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Reduced herbivore resistance in introduced smooth cordgrass (*Spartina alterniflora*) after a century of herbivore-free growth

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Abstract We compared resistance to insect herbivory in two introduced populations of smooth cordgrass (*Spartina alterniflora*) differing in their history of herbivory. One population in Willapa Bay, Washington, has spread in the absence of herbivory for more than a century, while another population in San Francisco, California, was introduced 20 years ago and is fed upon by the *Spartina*-specialist planthopper, *Prokelisia marginata*. The planthopper is a sap-feeder common on the Atlantic and Gulf coasts of North America, where smooth cordgrass is native. Smooth cordgrass plants from Willapa Bay (WB), San Francisco Bay (SFB), and Maryland (the source of the SFB introduction) were exposed to *P. marginata* herbivory over two consecutive summers in a common greenhouse environment, and their growth was compared with that of control plants that were grown herbivore-free. The planthoppers had relatively little effect on the growth of SFB plants, with plants exposed to herbivores averaging 77% and 83% of the aboveground biomass of herbivore-free controls after the first and second season of herbivory, respectively. The growth of plants from Maryland was similarly little-affected by the planthoppers, with the plants exposed to herbivores averaging near 100% of the biomass of herbivore-free controls after two seasons. In contrast, the growth of the WB plants was greatly reduced by the planthopper, with the plants exposed to planthopper herbivory averaging only 30% and 12% of the aboveground biomass of herbivore-free controls after the first and second seasons of herbivory, respectively. By the end of the second season of herbivory, 37% of the WB plants exposed to herbivory had died, while none of the SFB plants exposed to herbivores had died. Among WB clones, there was variation in resistance; one WB clone suffered 0% mortality while another suffered 100% mortality when exposed to herbivores. Short-term herbivory experiments with the putative founder clone for the WB population suggested that the WB founder was similar to the more resistant WB clones in its susceptibility to planthopper herbivory. Nitrogen analyses of green leaf tissue indicated that WB plants, including the WB founder clone, averaged 70% more total leaf nitrogen than SFB and Maryland plants. In a planthopper choice experiment, more planthoppers were observed on WB plants than SFB plants after 95 days of exposure to herbivory. Planthopper preference for WB plants may have contributed to the lower resistance of WB plants to herbivory; however, even before planthoppers had become more abundant on the WB plants, the proportion of leaves with 50% or more dead tissue averaged significantly greater on the WB plants, suggesting a difference between populations in tolerance to herbivory as well. Multiple factors, including a founder effect, further loss of herbivore tolerance, and herbivore preference for WB plants, appear to account for the reduced planthopper resistance in the WB population.

Key words Herbivore resistance · Herbivore tolerance · Herbivore preference · Founder effect · Invasive plants

Introduction

Founder effects, genetic drift, and natural selection can all lead to genetic differentiation in populations of an invading species, relative to the invader's source population. Genetic differences between introduced populations and putative native source populations of plant species have been reported at the level of allozymes (Schwaegerle and Schaal 1979; Brown and Marshall 1981; Barrett and Shore 1989; Rejmanek et al. 1991; Novak and Mack 1993), DNA markers (Rejmanek et al. 1991), phenotypic traits (Wagner 1983; Glover and Barrett 1987; Olivieri et al. 1991), and mating systems (Glover and Barrett 1986; Bailey 1994), but genetic differentiation for specific ecological traits important in interspecific interactions has rarely been investigated in introduced plant

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populations (but see Jain and Martins 1979; Olivieri 1984; Blossey and Notzöld 1995). An ecological character of particular interest in introduced plant populations is response to herbivory. Because many introduced plant species invade new geographic sites that lack many or all of their native insect herbivores (DeBach and Rosen 1991), there is the possibility that a population freed from herbivory might lose adaptations to herbivory over time (Janzen 1975), as has been suggested for some grass populations that were excluded from mammalian grazing pressures for several decades (Painter et al. 1989) and purple loosestrife introduced to the United States from Europe (Blossey and Notzöld 1995). In this study, we tested for genetic differentiation with respect to herbivore resistance between two introduced Pacific populations of smooth cordgrass (*Spartina alterniflora*) with differing histories of herbivory at their sites of invasion.

Smooth cordgrass, a 1- to 2-m-tall perennial saltmarsh grass native to the Atlantic and Gulf Coasts of North America, is presently invading open-mud intertidal habitats in both Willapa Bay, Washington and San Francisco Bay, California. In Willapa Bay, smooth cordgrass was introduced in 1894 with oyster shipments from either the New York City area or Chesapeake Bay (Sayce 1988); the founding propagule may have consisted of a single clone. This putative founding clone, identified as a homogeneous circular patch from early aerial photographs, still survives today at its original site of establishment (Stiller and Denton 1995). A comparison of DNA markers between the putative founding clone and other plants in Willapa Bay suggested that all plants were descendants of the putative founding clone, although the descendants were not all genetically identical due to sexual recombination in this hexaploid species (Stiller and Denton 1995). Over the past century, smooth cordgrass in Willapa Bay has spread to occupy over 1000 ha of open mud, growing in the absence of insect herbivores. In contrast, smooth cordgrass in San Francisco Bay is fed upon by the planthopper, *Prokelisia marginata* (Homoptera), a *Spartina* specialist that is also common where smooth cordgrass is native, along the Atlantic and Gulf Coasts of North America (Denno et al. 1980; Strong and Stiling 1983). The San Francisco Bay smooth cordgrass population was introduced in the mid-1970s via seeds from a native population in Maryland (Daehler and Strong 1994), and the planthopper was present in San Francisco Bay before the introduction of smooth cordgrass (Lane 1969).

In this study, we compared resistance to planthopper herbivory between the Willapa Bay smooth cordgrass population, which has not experienced insect herbivory for more than a century, and the San Francisco Bay and Maryland populations, which have historically been exposed to insect herbivory. The goals of our study were: (1) to test whether plants from Willapa Bay are less resistant to planthopper herbivory than plants from San Francisco Bay and Maryland; (2) to test planthopper preference as a possible mechanism responsible for differences in resistance between populations; and (3) to address the possibility of a founder effect in the Willapa Bay population by comparing resistance in the putative Willapa Bay founder clone to that of other plants. Because smooth cordgrass in Pacific estuaries reduces native shorebird feeding habitats, threatens oystering grounds, and clogs flood control channels (Daehler and Strong 1996), we were also interested in implications of genetic differentiation among populations for the prospects of biological control of this noxious invader.

Materials and methods

Measuring the effect of herbivores

Seeds of smooth cordgrass (*Spartina alterniflora*) were collected in fall 1992 from four sites spanning the plant's current distribution in Willapa Bay, Washington (Diamond Point, Leadbetter Point, Oysterville, and Palix River). Seeds from the San Francisco Bay population were collected from about 100 individual smooth cordgrass clones located near the species' original site of introduction at Coyote Hills Slough in south San Francisco Bay. All seeds were stored over winter in 50% sea water at 4°C and germinated in spring 1993. Five seedlings from each population were vegetatively propagated for herbivory experiments. The five seedlings from the San Francisco Bay population were randomly chosen, one from each of five different maternal plants, while the five Willapa Bay seedlings were also chosen randomly with the restriction that one seedling was chosen from each of the four collection sites (two from Diamond Point were used, for a total of five seedlings).

The seedlings were grown over summer 1993 and winter 1994 in 30x45x13 cm pans containing a mixture of 25% Bodega Bay intertidal mud and 75% vermiculite (by volume). These plants were housed together in a greenhouse at Bodega Bay in northern California. In March 1994, each plant was divided into 12 clonal replicates consisting of three to five shoots. We attempted to allocate equal root biomass to each replicate. These replicates were individually transferred to 2.8-l pots containing the same mixture of vermiculite and mud. Extra clonal replicates were also reserved for later use in the planthopper choice experiment. In fall 1993, smooth cordgrass seeds were obtained directly from a Maryland population (Environmental Concerns Inc., St. Michaels, Md.), and eight random seedlings that germinated in spring 1994 were transferred to 2.8-l pots for use in the herbivory experiment.

In May 1994, the aboveground biomass of each clonal replicate was estimated by measuring the length of all stems. Aboveground dry biomass is highly correlated with stem length in smooth cordgrass (r=0.98, Daehler and Strong 1995), allowing accurate, non-destructive estimates of aboveground biomass to be made from measurements of stem lengths. Within each clone, the 12 replicates were paired for equal size, and one replicate was randomly assigned to the herbivory treatment, while the other was deferred to the no-herbivory control group. The eight plants from Maryland were also paired by size and randomly assigned to the herbivory treatment or the no-herbivory control group. All plants were then transferred to a 2.5x3.5 m greenhouse that had been divided in half with a fine mesh fabric (American Agrifabrics Inc., Alpharetta, Ga.) that prevented most planthopper dispersal between sides. Each half of the greenhouse held a large water table containing 33% seawater, and all plants that had been assigned to the herbivory treatment were placed in the water table on one side of the greenhouse. The initial side to be used for the herbivory treatment was chosen randomly, and a total of approximately 300 adult planthoppers (*P. marginata*) collected from San Francisco Bay were evenly sown onto plants in the herbivory treatment. All pots in the herbivory treatment were in close proximity, allowing planthoppers to move freely from pot to pot within the herbivory treatment. The herbivore-free control plants were placed in the identical water table on the opposite side of the greenhouse.

Every week during the summer and every 2 weeks during the winter, the location of plants within each treatment was re-randomized to minimize position effects, while every 2 weeks (every 3–4 weeks in the winter), the side of the greenhouse used for the herbivory treatment was switched by moving all plants in the herbivory treatment (and their associated planthoppers) to the opposite side of the greenhouse and placing the no-herbivory plants where the herbivory treatment had been. Throughout the summer, the water tables were maintained with approximately 4 cm of standing water by adding fresh water as needed, and every 4 weeks, 32 g Plantex 20-20-20 fertilizer dissolved in water was added to each water table. Over the summer, completely dead, shedding leaves (usually the oldest leaves) were removed by hand, simulating the natural removal of dead leaves by tidal action under field conditions and allowing natural growth of the plants. Every few weeks, we counted the number of planthoppers on three to five plants to obtain rough estimates of planthopper densities in the herbivory treatment over time. Planthoppers occasionally found on plants in the no-herbivory treatment were removed by hand, and planthopper densities on the no-herbivory treatment always averaged fewer than one planthopper per two plants. With this experimental design, each plant pair is treated as a replicate, since plants making up pairs were identified before the start of the experiment, and rotation was applied to all pairs in the same fashion (C. M. Drake, personal communication).

In mid-October 1994, the aboveground biomass of all plants was estimated by measuring the length of all stems and shoots. The planthoppers were then removed by spraying each plant with a watering hose outside the greenhouse. The herbivore-free control plants were also sprayed with water. This October herbivore removal simulated a natural die-back of the planthopper population that occurs over winter in the field (Roderick 1987; Daehler and Strong 1995).

In mid-April 1995, the stems of all plants were again measured to estimate combined over-winter and early spring growth. Approximately 300 adult planthoppers were then added to the herbivory treatment, simulating the pulse in the planthopper population that occurs in the field in spring (Roderick 1987; Daehler and Strong 1995). As in 1994, the herbivory treatment plants were exposed to planthopper herbivory from April until late September 1995. All stems and shoots from all plants were then harvested and dried to a constant mass at 55°C to determine final biomass.

Throughout the experiment, plants in the greenhouse were exposed to natural sunlight, and large open vents at the sides and top of the greenhouse provided a semi-open-air environment. Greenhouse temperatures averaged around 23°C by day and 12°C by night in the summers. Winter temperatures averaged about 2°C lower, both day and night. On two sunny summer days, temperatures in the greenhouse peaked at 33°C, while on the coldest winter nights, temperatures dropped as low as 3°C. This range of temperatures is similar to that in both San Francisco and Willapa Bays (National Ocean Service 1994), although peak summer temperatures in both Willapa Bay and San Francisco Bay exceeded those in our greenhouse, and extreme low temperatures in Willapa Bay were lower than those in our greenhouse.

Impact of herbivory on the Willapa Bay founding clone

Clonal fragments dug from the putative founding clone (Stiller and Denton 1995) were obtained from K. Sayce (Willapa Bay, Wa.) in early April 1995 and grown out in greenhouse pans in the same mixture of intertidal mud and vermiculite used for the other plants. April was the earliest date that the putative founder clone could be sampled and shipped to us. After 3 months, the founder clone had grown sufficiently to be divided into ten clonal replicates. The clonal replicates consisted of two to five stems each and were transferred to 2.8-l pots. After 1 month of growth in the pots, the replicates were measured, paired by size, and added to the herbivory experiment, with one clonal replicate from each pair exposed to planthopper herbivory and the other kept herbivore-free. The herbivory treatment plants were exposed to the planthoppers for 8 weeks, from August through September and their aboveground biomass was then compared to that of the herbivore-free controls.

Planthopper choice experiment

In January 1995, extra clonal replicates from April 1994 were further divided in replicates containing five to seven stems each and transferred to 2.8-l pots. Aboveground biomass of the plants was estimated by measuring all stems in April, and ten plants from Willapa Bay (four different clones) were paired by size with ten plants from San Francisco Bay (five different clones). Pots were transferred to a small (2x2 m) greenhouse and plant pairs were placed in close proximity so that the leaves of plant pairs intermingled. In early May, each plant was inoculated with seven third- or fourth-instar planthopper nymphs, and the number of planthoppers on each plant was counted every 1–2 weeks over the summer to determine whether the planthoppers favored the Willapa Bay plants over the San Francisco Bay plants. The position of plant pairs in the greenhouse was rotated every week to minimize position effects, and each plant pair was fertilized every 4 weeks with 2 g of Plantex 20-20-20 dissolved in water. Individual planthopper feeding scars on leaves are usually difficult to score; however, collective feeding by multiple planthoppers can accelerate leaf senescence and kill tissue directly (C. C. Daehler, personal observation). As an estimate of the impact of herbivory, in late August we counted the proportion of leaves on each plant with 50% or more dead (brown) tissue. If a plant contained more than 100 leaves, only the first 100 leaves encountered were scored.

In early August 1995, six fresh Willapa Bay-San Francisco Bay plant pairs that had grown throughout the summer without herbivores were added to the herbivore choice experiment to determine whether planthopper preference differed in mid-summer. No planthoppers were placed on these fresh plants, rather they were positioned in pairs in close proximity to infested plants, and the planthoppers were allowed to colonize these plants naturally. Planthopper counts on these new plants were made from August through September.

Nitrogen analyses of leaf tissues

Green leaves from herbivory and herbivore-free plants that were harvested in early September 1995 were dried at 55°C, ground to a powder in an Alpine mill, passed through a #40 mesh and analyzed for total nitrogen content by the University of California Davis DANR Analytical Laboratory (Davis, Calif.) using a nitrogen gas analyzer (Sweeney 1989). Where possible, leaves from two replicate pots of each clone were analyzed separately from both the herbivory and herbivore-free treatments; however, for two of the five Willapa clones, leaves from herbivory treatment replicates had to be pooled to obtain sufficient material for analysis. Total nitrogen content was also measured from green leaves of all eight Maryland plants and four replicates of the Willapa founder clone.

Statistical analyses

All statistical analyses were conducted using Systat version 5.01 for Windows (Systat Inc., Evanston, Ill.). Differences between San Francisco Bay and Willapa Bay plants in resistance to herbivory were statistically analyzed using the difference between the logarithms of biomass of the herbivore-free plant and the herbivory treatment plant for each plant pair as the response variable. This measure has been employed in comparing the fitness of selfed and outcrossed progeny in studies of inbreeding depression (Johnston and Schoen 1994). As in analyses of population differences in inbreeding depression, it is the ratio between the biomass of the herbivory and herbivore-free plants that is of interest in comparing the *response to herbivory* between populations, not the absolute, scale-dependent differences in biomass between herbivory and herbivore-free plants (Johnston and Schoen 1994). The logarithmic transformation makes the ratio between herbivory and herbivore-free plants additive in the ANOVA and thus appropriate for comparing populations and interpreting significant population effects in the ANOVA as differences in response to herbivory between the two populations (Johnston and Schoen 1994). In the ANOVA, biomass measurements were treated as repeated measures over time (fall 1994, spring 1995, and fall 1995), place of origin (San Francisco Bay or Willapa Bay) was treated as a fixed factor, and clone nested within origin was considered a random factor (Neter et al. 1990). Although differences in log-transformed biomasses were used for all statistical analyses, untransformed ratios between biomass of herbivory and herbivore-free plants are presented in the figures for their relative ease of interpretation.

For the planthopper choice experiment, differences in planthopper numbers on Willapa Bay versus San Francisco Bay plants were analyzed using ANOVA, with number of planthoppers counted on a plant at each census date as a repeated measure, plant origin as a fixed factor and plant pair as a random factor. Significant differences between Willapa bay and San Francisco Bay plants at each census period were then tested for using univariate *F*-tests (Wilkinson 1992).

Differences in nitrogen content of leaf tissues were compared with analysis of variance using log-transformed values for nitrogen content to improve normality. Origin of the plant (San Francisco Bay, Willapa Bay, Willapa Bay founder, or Maryland) and herbivory treatment were both assigned as fixed factors in the ANOVA (Neter et al. 1990). All tests for significance based on ANOVAs used type III sums of squares (Wilkinson 1992).

Results

Herbivore loads

By May 1994, plants in the herbivory treatment averaged about 10 planthoppers per plant. Cohorts of eggs hatched almost synchronously approximately every 3 weeks, increasing the population over time. In June, planthopper densities averaged around 30 per plant and between July and August planthopper densities ranged from 50 to 100 per plant. By September, a few weeks before removal of the planthoppers, densities were estimated at above 200 per plant (mostly nymphs). The buildup of the planthopper population observed in the greenhouse over summer is also characteristic of planthopper populations in the field, and the densities of planthoppers in the greenhouse experiment were similar to densities that commonly occur in the field in San Francisco Bay (Daehler and Strong 1995). The greenhouse planthopper population densities in 1995 followed a similar pattern to that in 1994.

Growth response to herbivores

There was a highly significant effect of plant origin on growth response to herbivores (Table 1), reflecting a much greater impact of herbivores on Willapa Bay plants than on San Francisco Bay plants. The significant effect of clone nested within origin (Table 1) indicated variation among clones within each population in their response to herbivory (Figs. 1 and 2). A significant origin-by-time interaction was due to an increasing difference between the Willapa Bay plants and the San Francisco Bay plants in their response to herbivory over time. The longer the exposure to herbivory, the greater the difference between the response of the Willapa Bay and San Francisco Bay populations (Figs. 1 and 2).

All of the Willapa Bay clones were less resistant to herbivory than the San Francisco Bay clones (Fig. 2). At the end of the first season, San Francisco Bay herbivory plants averaged 77% of the biomass of herbivore-free plants, while Willapa Bay herbivory plants averaged only

Table 1 Repeated-measures analysis of variance on the difference between log-transformed biomasses of herbivore-free and herbivore-exposed plants. *Origin* is the place of plant origin (San Francisco or Willapa Bay). A significant effect of origin indicates a difference between Willapa Bay and San Francisco Bay populations in their response to herbivory. *Time* represents the time of biomass measurement (fall 1994, spring 1995, or fall 1995). Greenhouse-Geisser ε=0.86; conclusions are not affected by Greenhouse-Geisser correction for non-sphericity

	df	MS	*F*	*P*
Between subjects				
Origin	1	51.17	36.11	<0.001
Clone (Origin)	7	1.42	2.55	0.03
Error	37	0.56		
Within subjects				
Time	2	4.30	10.0	<0.001
Time×Origin	2	4.70	10.9	<0.001
Time×Clone (Origin)	14	0.43	2.97	0.001
Error	74	0.15		

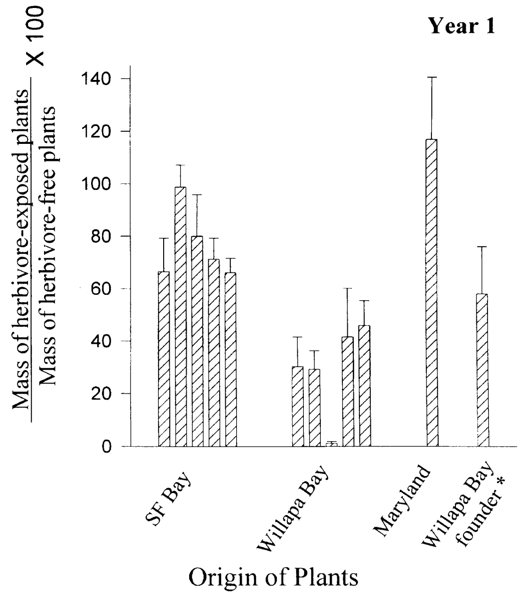

Fig. 1 Mass of herbivore-exposed plants relative to no-herbivory plants after one summer (22 weeks) of exposure to herbivory by the planthopper *Prokelisia marginata*. From *left* to *right*, the five *bars* in the San Francisco Bay population represent clones coy84, coy70, coy140, coy81, and coy106, while the five *bars* in the Willapa Bay population represent clones diam10, lead2, oyst10, palix5, and diam5. *The response of the Willapa Bay founder clone to 22 weeks of herbivory was estimated by linear extrapolation of the response to 8 weeks of herbivory (see Results). *Error bars* represent 1 SE

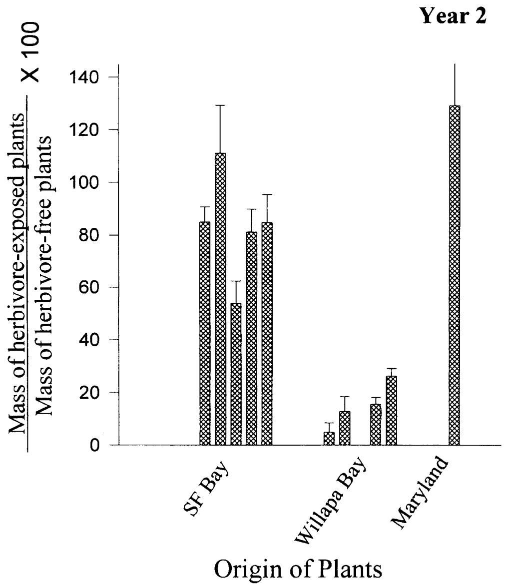

Fig. 2 Final mass of herbivory plants relative to non-herbivory plants after two summers of exposure to herbivory by the planthopper *P. marginata*. From *left* to *right*, the five *bars* in the San Francisco Bay population represent clones coy84, coy70, coy140, coy81, and coy106, while the five *bars* in the Willapa Bay population represent clones diam10, lead2, oyst10 (blank), palix5, and diam5. All plants in the oyst10 herbivory treatment died. *Error bars* represent 1 SE

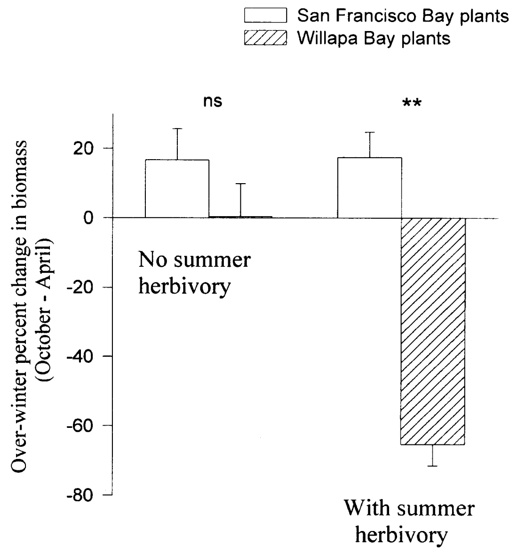

Fig. 3 Over-winter percent change in biomass for Willapa Bay and San Francisco Bay plants. All treatments were free of planthoppers over the winter. Willapa Bay summer herbivory plants lost biomass over winter, while San Francisco Bay summer herbivory plants gained biomass (**significant difference between populations, $P<0.001$, *ns* no significant difference between populations, $P>0.05$) *Error bars* represent 1 SE. We also tested whether the difference in proportional change in biomass between the no-herbivory and herbivory treatments differed between populations. The difference in change in biomass was significantly greater for the Willapa Bay population, indicating a significantly stronger effect of herbivory on the over winter growth of the Willapa Bay plants (t-test on clone means, $t=3.05$, Bonferroni-corrected $P=0.03$)

Table 2 Mortality of smooth cordgrass after two seasons of greenhouse growth in herbivore-exposed plants and herbivore-free controls

	Mortality (% of clonal replicates)	
	Herbivory	No herbivory
San Francisco Bay	0	0
Willapa Bay	37	3
Willapa Bay – by clone		
Diam5	0	0
Palix8	17	17
Lead2	17	0
Diam10	50	0
Oyst2	100	0

30% of the biomass of herbivore-free plants (Fig. 1). At the end of the second summer of herbivory, San Francisco Bay herbivory plants averaged 83% of the biomass of herbivore-free controls, while Willapa Bay herbivory plants averaged less than 20% of the biomass of herbivory-free controls (Fig. 2).

Between October 1994 and April 1995, all plants grew without herbivores, and San Francisco Bay plants increased in biomass by an average of 17% during this period (Fig. 3). During the same period, the Willapa Bay plants that had been herbivore-free over the summer increased in biomass by an average of 1% (not significantly different from the San Francisco Bay plants), while the Willapa Bay plants that had been exposed to herbivory during the summer lost an average of 65% of their biomass, even though the herbivores had been removed (Fig. 3).

After two seasons of herbivore exposure, none of the San Francisco Bay plants in either of the treatments had died (Table 2). Among the Willapa Bay plants, a single plant in the no-herbivory treatment died, while 11 plants (37%) in the herbivory treatment died (Table 2). There was variation in mortality rate among the Willapa Bay herbivory clones, with one clone suffering 100% mortality and another 0% mortality (Table 2).

The response of Maryland plants to herbivores was similar to that of the San Francisco Bay plants, with plants exposed to herbivory attaining similar biomass to herbivore-free plants (Figs. 1 and 2). The replicates of the Willapa Bay founder clone were only exposed to 8 weeks of herbivory, and after that period of exposure, the herbivory plants averaged 85% of the biomass of herbivore-free plants. A linear extrapolation for the effect of 22 weeks of herbivory (as experienced by the other Willapa Bay plants during the first year) gives an estimated response of the Willapa Bay founder clone herbivory plants having only 58% of the biomass of herbivore-free plants, which is a stronger response to herbivory than observed among the San Francisco Bay plants (Fig. 1). This estimate of the Willapa founder clone's response to herbivory may be conservative because the detrimental effects of herbivory on growth are probably compounded over time to some degree, rather than simply linear.

Growth of herbivore-free control plants

By the end of the two seasons of greenhouse growth, the herbivore-free Willapa Bay plants had increased in biomass by 7.2 times on average, while the San Francisco Bay herbivore-free plants had increased in biomass by an average of 24 times. This overall difference in means between populations was significant (*t*-test, t=6.13, P<0.01); however, there was also overlap between the Willapa Bay and San Francisco Bay clones in net herbivore-free growth. For example, San Francisco Bay clone coy81 and Willapa Bay clone lead2 both averaged a 13-fold increase in biomass after two seasons of growth. The Willapa Bay clone that suffered 100% mortality in the herbivory treatments did not grow less in the absence of herbivory than other Willapa Bay clones (Tukey test after ANOVA, P>0.56 for all comparisons). In fact, two other Willapa Bay clones (diam10 and palix8) averaged smaller increases in biomass when not exposed to herbivores.

Planthopper choice experiments

Following the introduction of 7 planthoppers to each plant in May, the planthoppers multiplied over the summer to densities exceeding 100 per plant by September (Fig. 4). Planthoppers were observed to move freely and frequently between leaves of Willapa Bay and San Francisco Bay plants. There were no significant differences in number of planthoppers on San Francisco Bay versus Willapa Bay plants during the first 66 days of the initial experiment, however by days 95 and 108, the Willapa Bay plants had attracted significantly more planthoppers than the San Francisco Bay plants (univariate F-tests, P<0.05). On the final census (day 122), the Willapa Bay plants continued to average more planthoppers than San Francisco Bay plants, but this difference was not significant at the P=0.05 level ($F_{1,8}$=3.70, P=0.09). For the plants exposed to planthoppers beginning in August, there was a similar trend of initially similar numbers of planthopper on both San Francisco Bay and Willapa Bay, followed by higher average numbers of planthoppers on Willapa Bay plants after 28 days (Table 3), but these differences were not statistically significant for any census period (univariate F-tests, P>0.26).

At day 95, the percentage of leaves with 50% or more dead (brown) tissue on the Willapa Bay plants averaged 64% and was significantly greater than San Francisco Bay plants, which only averaged 28% of leaves with 50% or more dead tissue (paired t-test, t=6.5, P<0.001). The difference in the proportion of dead or dying leaves was a record of *Prokelisia* damage up to around day 85, since tissue does not completely brown until 7–10 days after feeding (C. C. Daehler, unpublished work). The greater damage to Willapa Bay plants was therefore observed before differences in planthopper densities between San Francisco Bay and Willapa Bay plants had developed, suggesting the Willapa Bay plants are less tolerant of planthopper herbivory, in addition to being preferred by the planthoppers over time.

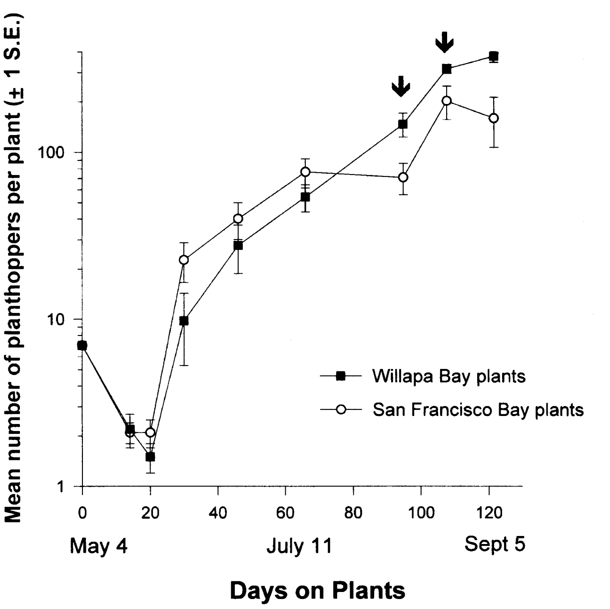

Fig. 4 Comparison of mean number of planthoppers on Willapa Bay and San Francisco Bay plants over time, when inoculated in May. *Arrows* indicate significant differences between populations. There were no significant differences between populations until after day 94 when Willapa Bay plants had significantly higher planthopper loads than San Francisco Bay plants

Table 3 Planthopper counts on smooth cordgrass when planthoppers were given a choice between plants from San Francisco Bay and Willapa Bay, beginning in August

Days of exposure	Average number of planthoppers per plant	
	San Francisco Bay	Willapa Bay
6	38±15	37±12
13	79±23	77±25
28	162±40	213±32
43	117±24	143±20

Nitrogen analyses

Throughout the summer, the greenest leaves among the Willapa Bay plants were a darker green than the greenest leaves on San Francisco Bay plants, suggesting higher leaf nitrogen content in Willapa Bay plants (Munsell 1977 color reference 7.5 GY 4/2 to 4/4 for Willapa Bay versus 7.5 GY 4/6 to 5/6 for San Francisco Bay). The nitrogen analyses showed that planthopper herbivory did not affect total green leaf nitrogen content, however total leaf nitrogen content did differ significantly between the San Francisco Bay and the Willapa Bay plants (Table 4). The Willapa Bay clones averaged 70% more nitrogen in their leaf tissues than San Francisco Bay clones. The Maryland plants were similar to the San Francisco Bay plants in nitrogen content, while the Willapa Bay foun-

Table 4 Comparison of average percent nitrogen content in green leaf tissue for smooth cordgrass plants from San Francisco Bay, Willapa Bay and Maryland in herbivore-exposed and no-herbivory treatments (±1 SE). Values with different *superscripts* are significantly different (Bonferroni-corrected linear contrasts, $P<0.05$)

	Origin of plants			
	San Francisco Bay	Willapa Bay founder	Willapa Bay	Maryland
Herbivory	0.884 ± 0.056^{a}	1.656 ± 0.092^{b}	1.437 ± 0.015^{b}	0.947 ± 0.027^{a}
No Herbivory	0.946 ± 0.048^{a}	1.432 ± 0.056^{b}	1.304 ± 0.160^{b}	0.993 ± 0.090^{a}

der clone was similar to other Willapa Bay clones in nitrogen content (Table 4). Although leaf nitrogen measurements were made only in September, the consistent color difference between the Willapa Bay and San Francisco Bay plants suggested that they differed in nitrogen content throughout the summer.

Discussion

Herbivore resistance refers to any genetically controlled quality that results in one population (or other unit of interest) being less damaged by a particular herbivore than another (Kennedy and Barbour 1992). Based on above-ground biomass measurements from five clones, the Willapa Bay, Washington population was less resistant to herbivory than the San Francisco Bay and Maryland populations. Below-ground biomass measurements were not made in this study because of difficulty separating roots from vermiculite; however, in previous studies of herbivory on smooth cordgrass, above-ground biomass was strongly correlated with below-ground biomass (r=0.95, Daehler and Strong 1995). In an earlier trial herbivory experiment, shoot-root ratios for 4-month-old seedlings averaged near 1 for both Willapa Bay and San Francisco Bay plants (C. C. Daehler, unpublished work). General observations of root and rhizome growth in the present study gave no indication of a difference in shoot-root ratios between the two populations (C. C. Daehler, personal observation). The higher sensitivity of the Willapa Bay population to herbivory could be the result of a founder effect, genetic drift, natural selection in Willapa Bay, or any combination of these factors.

Evidence for a founder effect

The sensitivity of the putative Willapa Bay founder clone to short-term herbivory suggested that the difference in resistance between the Willapa Bay and San Francisco Bay populations may in part be due to a founder effect. The difference in total leaf nitrogen between the Willapa founder clone and San Francisco and Maryland plants also suggests a founder effect, since all other Willapa Bay clones tested shared the higher leaf nitrogen content of the founder clone. The Willapa Bay founder clone and other Willapa Bay clones also differed from the San Francisco Bay and Maryland plants in that they generally produced smaller leaves, but more numerous shoots (C. C. Daehler, unpublished work). Sayce (1988) reported "dense-type" growth among seedlings and older smooth cordgrass plants in the field in Willapa Bay. These "dense-type" plants were similar in morphology to our greenhouse-grown Willapa Bay plants, suggesting that the growth characteristics observed in the greenhouse may be common in the field. Under common growing conditions, clones of smooth cordgrass collected at various sites along the Atlantic coast of North America vary greatly in growth characteristics like stem density and size (Anonymous 1992), indicating that geographic differences between the original source populations of smooth cordgrass in Willapa Bay and San Francisco Bay may be one cause of differences between these populations.

Evidence for further loss of resistance to herbivory by drift or selection

High variation in resistance to herbivory among the Willapa Bay clones suggests that some plants in Willapa Bay have lower resistance to planthopper herbivory than others as a result of either drift or selection. In particular, clone oyst10 suffered 100% mortality under planthopper herbivory, indicating an extremely low herbivore resistance relative to other clones tested and relative to the Willapa Bay founder clone. This especially low resistance to herbivory was not due to an intrinsically slower growth rate relative to other Willapa Bay clones, nor could the lower resistance be attributed to unusually high leaf nitrogen content (oyst10 averaged 1.56% nitrogen, compared to the overall average of 1.43% for all Willapa Bay clones). The geographic location of the oyst10 maternal plant at Oysterville, relatively close to the site of the founder clone (approximately 14 km across the bay) compared to the other sites from which plants were tested, does not provide evidence for a cline of decreasing resistance to herbivory with increasing distance from the founder clone. Oyst10 would not be predicted to survive in a habitat where *P. marginata* is present, and the finding of a clone with such a low level of resistance in the Willapa Bay population suggests that some plants in Willapa Bay now have lower herbivore resistance than the founder.

Possible causes of differences in resistance

Many sap-feeding insects are known to transmit viral diseases to their host plants (Raccah and Irwin 1988; Nault and Ammar 1989), and one possibility is that the Willapa Bay plants were highly susceptible to an unknown debilitating virus transmitted by *Prokelisia*, resulting in apparently low resistance to herbivory among Willapa Bay plants. We find this hypothesis unlikely since planthoppers were occasionally found on the no-herbivory plants and they would probably have inoculated the no-herbivory Willapa Bay plants with the virus as well. The herbivore-free Willapa Bay plants appeared healthy in all respects.

Kennedy and Barbour (1992) list four plant strategies for reducing damage by herbivores: (1) association with other species; (2) escape in time or space; (3) tolerance; or (4) physical or chemical defenses, including resistance due to non-preference herbivores. In the case of smooth cordgrass, which generally grows in monospecific stands, the differential response to herbivory between the two populations probably involves a combination of differences in planthopper preference and differences in plant tolerance to herbivory.

The Willapa Bay plants were preferred by the planthoppers over San Francisco Bay plants after an initial period of planthopper colonization. This delayed preference was probably not due to seasonal changes in the plants because when fresh plants were added to the experiment in mid-summer, preference again required several weeks to develop. The planthoppers may have been attracted to the higher nitrogen content of Willapa Bay plant leaves, and this high nitrogen content may have become more apparent to the planthoppers either following a period of sampling different plants (Sogawa 1982) or following some initial plant damage that increased free amino acid concentrations in the leaf tissue (Bacheller 1990). In a New Jersey saltmarsh, Denno et al. (1980) found the highest densities of *P. marginata* on leaves that had the highest crude protein content. Crude protein is correlated with total leaf nitrogen (Denno 1983), suggesting that the planthoppers prefer leaf tissue with high nitrogen. *Prokelisia* densities were also higher in those smooth cordgrass stands having higher overall levels of crude protein (Denno et al. 1980), and in experimentally fertilized smooth cordgrass plots (Vince et al. 1981; Stiling et al. 1991), suggesting that the planthoppers are attracted to high-nitrogen stands. Other planthopper species are similarly attracted to higher nitrogen plants in the field (Kushwaha and Chand 1988). These observations suggest the *Prokelisia* planthoppers prefer leaf tissue that is higher in nitrogen, and this is a likely reason for the planthopper preference for Willapa Bay plants observed in this study.

Herbivore preference is probably not the sole cause of differences in resistance between the populations because a higher proportion of dead or dying leaves were observed on Willapa Bay plants even before differences in the number of planthoppers between Willapa Bay and San Francisco Bay plants had developed. The differences in morphology between plants of the Willapa Bay and San Francisco Bay populations may also have contributed to differences in planthopper tolerance. The shorter, thinner stems and leaves, on average, of the Willapa Bay plants probably allowed a relatively greater proportion of the plant's tissue to be physically accessible by the planthoppers' short feeding stylets.

Blossey and Notzöld (1995) hypothesized that if resources are limited and resistance to herbivory is costly, then introduced plant populations growing in the absence of herbivory may evolve increased allocation to biomass at the expense of allocation to resistance to specialized herbivores. In contrast, we found that plants with faster growth rates had higher resistance to herbivory. Even within populations, the correlation between plant resistance and plant performance without planthoppers was positive (r=0.65 and r=0.95 for the San Francisco and Willapa Bay populations, respectively). In a comparison of two populations of the grass *Agropyron smithii* differing in grazing history, plants from the historically defoliated population grew faster and may have been better able to capture resources and store them below-ground (Polley and Detling 1988). Slower overall growth of the herbivore-free Willapa Bay plants compared to the herbivore-free San Francisco Bay plants could have led to decreased herbivore tolerance in the Willapa Bay plants, since plants with more rapid growth may often be more tolerant to herbivory (Rosenthal and Kotanen 1994).

The "trade-offs" approach to predicting the relationship between resistance and growth rate (Blossey and Notzöld 1995) does not consider effects of inbreeding (Charlesworth and Charlesworth 1987; Husband and Schemske 1996), which could act to reduce the average biomass of plants in introduced populations, especially if populations are established from a single introduction of a few founders. Inbreeding depression is likely to be an important factor affecting plant growth rates in introduced populations of smooth cordgrass since inbred (selfed) progeny have 30–90% slower growth rates than outbred progeny (Daehler 1996). Extreme inbreeding in the Willapa Bay population would have been unavoidable given that the population was founded from a single clone, and the slower growth of Willapa Bay plants in the absence of herbivory could be due to greater effects of inbreeding in that population, relative to the more recently introduced San Francisco Bay plants. Inbred individuals of *Impatiens palida* had slower growth rates than outcrossed individuals and also suffered greater mortality under herbivory (McCall et al. 1994). If inbreeding depression is responsible for slower growth in the Willapa Bay plants, then the founder (presumably outcrossed) should be more vigorous than the average inbred progeny now established in Willapa Bay. Unfortunately this test has very low power using the data from this experiment, and a larger experiment would be needed to provide conclusive evidence of more rapid growth in the Willapa Bay founder. An alternative explanation for the observed population growth rate differences could be that the Willapa Bay plants were less well adapted than San Francisco Bay plants to our greenhouse growing conditions, despite our maintenance of greenhouse temperatures within the normal range experienced by the plants in southern Washington.

Implications for biocontrol

Current efforts to control established smooth cordgrass in Willapa Bay involve herbicides or cutting, and the effectiveness of these expensive methods over large areas is questionable (Aberle 1993). Previous field studies of the effects of *P. marginata* herbivory on smooth cordgrass in San Francisco Bay indicated that the planthopper would be unlikely to limit the spread of smooth cordgrass there due to its minor impact on the plants (Daehler and Strong 1995). However, the low resistance of the Willapa Bay population to herbivory by *P. marginata*, as observed in this greenhouse study, suggests biocontrol of smooth cordgrass in Willapa Bay may be an option worth exploring in Washington State.

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References

Aberle B (1993) The biology and control of introduced *Spartina* (cordgrass) worldwide and recommendations for its control in Washington. Masters Thesis, Evergreen State College, Olympia, Washington

Anonymous (1992) Annual report, Cape May plant materials center. US Department of Agriculture Soil Conservation Service, Cape May, New Jersey

Bacheller JD (1990) The effect of salinity, soil aeration, and insect predation on the free amino acid, glyclinebetaine, and soluble protein levels of the salt marsh cordgrass, *Spartina alterniflora*. MS Thesis, University of South Florida, Tampa

Bailey JP (1994) Reproductive biology and fertility of *Fallopia japonica* (Japanese knotweed) and its hybrids in the British Isles. In: Wall LC de, Child LE, Wade PM, Brock JH (eds) Ecology and management of invasive riverside plants. Wiley, New York, pp 141–158

Barrett SCH, Shore JS (1989) Isozyme variation in colonizing plants. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides, Portland, pp 106–126

Blossey B, Notzöld R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83:887–889

Brown AHD, Marshall DR (1981) Evolutionary changes accompanying colonization in plants. In: Scudder GGT, Reveal JL (eds) Evolution today. Proceedings of the second international congress of systematic and evolutionary biology. Carnegie-Mellon University, Pittsburgh, pp 351–363

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Daehler CC (1996) Seed set variability, inbreeding depression, and effects of herbivory in introduced smooth cordgrass (*Spartina alterniflora*) invading Pacific estuaries. PhD Dissertation, University of California, Davis

Daehler CC, Strong DR (1994) Variable reproductive output among clones of *Spartina alterniflora* (Poaceae) invading San Francisco Bay, California: the influence of herbivory, pollination, and establishment site. Am J Bot 81:307–313

Daehler CC, Strong DR (1995) Impact of high herbivore densities on introduced smooth cordgrass, *Spartina alterniflora*, invading San Francisco Bay, California. Estuaries 18:409–417

Daehler CC, Strong DR (1996) Status, prediction, and prevention of introduced cordgrass (*Spartina* spp.) invasions in Pacific estuaries, USA. Biol Conserv 78:51–58

DeBach P, Rosen D (1991) Biological control by natural enemies. Cambridge University Press, Cambridge

Denno RF (1983) Tracking variable host plants in space and time. In: Denno RF, McClure MS (eds) Variable plants and herbivores in natural and managed systems. Academic Press, New York, pp 291–341

Denno RF, Raupp MJ, Tallamy DW, Reichelderfer CF (1980) Migration in heterogeneous environments: differences in habitat selection between the wing forms of the dimorphic planthopper, *Prokelisia marginata* (Homoptera: Delphacidae). Ecology 61:859–867

Glover DE, Barrett SCH (1986) Variation in the mating system of *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). Evolution 40:1122–1131

Glover DE, Barrett SCH (1987) Genetic variation in continental and island populations of *Eichhornia paniculata* (Pontederiaceae). Heredity 59:7–17

Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70

Jain SK, Martins PS (1979) Ecological genetics of the colonizing ability of rose clover (*Trifolium hirtum* All.). Am J Bot 66:361–366

Janzen DH (1975) Behavior of *Hymenaea courbaril* when its predispersal seed predator is absent. Science 189:145–147

Johnston MO, Schoen DJ (1994) On the measurement of inbreeding depression. Evolution 48:1735–1741

Kennedy GG, Barbour JD (1992) Resistance variation in natural and managed systems. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens ecology, evolution and genetics. University of Chicago Press, Chicago, pp 13–41

Kushwaha KS, Chand H (1988) Effect of transplanting date and nitrogen level on the incidence of white backed planthopper in paddy. Indian J Entomol 50:12–16

Lane RS (1969). The insect fauna of a coastal salt marsh. Masters Thesis, San Francisco State College, San Francisco

McCall C, Waller DM, Mitchell-Olds T (1994) Effects of serial inbreeding on fitness components in *Impatiens capensis*. Evolution 48:818–827

Munsell Color (1977) Munsell color charts for plant tissues. Munsell Color, New Windsor, New York

Nault LR, Ammar ED (1989) Leafhopper and planthopper transmission of plant viruses. Annu Rev Entomol 34:503–529

National Ocean Service (1994) United States coast pilot 7. Pacific Coast, California, Oregon, Washington, and Hawaii. US Government Printing Office, Washington

Neter J, Wasserman W, Kutner MH (1990) Applied linear statistical models. Irwin, Boston

Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* (Poaceae): comparison between native and introduced populations. Heredity 71:167–176

Olivieri I (1984) Effect of *Puccinia cardui-pycnocephali* on slender thistles (*Carduus pycnocephalusa* and *C. tenuiflorus*). Weed Sci 32:508–510

Olivieri I, Gouyon PH, Prosperi IM (1991) Life cycles of some Mediterranean invasive plants. In: Groves RH, DiCastri F (eds) Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, pp 145–157

Painter EL, Detling JK, Steingraeber DA (1989) Grazing history, defoliation, and frequency-dependent competition: effects on two North American grasses. Am J Bot 76:1368–1379

Polley HW, Detling JK (1988) Herbivory tolerance of *Agropyron smithii* populations with different grazing histories. Oecologia 77:261–267

Raccah B, Irwin ME (1988) Techniques for studying aphid-borne virus epidemiology. In: Kranz J, Rotem J (eds) Experimental techniques in plant disease epidemiology. Springer, Berlin Heidelberg New York, pp 209–222

Rejmanek M, Thomsen CD, Peters ID (1991) Invasive vascular plants of California. In: Groves RH, DiCastri F (eds) Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, pp 81–101

Roderick GK (1987). Ecology and evolution of dispersal in Californian populations of a salt marsh insect, *Prokelisia marginata*. PhD Dissertation, University of California, Berkeley

Rosenthal JP, Kotanen PM (1994) Terrestrial plant tolerance to herbivory. Trends Ecol Evol 9:145–148

Sayce K (1988) Introduced cordgrass, *Spartina alterniflora* Loisel. in salt marshes and tidelands of Willapa Bay, Washington (US Fish and Wildlife Service report). Willapa Bay National Wildlife Refuge, Ilwaco

Schwaegerle KE, Schaal BA (1979) Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. Evolution 33:1210–1218

Sogawa K (1982) The rice brown planthopper: feeding physiology and host plant interactions. Annu Rev Entomol 27:49–73

Stiling P, Brodbeck BV, Strong DR (1991) Population increases of planthoppers on fertilized salt-marsh cordgrass may be prevented by grasshopper feeding. Fla Entomol 74:88- 97

Stiller JW, Denton AL (1995) One hundred years of *Spartina alterniflora* (Poaceae) in Willapa Bay, Washington: random amplified polymorphic DNA analysis of an invasive population. Mol Ecol 4:355–363

Strong DR, Stiling P (1983) Wing dimorphism changed by experimental density manipulation in a plant hopper *Prokelisia marginata* (Homoptera, Delphacidae). Ecology 64:206–209

Sweeney RA (1989) Generic combustion method for determination of crude protein in feeds: collaborative study. J Assoc Off Anal Chem 72:770–774

Vince SW, Valiela I, Teal JM (1981) An experimental study of the structure of herbivorous insect communities in a salt marsh. Ecology 62:1662–1678

Wagner LK (1983) The population biology of an introduced weedy annual: *Spergula arvensis*. Botany. PhD Dissertation, University of California, Berkeley

Wilkinson, L (1992) Systat for Windows: statistics, version 5 edn. Systat, Evanston