; Tilman 2004).

The positive effect of increased late-season water availability on *Holcus* performance in northern California is particularly significant because several models of global climate change predict increased spring and summer precipitation for the region within the next 50 years (National Assessment Synthesis Team. 2000). Although the more recent Hadley Centre Model (v. 3) moves away from this prediction (Pope *et al.* 2000), it remains a possibility. If low summertime water availability currently prevents *Holcus* invasion in some sites, global climate change could erode abiotic resistance and allow it to expand into new areas. Furthermore, El Niño events, which typically increase winter rainfall at the site (Fig. 3), may also facilitate *Holcus* establishment, since 11 individuals flowered in winter-watered plots in 2002 but none flowered in ambient plots. January to March rainfall in 1997–1998 was far greater than the winter water addition treatment in any year of the experiment, suggesting our treatment may underestimate the effect of El Niño years on *Holcus* invasion.

The effect of propagule density and its interaction with resistance

The species composition of California annual grassland sites is re-set each year as individuals germinate from the soil seed bank, making the system responsive to changes in propagule composition (Robinson *et al.* 1995; Seabloom *et al.* 2003); in this study, increasing seed density increased seedling numbers. We did not identify a threshold in seed density below which invasion is impossible, as adding seeds always resulted in more than zero seedlings (although the number of survivors at the two lowest densities in 2001 was not statistically distinguishable from zero). Furthermore, the proportional establishment of added seeds in 2001 and 2002, and their survival in 2002, were greatest at the lowest seed densities tested, opposite to the pattern associated with the stochastic vulnerability of small populations (Tilman 2004), perhaps due to intraspecific competition.

In 2001, we saw no evidence for a threshold density above which the number of established or surviving seedlings levelled off, and the proportional establishment and survival of added seeds remained constant rather than decreasing at the two highest seed densities. However, the number of established and surviving seedlings in 2002 appeared to plateau at the two highest densities, as the proportional establishment and survival of added seeds

decreased. This pattern could represent either the saturation of safe sites at high seed densities or density-dependent mortality among establishing seedlings (Andersen 1989). Regardless of the mechanism, when an upper threshold in seed density exists, management efforts to decrease seed rain will not affect invasion likelihood unless propagule supply is brought below the threshold level (D'Antonio *et al.* 2001).

Finally, we found strong evidence for an interaction between propagule density and resistance: regardless of seed density, seedlings in the winter and control plots died before the end of July because of strong abiotic constraints. In contrast, the numbers of surviving seedlings increased with seed density in spring water addition plots, suggesting that if resistance decreases, the likelihood of invasion increases with propagule pressure. The question of whether or not propagule inputs can swamp strong resistance is important from a conservation viewpoint: if so, high propagule inputs from neighbouring populations of exotic species could result in the eventual invasion of sites initially considered very resistant. However, our results suggest that for dry coastal prairie sites such as South Meadow, even high propagule inputs of *Holcus* are unlikely to result in invasion unless the rainy season is lengthened.

In conclusion, we found evidence for weak biotic resistance (competition from resident exotic annual grasses) and strong abiotic resistance (summer drought) to the invasion of *Holcus* into a California coastal prairie. These findings improve our understanding of resistance, by illustrating how climatic variation can affect abiotic resistance. Furthermore, they demonstrate a strong interaction between propagule supply and abiotic resistance, since springtime water addition resulted in an alteration of the relationship between the numbers of surviving seedlings and seed density. The mechanistic understanding of an interaction of resistance and propagule supply provides new insights into the factors controlling invasive plant spread.

REFERENCES

Alpert, P., Bone, E. & Holzapfel, C. (2000). Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspect. Plant Ecol., Evol. Syst.*, 3, 52–66.

Andersen, A.N. (1989). How important is seed predation to recruitment in stable populations of long-lived perennials? *Oecologia*, 81, 310–315.

Anderson, L., DiTomaso, J., Hrusa, G.F. & Rejmanek, M. (1999). *The CalEPPC List: Exotic Pest Plants of Greatest Ecological Concern in California*. California Invasive Plant Council, Berkeley, CA.

Bartolome, J.W., Stroud, J.C. & Heady, H.F. (1980). Influence of natural mulch on forage production on differing California annual range sites. *J. Range Mgmt*, 33, 4–8.

Brown, R.L. & Fridley, J.D. (2003). Control of plant species diversity and community invasibility by species immigration: seed richness versus seed density. *Oikos*, 102, 15–24.

Burke, M.J.W. & Grime, J.P. (1996). An experimental study of plant community invasibility. *Ecology*, 77, 776–790.

Collins, K.A. (1979). *Geology of the Northern California Coast Range Preserve, Mendocino County, California*. The Nature Conservancy, California Chapter, San Francisco, CA.

Connell, J.H. & Slatyer, R.O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.*, 111, 1119–1144.

Corbin, J.D. & D'Antonio, C.M. (2004). Competition between native perennial and exotic annual grasses: implications for an historical invasion. *Ecology*, 85, 1273–1283.

Crawley, M.J. & Long, C.R. (1995). Alternate bearing, predator satiation and seedling recruitment in *Quercus robur* L. *J. Ecol.*, 83, 683–696.

D'Antonio, C.M. (1993). Mechanisms controlling invasion of coastal plant communities by the alien succulent *Carprobrotus edulis*. *Ecology*, 74, 83–95.

D'Antonio, C., Levine, J. & Thomsen, M. (2001). Ecosystem resistance to invasion and the role of propagule supply: a California perspective. *J. Med. Ecol.*, 2, 233–246.

Davis, M.A. & Pelsor, M. (2001). Experimental support for a resource-based mechanistic model of invasibility. *Ecol. Lett.*, 4, 421–428.

Dyer, A.R. & Rice, K.J. (1999). Effects of competition on resource availability and growth of a California bunchgrass. *Ecology*, 80, 2697–2710.

Elton, C.S. (1958). *The Ecology of Invasions by Animals and Plants*. University of Chicago Press, Chicago, IL.

Field, C.B., Daily, G.C., Davis, F.W., Gaines, S., Matson, P., Melack, J. *et al.* (1999). *Confronting Climate Change in California: Ecological Impacts on the Golden State*. Union of Concerned Scientists and Ecological Society of America, Cambridge and Washington.

Hamilton, J.G., Holzapfel, C. & Mahall, B.E. (1999). Coexistence and interference between a native perennial grass and non-native annual grasses in California. *Oecologia*, 121, 518–526.

Harper, J.L. (1977). *Population Biology of Plants*. Academic Press, New York, NY.

Heady, H.F., Foin, T.C., Hektner, M.M., Taylor, D.W., Barbour, M.G. & Barry, W.J. (1977). Coastal prairie and northern coastal scrub. In: *Terrestrial Vegetation of California* (eds Barbour, M.G. & Major, J.). Wiley-Interscience, New York, NY, pp. 733–760.

Herring, M.L. (1997). *Heath and Marjorie Angelo Coast Range Preserve*. University of California, Oakland, CA.

Jesson, L., Kelly, D. & Sparrow, A. (2000). The importance of dispersal, disturbance, and competition for exotic plant invasions in Arthur's Pass National Park, New Zealand. *N. Zeal. J. Bot.*, 38, 451–468.

Johnson, S.P. (1979). Forest encroachment into abandoned fields in the northern California Coast Range. MA Thesis, San Francisco State University, San Francisco, CA.

Keddy, P.A. (1981). Experimental demography of the sand-dune annual, *Cakile edentula*, growing along an environmental gradient in Nova Scotia. *J. Ecol.*, 69, 615–630.

Kolb, A., Alpert, P., Enters, D. & Holzapfel, C. (2002). Patterns of invasion within a grassland community. *J. Ecol.*, 90, 871–881.

Kotanen, P.M. (1997). Effects of experimental soil disturbance on revegetation by natives and exotics in coastal Californian meadows. *J. Appl. Ecol.*, 34, 631–644.

Kotanen, P.M. (2004). Revegetation following soil disturbance and invasion in a Californian meadow: a 10-year history of recovery. *Biol. Invasions*, 6, 245–254.

Kriticos, D.J., Sutherst, R.W., Brown, J.R., Adkins, S.W. & Maywald, G.F. (2003). Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp *indica* in Australia. *J. Appl. Ecol.*, 40, 111–124.

Leung, B., Drake, J.M. & Lodge, D.M. (2004). Predicting invasions: propagule pressure and the gravity of allee effects. *Ecology*, 85, 1651–1660.

Levine, J.M. (2000). Species diversity and biological invasions: relating local process to community pattern. *Science*, 288, 852–854.

Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecol. Lett.*, 7, 975–989.

Lonsdale, W.M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, 80, 1522–1536.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.*, 10, 689–710.

Mortimer, A.M. (1976). Aspects of the seed population dynamics of *Dactylis glomerata* L., *Holcus lanatus* L., *Plantago lanceolata* L., and *Poa annua* L. *Proceedings 1976 British Crop Protection Conference–Weeds*, British Crop Protection Council, London, pp. 687–694.

National Assessment Synthesis Team (2000). *Climate Change Impacts on the United States: the Potential Consequences of Climate Variability and Change*. US Global Change Research Program, Washington, DC.

Peart, D.R. (1982). Experimental analysis of succession in a grassland at Sea Ranch, California. PhD Thesis, University of California, Davis, CA.

Peart, D.R. (1989). Species interactions in a successional grassland. II. Colonization of vegetated sites. *J. Ecol.*, 77, 252–266.

Pitcher, D. & Russo, M.J. (1988). *Element Stewardship Abstract for Holcus lanatus*. The Nature Conservancy, Arlington, TX.

Pitt, M.D. & Heady, H.F. (1978). Responses of annual vegetation to temperature and rainfall patterns in northern California. *Ecology*, 59, 336–350.

Pope, V.D., Gallani, M.L., Rowntree, P.R. & Stratton, R.A. (2000). The impact of new physical parameterizations in the Hadley Centre climate model: HadAM3. *Clim. Dyn.*, 16, 123–146.

Robinson, G.R., Quinn, J.F. & Stanton, M.L. (1995). Invasibility of experimental habitat islands in a California winter annual grassland. *Ecology*, 76, 786–794.

Rouget, M. & Richardson, D.M. (2003). Inferring process from pattern in plant invasions: a semimechanistic model

incorporating propagule pressure and environmental factors. *Am. Nat.*, 162, 713–724.

Seabloom, E.W., Harpole, W.S., Reichman, O.J. & Tilman, D. (2003). Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proc. Natl Acad. Sci. USA*, 100, 13384–13389.

Stromberg, M.R. & Griffin, J.R. (1996). Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. *Ecol. Appl.*, 6, 1189–1211.

Thompson, J.D. & Turkington, R. (1988). The biology of Canadian weeds 82: *Holcus lanatus* L. *Can. J. Plant Sci.*, 68, 131–147.

Tilman, D. (2004). Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proc. Natl Acad. Sci. USA*, 101, 10854–10861.

Turnbull, L.A., Crawley, M.J. & Rees, M. (2000). Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, 88, 225–238.

Tyser, R.W. & Worley, C.A. (1992). Alien flora in grasslands adjacent to road and trail corridors in Glacier National Par, Montana (U.S.A.). *Conserv. Biol.*, 6, 253–262.

Von Holle, B. & Simberloff, D. (2004). Testing Fox's assembly rule: does plant invasion depend on recipient community structure? *Oikos*, 105, 551–563.

Watt, T.A. (1978). The biology of *Holcus lanatus* L. (Yorkshire fog) and its significance in grassland. *Herb. Abstr.*, 48, 195–204.

Williamson, J. & Harrison, S. (2002). Biotic and abiotic limits to the spread of exotic revegetation species. *Ecol. Appl.*, 12, 40–51.

Zar, J.H. (1999). *Biostatistical Analysis*. Simon & Schuster, Upper Saddle River, NJ.