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Davis, Heather G.

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The Ecology of an Invasive Grass, *Spartina alterniflora*

By

HEATHER GRAHAM DAVIS
B.A. (University of California, Santa Barbara) 1987
M.A. (California State University, San Francisco) 1998

DISSERTATION

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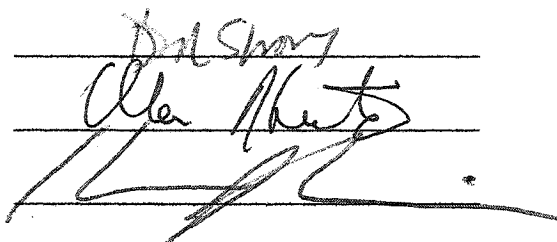
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of the

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Heather Graham Davis
September 2004
Population Biology

The Ecology of an Invasive Grass, *Spartina alterniflora*

Abstract

There are a wide variety of ecological and genetic factors that influence the rate of

population growth. As an invading species changes its habitat, varying selective regime

may change it in turn. *Spartina alterniflora* Loisel. has been introduced around the world

and is a clonal estuarine weed of both environmental and economic concern. Recruitment

by seed is a relatively uncommon event in the native range of this plant where fitness is

enhanced by vegetative persistence. Invaded regions provide an essentially unoccupied

niche facilitating rapid propagation by seed. The invaded community and the source

location may differ in selective pressure leading to a distinct response trajectory in the

invader. Genetic adaptation can occur as a result of intrinsic ecological selective

pressures, including Allee effects, reduction of some component of fitness with

decreasing population density, and low genetic variability. This wind-pollinated exotic

suffers from an Allee effect, caused by pollen limitation. The Allee effect reduces

fecundity at a more than a ten-fold magnitude at low density, contrasting with the

competitive, highly dense nature of the native range. Knowledge of density effects is a

critical, though often omitted, requirement for empirical testing of much life-history

evolution theory. Changes in life history traits following selection can then affect the rate

and characteristics of population growth. I found the invader to have much greater

reproductive effort, initiate reproduction both younger and smaller, has a greater risk of

death with greater reproductive effort and to have greater self-compatibility than natives. The study of the interaction of biology and life history evolution of an invading organism with the ecology of the recipient community provides an opportunity to address the consequences to subsequent population expansion.

Additionally, we assessed pathogen-vectoring risks of a planthopper, *Prokelisia marginata*, proposed as an agent of biological control. We identified a list of possible pathogens and amplified sections of bacterial DNA and sequenced the fragments to assay whether bacterial DNA extracted from both plants and insects, is potentially pathogenic. We found no evidence of transmissible pathogens but found that putatively specific PCR primers for *Pseudomonas* bacteria amplified a variety of other bacteria.

The use of molecular assays to identify plant pathogenic organisms vectored by biological control agents

Heather G. DAVIS[1,2,*], Dino GARCIA-ROSSI[2], Melissa DAOUST[2] and Donald R. STRONG[2,3]

[1]*Center for Population Biology, University of California, Davis, CA 95616, USA;* [2]*Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923, USA;* [3]*Section of Evolution and Ecology, University of California, Davis, CA 95616, USA*
*Author for correspondence; e-mail: hgdavis@ucdavis.edu

Abstract. The planthopper *Prokelisia marginata* Van Duzee (Homoptera: Delphacidae) has been considered for the biological control of the weed *Spartina alterniflora* Loisel (Poaceae) in Willapa Bay, Washington, U.S.A. *Prokelisia marginata* is a stenophagous phloem-feeding insect with the potential to transmit bacterial plant diseases that could be moved by less-specific vectors to other plant species. Initial assays with PCR primers that are putatively specific for phytoplasmas gave positive results in *Spartina*. However, subsequent analyses did not indicate the transmission of the pathogen by the planthopper. We sequenced the 16S ribosomal RNA (rRNA) gene of the bacterial species that gave positive results in PCR. Comparisons with sequences available in GenBank suggested that the positive results using the putatively specific PCR primers were due to the presence of such bacteria as *Pseudomonas*, *Holomonas*, *Vibrio*, and *Acinetobacter*. We did not find phytoplasmas in either *Spartina* or the planthopper *P. marginata*.

Key words: biological control, ecological safety, Homoptera, indirect effects of introductions, invasive plant, pathogen, phytoplasma, *Prokelisia marginata*, *Spartina alterniflora*, vector

Introduction

Ecological safety is an important element of biological control (Thomas and Willis, 1998; Strong and Pemberton, 2000; Pemberton and Strong, 2000). Recent developments in molecular techniques now allow assessment of the infrequently considered consequence that plant diseases can accompany introductions of biological control insects. The introduction of phytophagous insects as control agents can be a source of introduced microbes due to the vectoring of novel plant diseases. Homoptera, which feed by means of stylet insertion into the plant vascular system, vector more than 90% of insect-transmitted diseases in plants (Eastop, 1977). Introduced Homoptera can

carry exotic, and potentially cryptic (Lee et al., 1998), diseases into a new region; they also can spread pathogens already present (see Bezark, 1999). The use of highly specific PCR assays that do not depend on obvious symptomology in plant hosts has great potential to assist in the appraisal of the risk of disease transmission by biological control insects.

Spartina alterniflora (smooth cordgrass) is a tall, dense grass that grows in the intertidal habitat of estuaries in extensive monocultures (Radford et al., 1968). It is native to the East and Gulf coasts of North America and has become a highly invasive weed in some Pacific estuaries. In San Francisco Bay, California and Willapa Bay, Washington, it has spread rapidly, invading mud flats that are largely unoccupied by vascular plants. The Washington Department of Natural Resources, the Washington Department of Agriculture, and both State and Federal Departments of Fish and Wildlife have attempted both chemical control with glyphosate and mechanical control by mowing. These expensive methods have been only modestly successful.

The phloem feeding planthopper *Prokelisia marginata* Van Duzee (Homoptera: Delphacidae) has been studied as a possible biological control agent for *Spartina alterniflora* at Willapa Bay, WA. This insect is native to the East and Gulf coasts of North America as well as to California, where it is a monophagous herbivore of the native California cordgrass, *Spartina foliosa* (Denno et al., 1987). In greenhouse experiments, *S. alterniflora* from Willapa Bay, Washington was shown to be susceptible to stunting and death when exposed to *P. marginata* from San Francisco Bay (Daehler and Strong, 1997).

Of possible relevance for the contemplated biological control of *Spartina alterniflora* by *Prokelisia marginata* in Willapa Bay, WA are non-culturable phloem-limited phytoplasmas. Phytoplasmas were previously known as mycoplasma-like organisms (MLO's) and phytopathogenic mollicutes (Purcell, 1982). Phytoplasmas inhabit plant phloem and, though infections are systemic, pathological effects are seen in plant parts where concentrations of bacteria are highest (Kuske and Kirkpatrick, 1992). The effects of phytoplasma diseases can be severe, including plant death, but not all plant species infected with and supporting phytoplasmas have disease symptoms. Some phloem-feeding leafhoppers, planthoppers and treehoppers acquire these pathogens by feeding upon plants, and plants acquire them only by being fed upon by infected insects (Purcell, 1982).

Preliminary work in the greenhouses at the Bodega Marine Laboratory (BML) suggested that phytoplasmas could be involved with pathology associated with *Spartina alterniflora* from Willapa Bay that was fed on by *Prokelisia marginata*. The symptoms of shortened internode length, reduced root biomass, narrower leaves, shorter leaves, and proportionally fewer large

vascular bundles that we observed (Daehler and Strong, 1997; personal observation) are reminiscent of those produced by phytoplasmas (Kirkpatrick, personal communication). Our assays with PCR primers that are used as diagnostic tools for phytoplasmas (Smart et al., 1996) indicated this bacterial pathogen could be associated with a disease in *Spartina*. Here we present the results of the screening for potential transmissible plant pathogens on a phloem-feeding planthopper, *P. marginata*. We illustrate that the molecular primers available for detecting these pathogens are not sufficiently specific to distinguish the pathogens from related, apparently benign microbes. We also underscore the impossibility of assessment of all risks; it is not feasible to consider every potential pathogen.

Materials and methods

PCR detection for phytoplasmas. We used two pairs of putatively pathogen specific molecular primers to screen for phytoplasmas. A CTAB (cetyltrimethylammonium bromide) method of extraction (Zhang et al., 1998) and amplification of diagnostic DNA method (Smart et al., 1996) were modified from the literature. Differences in extraction technique included: leaf samples were ground in liquid nitrogen, sterile materials were used for all stages of the extraction and pellets were dried in a laminar flow hood.

For PCR testing for phytoplasmas, each reaction had a volume of 30 μl. For each reaction, the following volumes were used: H_2O 17.35 μl; dNTPs 0.45 μl (1.5 mM); 10X $MgCl_2$-free PCR buffer (Promega, Madison, Wisconsin) 3 μl; $MgCl_2$ (Promega, Madison, WI) 3 μl (3 mM); primer P1 (Deng and Hiruki, 1991) 3 μl (5 μM); primer Tint (Smart et al., 1996) or P7 (Schneider et al., 1995); 3 μl (5 μM); Taq polymerase 0.2 μl (5U/μl) (Promega, Madison, WI); DNA 2 μl (approximately 12 ng genomic DNA). Polymerase chain reaction was done using a Perkin Elmer 9600 thermocycler (Norwalk, CT). Amplification cycles were: 95 °C for 5 min.; 35 cycles of 95 °C for 1 min.; 56 °C for 1 min.; 72 °C for 2 min.; 72 °C for 10 min.; 4 °C until samples are removed from PCR. 15 μl of each sample was run on a 1.5% agarose gel. PCR products were stained with ethidium bromide and visualized by UV transillumination. Samples were scored positive if there was a band of the diagnostic length 1.7 kb (P1/Tint) and negative if there was no band at 1.7 kb and a non-diagnostic 200 kb band was present (see Smart et al., 1996). For primer pair (P1/P7), a sample was scored positive if there was a band of the diagnostic length 1.8kb (see Schneider et al., 1995) (Figure 1).

Plant species screened for the presence of phytoplasmas (P1/Tint and P1/P7) are: *Spartina alterniflora*, *S. foliosa*, *S. foliosa* × *alterniflora*, *S. anglica* Hubbard and a positive control of Elm Yellows extracted from *Cath-*
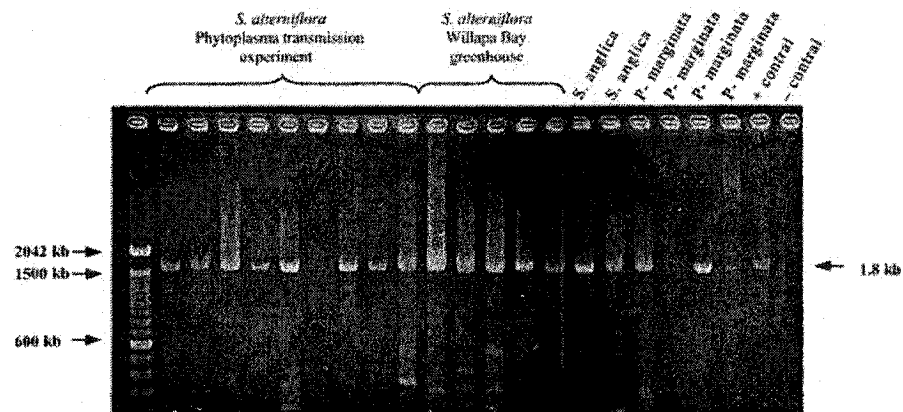
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Figure 1. Phytoplasma amplification. Ethidium bromide-stained agarose gel of PCR amplification products using primer pair P1/P7. Diagnostic band is at 1.8 kb. Size standard at left is a 100 bp DNA Ladder (GibcoBRL, Rockville, MD). Species extracted are included above each lane.

aranthus roseum acquired from the Kirkpatrick Laboratory at U.C. Davis (Table 1). Multiple extractions were performed on some plant individuals. *Prokelisia marginata* screened included: from the BML greenhouse, caged on yellowed and stunted plants in the BML greenhouse and from the field (San Francisco Bay) (Table 2). 10 or 30 planthoppers were pooled for each extraction.

Phytoplasma transmission by Prokelisia marginata. The objectives for this experiment were: (1) test the effectiveness of *Prokelisia marginata* to transmit phytoplasmas to *Spartina alterniflora* seedlings by molecular assay, and (2) test for symptoms of disease on infected *S. alterniflora* seedlings.

Spartina alterniflora seed from Virginia (Environmental Concern Inc., St. Michaels, MD) were germinated and grown in an insect free greenhouse. One hundred plants from the Virginia seedlings were randomly selected and grown for three months until they had developed sufficient roots, shoots and leaf tissue to sustain planthopper growth. Prior to the experiment a set of 20 randomly selected plants from the same cohort tested negative for phytoplasma by the molecular assay using PCR primers P1/Tint. At the end of the three months, vigorous plants were sorted into five groups of twenty; within groups, 10 plants were designated as control and the other 10 as experimental.

One hundred and fifty greenhouse reared 3rd instar *Prokelisia marginata*, originally from the San Francisco Bay, were placed in plastic cages on 10 *Spartina alterniflora* plants from Willapa Bay, WA, and one *S. anglica* from Puget Sound. Each plant received about 14 planthoppers. Each plant had previously tested consistently positive for phytoplasmas by PCR using

Table 1. Plant species, with location of origin (original field collection site) and collection and exposure to Prokelisia marginata, with number of accessions screened for phytoplasmas and testing positive (primers P1/Tint and P1/P7)

Species	Location of Collection	Location of Origin	Exposed to P. Marginata	P1/Tint		P1/P7	
				Screened	Positive	Screened	Positive
S. alterniflora	BML gh	CB	no	4	0	4	0
S. alterniflora	BML gh	CB	yes	100	27	100	13
S. alterniflora	BML gh	CB	no	10	1	10	1
S. alterniflora	BML gh	WB	no	15	1	15	3
S. alterniflora	BML gh	WB	yes	75	12	75	6
S. anglica	BML gh	PS	yes	17	7	17	4
S. anglica	PS	PS	no	5	1	5	0
S. foliosa × alterniflora	SFB	SFB	yes	19	2	19	1
S. patens	BML gh	unknown	yes	2	0	2	0
Catharanthus roseus*	UCD gh	UCD gh	N/A	7	7	7	7

CB – Chesapeake Bay, VA.
PS – Puget Sound, WA.
SFB – San Francisco Bay, CA.
WB – Willapa Bay, WA.
BML gh – Bodega Marine Laboratory greenhouse.
UCD gh – University of California Davis greenhouse.
*Positive control, infected with phytoplasma disease Elm Yellows.

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Table 2. Number of amplifications from pooled planthoppers, with location of origin (original field collection site) and collection, screened for phytoplasmas and testing positive (primers P1/Tint and P1/P7).

Species	Location of collection	Location of origin	P1/Tint		P1/P7	
			Screened	Positive	Screened	Positive
P. marginata	SFB	SFB	18	5		
P. marginata	BML gh	SFB	99	2	99	0
*P. marginata**	BML gh	SFB	15	0	15	0

CB – Chesapeake Bay, VA.
PS – Puget Sound, WA.
SFB – San Francisco Bay, CA.
WB – Willapa Bay, WA.
BML gh – Bodega Marine Laboratory greenhouse.
UCD gh – University of California Davis greenhouse.
*From eggs excised from leaves and reared on *S. alterniflora* raised from seed.

primers P1/Tint. In this experiment *P. marginata* were fed on 'infected' plants for a total of 15 days; this is ample time for a competent insect to acquire the pathogen (Purcell, 1982). At the end of the fifteen-day acquisition access period (AAP), the surviving *P. marginata* were pooled and randomly sorted into 10 groups of 10.

Prokelisia marginata were fed on the leaves of each group of 10 experimental plants for a total of seven days, confined inside a ventilated tube two cm in diameter. Each of the 10 experimental plants and 10 control plants was rigged with a plastic cage with one leaf per cage. Two days prior to the start of the incubation access period, number of shoots, number of leaves, chlorosis (cm of yellowing, measured from tip to base of each leaf contained within the plastic cages) and total shoot length were counted and measured for control and experimental plants. Every seven days the same planthoppers were serially transferred to new sets of 10 plants. In this manner, five transfers were performed.

At the end of a 40 day incubation period, the shoot number, leaf number, chlorosis and total shoot length of each group of control and experimental plants were measured. The data were analyzed as a 2-way MANCOVA (multiple analysis of covariance) (SAS 7.0) with treatments of insect pathogen acquisition and latent period. The covariates were the measurements of the response variables before application of the treatments. Finally, all *Spartina* plants and planthoppers were again tested with PCR for the presence of phytoplasmas.

Sequencing of 16S rRNA gene. As the results from the efforts described above were inconsistent for phytoplasmas (Table 1), we sequenced the PCR

product from the phytoplasma reactions. The 16S rRNA gene was sequenced from products of the diagnostic length of amplified DNA (examples of product in Figure 1) from 18 *Spartina* plants and planthoppers (5' end only, P1).

The DNA was amplified using the protocol described above with primer pair P1/Tint or P1/P7. The reaction volumes were increased to 50 μl. The concentration of the PCR product was visually estimated by running 5 μl of product from each reaction on a 1.5% agarose gel along with Gibco DNA mass ladder as a standard. PCR products from multiple reactions from an individual DNA extraction were combined as needed and directly purified using the Bio-Rad Prep-A-Gene DNA purification kit (Hercules, CA). The concentration of the purified PCR product was estimated as previously described and 15 μl of purified PCR product was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide and visualized by UV transillumination to check for the presence of non-specific bands. Samples that showed non-diagnostic bands then underwent gel purification to isolate the diagnostic bands. In such cases the bands were excised from a 1% agarose gel and purified using the BioRad Prep-A-Gene DNA purification kit. The final purified PCR generated products were sequenced at the Department of Biological Sciences (DBS) Automated DNA Sequencing Facility at the University of California at Davis using a Perkin Elmer ABI 377 Automated Sequencer. Eighteen sequences were submitted to GenBank with the accession numbers of AF288709 – AF288726. We performed BLAST searches (Altschul et al., 1997) on each of these sequences at the website supported by GenBank and recorded the 10 hits with the greatest similarities.

PCR amplification products were sequenced from extractions of: (1) plants from the phytoplasma transmission experiment. Two dead experimental (inoculated by *Prokelisia marginata*) plants (AF288709, AF288721), two live experimental plants (AF288710, AF288722), two dead control plants (AF288711, AF288713) one live control plant (AF288712), (2) one dead *S. alterniflora* seedling from seed collected in Virginia, raised in greenhouse with no planthoppers (AF288714), (3) five yellowed and stunted *S. alterniflora* from Willapa Bay, WA from BML greenhouse with *P. marginata* present (AF288715, AF288716, AF288717, AF288723, AF288724), (4) two *S. anglica* from BML greenhouse with *P. marginata* present (AF288718, AF288719), (5) one hybrid *S. foliosa* × *alterniflora* collected from San Lorenzo Marsh, San Francisco Bay, CA (AF288725), (6) *P. marginata*. One field collection of 30 planthoppers pooled extraction from San Lorenzo Marsh (AF288720) and one greenhouse pooled sample of 10 *P. marginata* that had been caged on yellowed and stunted Willapa *S. alterniflora* for 2 weeks (AF288726).

Results

Screening for phytoplasmas. Screening for the presence of phytoplasmas produced results inconsistent with the hypothesis and the mechanism of the hypothesis, including: individual plants extracted multiple times produced both positive and negative results, some plants never exposed to planthoppers gave positive results, plants 'inoculated' by planthoppers exposed to plants exhibiting symptoms did not produce positive results, plants that had extractions from both root and culm did not yield consistent results, planthoppers reared from eggs excised from leaves and reared on *Spartina alterniflora* raised from seed tested positive. Multiple overlapping bands were frequently produced from plants that were dead when extracted. This suggests that the amplified products may have been from multiple origins, possibly due to external or internal populations of saprophytic bacteria. We postulated, therefore, that the amplification of product was yielding false-positives. However, there was no indication that the technique produced false-negatives. The positive control lane contained a band of the appropriate weight in every gel with amplified product. This supports the conclusion of Smart et al. (1996) that the primer pairs P1/Tint and P1/P7 are reliable in not producing false-negatives.

Phytoplasma transmission by Prokelisia marginata. There were no overall significant differences between control and experimental plants for treatment effect of pathogen acquisition by *Prokelisia marginata* (Wilks' Lambda P = 0.9352). There was an overall significant effect of latent period (Wilks' Lambda P < 0.0001) and no overall significant effect of interaction between pathogen acquisition and latent period (Wilks' Lambda P = 0.2218). Results of screening of experimental and control plants and planthoppers using PCR and primer pairs P1/Tint and P1/P7 showed no treatment effect.

Sequencing results. Our results from analyses of fragment sequences amplified from the 16S rRNA bacterial gene isolated from *Spartina* and *Prokelisia marginata* did not indicate the presence of phytoplasmas. Sequence fragments with a mean number of 350 nucleotides generated by PCR amplification (using primers P1/Tint and P1/P7) were subjected to BLAST searches of GenBank (Altschul et al., 1997). The results of the searches indicated these sequences showed greatest similarity to 16S rRNA sequences from a number of common genera of gram-negative bacteria including *Halomonas*, *Pseudomonas*, *Acinetobacter* and *Vibrio*.

For sequences AF288709–AF288714 and AF288719–AF288721, all top ten hits were represented by *Pseudomonas spp.*, excepting between zero and four hits for each sequence were represented by unknown bacterial species. The Expect values (this parameter indicates the number of hits that would occur by chance when searching the database of a given size, decreasing exponentially with the score assigned between two sequences) were between –110 and –125. For sequences AF288715–AF288717, all top ten hits were represented by *Halomonas spp.*, excepting between two and four hits for each sequence were represented by unknown bacterial species. The Expect values were between –106 and –125. For sequences AF288718 and AF288722, both derived from insect extractions, all top ten hits were respectively represented by *Vibrio spp.* and *Acinetobacter spp.* excepting five and three hits represented by unknown bacterial species. The Expect values for the former ranged from –47 to –71 and the later from –41 to –53. All of the lowest scores denote perfectly matched sequences.

Discussion

In this experiment we tested the ability of *Prokelisia marginata* to vector phytoplasma pathogens. Ultimately, by analysis of nucleotide sequences of the evolutionarily conserved 16S rRNA gene, we showed that the bacteria in and upon *Spartina* that were detected by PCR were not derived from phytoplasmas. Seemueller and Kirkpatrick (1996) stress that the alternative methods they describe to identify phytoplasmas are similarly not foolproof. These results indicate that putative positive PCR results must be confirmed by sequencing the PCR products. The primer pair P1/P7 produced fewer positive results than the primer pair P1/Tint (Tables 1 and 2), suggesting P1/P7 has the greater specificity. There was no indication that the use of these primer pairs engendered false-negatives. Some error, consisting primarily of false-positives, in the use of these primers may be acceptable if the original identification of pathogen presence in a new species or population is confirmed via sequencing. Particular care should be applied to interpreting positive results from samples extracted from tissue with any decay present. Although we demonstrated that *P. marginata* did not transmit phytoplasmas, we did not test for other potential pathogens including viruses, fungi and other bacterial species. The decline in *S. alterniflora* from Willapa Bay, Washington (Daehler and Strong, 1997) after exposure to *P. marginata* is probably attributable to either the direct effects of the insect, possibly a toxin, or some unknown pathogenic agent.

The concern of false results of pathogen detection, both positive and negative, can hinder the implementation of biological control by insects.

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Ecological safety as well as agricultural safety requires assessment of the implications for introduction of novel pathogens and spread of already-present plant diseases by imported herbivorous insects. The risks associated with the introduction of *Prokelisia marginata* as a biological control agent to Willapa Bay, Washington has been researched far beyond that required under law. This effort also included host-specificity screening including types of the target species, species in the same genus as the target species, species in the same subfamily, species in the same family, species in other families of economic importance and threatened and endangered species. The proposal to introduce the Homopteran biological control agent, *P. marginata*, was submitted for a review to the Technical Advisory Group on the Biological Control of Weeds (TAG) (Grevstad et al., 2000). This review for the introduction of *P. marginata* from California to Washington is not obligatory. All 14 reviewers on the TAG committee recommended the release of *P. marginata* for the control of *Spartina alterniflora* to APHIS (the USDA's Animal and Plant Health Inspection Service) with approval number 46757.

Acknowledgements

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An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary

HEATHER G. DAVIS*¶, CAZ M. TAYLOR†, JANIE C. CIVILLE‡ and
DONALD R. STRONG§¶

*Population Biology Graduate Group, 2320 Storer Hall, University of California, Davis, California 95616, USA,
†Ecology Graduate Group, 2132 Wickson Hall, University of California, Davis, California 95616, USA, ‡Ecology
Graduate Group, 2320 Storer Hall, University of California, Davis, California 95616, USA, §Section of Evolution and
Ecology, 2320 Storer Hall, University of California, Davis, California 95616, USA, and ¶Bodega Marine Laboratory,
Box 247, Bodega Bay, CA 94923–0247, USA

Summary

1 *Spartina alterniflora* sets very little viable seed at the leading edges of an invasion in Willapa Bay, Washington, USA, where it was introduced c. 100 years ago. This largely outbreeding, rhizomatous grass recruits into previously unoccupied areas at low density, so young plants initially grow isolated from one another but eventually coalesce to form continuous meadows.

2 Isolated recruits set approximately one-tenth the seed of meadow plants at five sites, spread over the 230 km² of Willapa Bay mudflats, and this seed germinated at only one-third the rate observed in meadow plants.

3 The consistent patterns suggested that the low seed set in the isolated plants was largely due to the demographic effects of density. Differences between sites in the incidence and amount of seed set and germination rate indicated, however, that there was some environmental influence.

4 These data imply that plants in newly invaded, low-density areas produce little viable seed until rhizomatous growth brings them into close contact. This Allee effect could substantially reduce the rate of invasion.

Key-words: Allee effect, density, invasive species, reproduction, *Spartina alterniflora*

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Introduction

The demographic effects of density on individual reproductive success in exotic species can have profound environmental and economic consequences. Increasing density can raise the per capita rate of population growth when rare and thereby the rate of spatial spread (Hastings 1996). The Allee effect, defined as a positive relationship between any component of fitness and either numbers or density of conspecifics (Allee 1931; Stephens *et al.* 1999), could be a common, though largely uninvestigated, regulator of population growth in invasive species. Theory suggests, at the front of an invasion, where the diffusive effects of dispersal lead to low density, an Allee effect could slow or change the pattern of range expansion (Dennis 1989; Lewis & Kareiva 1993; Veit & Lewis 1996; Courchamp *et al.* 1999).

An Allee effect in plants could be caused by demographic, genetic or environmental mechanisms

(Courchamp *et al.* 1999; Stephens *et al.* 1999). For instance, reduced seed set, and thus recruitment, can result when pollen is limited due to low densities of pollen donors (Silander 1978; Antonovics & Levin 1980). This can be compounded by full or partial self-incompatibility when self-pollen is rejected (e.g. Menges 1991). Even wind-pollinated species can be limited by lack of pollen (Knapp *et al.* 2001; Koenig & Ashley 2003). But empirical evidence of an Allee effect caused by pollen limitation is confined to species with animal pollinators (e.g. Lamont *et al.* 1993; Groom 1998). Inbreeding depression and loss of heterozygosity can also reduce individual fitness when plants are forced to self-pollinate (Lande & Schemske 1985; Schemske & Lande 1985; Charlesworth & Charlesworth 1987; de Jong *et al.* 1993).

The perennial *Spartina alterniflora* Loisel. (smooth cordgrass), a native to the Atlantic and Gulf coasts of North America, grows on intertidal mudflats in extensive monocultures that often reach 1.5 m in height. Where it has invaded Pacific estuaries north of San Francisco, it is the sole emergent vascular plant. The inflorescence

Correspondence: Heather G. Davis (tel. +1 530 754 6456;
fax +1 530 752 1449; e-mail hgdavis@ucdavis.edu).

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Fig. 1 *S. alterniflora* inflorescence in male (upper) and female phase (lower). From top to bottom right: floret in male phase, female phase and with a ripe seed. Artwork by Jun Bando.

(Fig. 1) has loosely overlapping branching spikes and appressed florets (Hickman 1993). Each floret (also referred to as a spikelet or flower) contains an ovule that can form a single seed. Seeds do not separate from the floral structure, although they do detach from the inflorescence, and are dispersed, both locally and over long distances, in wrack mats on winter and spring tides, leaving no seedbank (Woodhouse 1979). Although *S. alterniflora* is wind pollinated and native populations are almost completely self-incompatible (Somers & Grant 1981), the population at our study site shows greater self-compatibility (H. G. Davis, unpublished data). Nevertheless, the invasive population in San Francisco Bay has substantial genetic load and suffers from inbreeding depression when self-pollinated (Daehler 1999).

This species was accidentally introduced to Willapa Bay, Washington, USA, *c*. 100 years ago and by 1997 it had colonized *c*. 60 of the 230 km² of previously bare intertidal mudflats. Each recruit germinates from a single seed and grows rhizomatously into a circular plant, comprised of a single genet, and with time, these merge to form dense continuous meadows. Due to the low density of recruits and lack of other emergent plants on the open mud, the progress of invasions, which is reminiscent of the growth of bacterial colonies on a Petri plate, is readily observed in aerial photographs (Fig. 2).

This study explores the effects of the isolation of recruits and of colonization history upon fecundity in the invading population, by comparing plants at the leading edge of the invasion, with those that have merged to produce continuous meadows. First we asked whether each plant could set any seed at all. If it did, then we investigated the effect of isolation on seed production and germination. Furthermore, we explored whether the site in Willapa Bay from which a plant originated affected the incidence and amount of seed set, and its germination rate.

Methods

We measured the effects of recruitment isolation of *S. alterniflora* plants on seed set during 2000 and 2001 and on germination rate in 2000 at Willapa Bay, Washington, USA. The Bay was divided into five sites: Long Island (LI), Peninsula (PN), South Bay (SO), Palix River (PX) and Shoalwaters (SH) (Table 1). We selected isolated plants, genets that had grown from a single seed into roughly circular clumps (Fig. 2), that were at least 1 m from each other and were often separated by tens of metres, and plants from dense meadows that had coalesced at different times (Table 1). The isolated and meadow plants occupied approximately the same range of tidal elevation at three of the sites (LI, PN, PX), but meadow plants were slightly higher at SO and slightly lower at SH.

Fig. 2 Oblique aerial photograph (courtesy of Fritzi Grevstad) of leading edge of *S. alterniflora* invasion at Willapa Bay.

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Table 1 History and location of sites

Site	Earliest evidence of colonization*	Earliest evidence of coalescence†	Latitude/ longitude
Long Island (LI)			
Meadow	1942‡	1985	46.48/–123.94
Isolated	1995	NA	46.47/–123.94
Peninsula (PN)			
Meadow	1955	1990	46.60/–124.03
Isolated	1970/95§	NA	46.60/–124.03
South Bay (SO)			
Meadow	1955	1995	46.38/–123.98
Isolated	1995	NA	46.39/–123.99
Shoalwaters (SH)			
Meadow	1990	2000	46.71/–123.83
Isolated	1995	NA	46.73/–123.97
Palix River (PR)			
Meadow	1985	2000	46.62/–123.92
Isolated	1995	NA	46.62/–123.94

NA = not applicable.
*Data from historical aerial photographs of Washington State Department of Natural Resources and US Army Corps of Engineers.
†Earliest dates that the sample sites were covered completely by *S. alterniflora* with no open mud visible in Washington State Department of Natural Resources historical aerial photographs.
‡Thomas Sheffer photo (1942, California Academy of Sciences Herbarium) shows *S. alterniflora* coalesced into a group *c.* 40 m diameter. Known growth rates would place the colonization date decades earlier.
§First date indicates earliest plants visible in photos. More plants colonized the area *c.* 1995. These plants had not coalesced by the time of this study (2000).

Germination, seedling growth and plant coalescence into meadows occurred between 1992 and 1999 (J. C. Civille, unpublished data). Thousands of seedlings characterized by attachment to the floret, filamentous root morphology and the absence of large rhizome attachments to other culms, were sampled during this period.

In October 2000, we collected inflorescences from 10 isolated plants and 10 meadow plants at each site. In meadows we chose individuals at least 20 m apart, walking perpendicularly to the tide gradient. We sampled within 1–2 m² areas to reduce the possibility of collecting from more than one genet. Where possible, we chose five inflorescences per plant that had not shattered and that were yellowing and snapped off easily (indicating that seeds were provisioned). We repeated sampling for four meadow and four isolated plants at three of the sites (LI, SO and PX) in autumn 2001.

To assess the role of increasing distance from the meadow, we laid out three parallel transects in 2000 approximately 50 m, 100 m and 150 m from the SO meadow, and collected inflorescences from 10 randomly selected plants per transect. *Spartina alterniflora* has recently colonized Gray's Harbor, *c.* 30 km north of Willapa Bay

(Sayce *et al.* 1997). We obtained up to five inflorescences from each of eight isolated plants at this site in 2000. No conspecific was visible; all were isolated by more than 250 m although most were separated by kilometres.

SEED SET AND GERMINATION RATE

Seed set was measured first by stripping and counting all florets from each inflorescence. Then, 50 florets (or fewer with small inflorescences) were chosen at random and screened for the presence of seeds. Seed set is expressed as the proportion of florets with seeds.

Florets collected in 2000 were placed in separate small zip-lock bags perforated with needle holes. Bags containing florets and seed were submerged in a single opaque plastic bin containing 1.75 L of autoclaved seawater and 5.75 L of distilled water to yield a salinity of *c.* 8‰. The bin was covered and placed in a refrigerator (4 °C) to simulate winter temperatures in the field. Every week the bags were drained for 2 hours and then resubmerged to oxygenate the water and reduce growth of microbes. After 2 months, by which time many of the seeds had begun to germinate, the bags were placed in a single layer in distilled water on a laboratory bench at room temperature. Number of germinations per inflorescence was scored 2 weeks later by counting the number of radicles. Germination rate for each inflorescence was calculated as the number of germinations per expected number of seeds (E_{seeds}) with E_{seeds} = (proportion of sampled florets with seeds) (total number of florets).

DATA ANALYSES

We used a logistic analysis with a binomial distribution to determine the likelihood of observing any seed set. Amongst those plants that did set seed, we asked whether the factors that are associated with zero seed set are also associated with lower numbers of seeds. To do this, we used an unbalanced ANOVA with the factors 'isolation' and 'site', the interaction, and the random variable 'plant', that is nested within the interaction. Germination rate was similarly analysed for all plants that had set seed. Differences between sites were explored using planned pairwise contrasts for the logistic analysis and post hoc Tukey pairwise comparisons for the ANOVAs. Seed set data were log transformed and 0.001 was added to the germination rate and then log transformed. As we are using percentages as outcomes in seed set and germination rate, inflorescences with more florets have more information than those with fewer. To compensate for this, the seed set ANOVA and logistic analysis weights the proportion with number of florets screened and the germination rate ANOVA weights data points proportional to the total number of florets of each inflorescence. This weighting scheme does not change the degrees of freedom. We used SAS version 8 for the analyses (SAS Institute Inc., Cary, NC, USA).
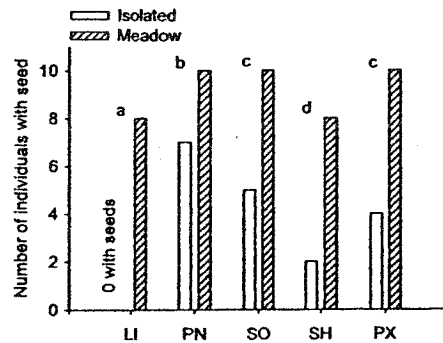
Fig. 3 Number of plants producing seed for each of the five sites. Twenty plants per site were observed, except for SH, where 10 meadow and nine isolated plants were observed. Sites (isolated and meadow) with different letters are statistically different at $P < 0.05$.

Table 2 Relationship between incidence of seed set (present or absent) and isolation and site in *S. alterniflora* in Willapa Bay, Washington, in 2000. Type III analysis of effects of logistic model

Source	d.f.	χ^2	P
Isolation	1	5603.72	< 0.0001
Site	4	3329.03	< 0.0001

All sites except PX and SO are statistically different from each other at $P < 0.05$ according to planned contrasts.
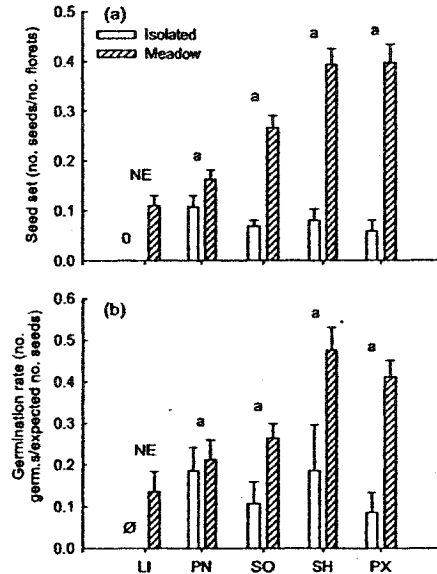

Fig. 4 Seed set (a) and germination rate (b) (means + 1 SE) of *S. alterniflora* in 2000 for isolated and meadow plants with at least some seed for each of the five sites. Untransformed data were used to generate graph. Sites with the same letter are not statistically different at $P < 0.05$. 'NE' denotes no least-squares means were estimable. '0' indicates no seed was set, 'Ø' indicates there were no seeds to test germination rate.

Results

RECRUITS

Spartina alterniflora has spread to new areas of Willapa Bay by seed that floats on the tide, with hundreds of seedlings observed each year from 1992 to 1999 (J. C. Civille, unpublished data) but with virtually no recruitment by rhizome fragments. Seedlings that survived the first winter were almost all spaced at intervals of 1 m to a few tens of metres.

SEED SET

In 2000, both the incidence of seed set and the quantity of seed produced were much greater in meadow plants than in recently established, isolated plants. The mean incidence differed among sites though the pattern remained consistent; overall 92% of meadow plants produced seed vs. 37% of isolated plants (Fig. 3, Table 2). The overall seed set was 0.2 (± 0.01, $n = 50$ plants) for meadow plants vs. 0.02 (± 0.004, $n = 49$ plants) in isolated plants.

Considering only those plants that had at least one seed, the proportion of florets containing a seed was 0.3 (± 0.02, $n = 46$) for meadow plants compared with 0.08 (± 0.01, $n = 18$) for recruits. In every site, meadow plants produced more seed than isolated plants, although some sites differed in mean seed set (Fig. 4, Table 3). The overall effect of site (Table 3) appears to be due solely to the very low production at LI (0.11 ± 0.02, $n = 8$) as there are no differences amongst the other sites (Fig. 4). The LSmean for site LI was not estimable, so direct comparisons were not possible.

The 2000 transects at SO isolates, showed that the mean seed set decreased with distance from the meadow, from 0.04 ± 0.01 near the meadow, to 0.02 ± 0.01, and 0.01 ± 0.004. Five of the eight very isolated plants sampled at Gray's Harbor set seed, with mean production 0.02 ± 0.01.

The seed set in 2001 was extremely low, but isolated plants again produced fewer seeds (0.004 ± 0.001, $n = 12$) than meadow plants (0.009 ± 0.005, $n = 12$).

GERMINATION

Germination rates paralleled the pattern of seed set (Fig. 4), with seeds from meadow plants more likely to germinate than seeds from isolates (0.34 ± 0.02, $n = 46$ vs. 0.13 ± 0.03, $n = 18$). While sites did differ in germination rate, this appears to be due solely to low germination at the oldest site, LI (0.01 ± 0.05, $n = 8$). As the LSmean was not estimable, comparisons with other sites were not possible. At SO, we found that the mean

Table 3 Relationship of seed set (seeds/50 or fewer florets) and germination rate (germinations/expected seeds) for the effects of isolation, site and plant for unbalanced nested ANOVAS

Analysis	Source	d.f.	Type III MS	F	P
Seed set	Isolation	1	1571.73	25.80	< 0.0001
	Site	4	304.80	5.02	0.0011
	Isolation × site	3	218.02	3.53	0.0186
	Plant (isolation × site)	55	80.74	2.80	< 0.0001
	Error	170	28.79		
	Total	233			
Germination rate	Isolation	1	120 686	14.54	0.0002
	Site	4	41 412	4.79	0.0012
	Isolation × site	3	10 173	1.21	0.3087
	Plant (isolation × site)	55	12 886	2.25	< 0.0001
	Error	170	5719.69		
	Total	233			

All observations with seed set equal to zero were removed from seed set and germination data sets. Seed set and germination rate data were log transformed.

germination rate, as well as seed set, decreased with distance from the meadow. For plants that set at least one seed, germination rate decreased from 0.02 (± 0.01 n = 6) at the closest transect to 0.01 (± 0.01, n = 2) at the other two. No seed from Gray's Harbor germinated.

Discussion

This study has documented the occurrence of an Allee effect during a large-scale invasion. The relatively isolated plants at the leading edge produce only c. one-tenth of the seed of well-established plants in high-density meadows; likewise, the germination was also reduced (c. 40% of the meadow plants). These differences were observed at all sites and were significant except for germination at PN.

Differences between sites indicate that some component of environmental variation probably affects seed set and germination rate. The ability to set any seed at all, showed only two of the sites to be the same (Fig. 3), whereas relative seed set and germination rate in plants that set at least some seed differed only between the longest site (LI) and the remainder (Figs 3 and 4). Despite these environmental differences, the Allee effect is consistent over the 43-km length of Willapa Bay, with isolated plants contributing much less to recruitment than plants in meadows.

The difference between seed set in meadow and isolated plants might be caused by pollen limitation. We have three observations that suggest that isolation decreases seed set. First, isolated plants were closer to the meadow at PN than at other sites, and more of these set seed, and those that did set more seed than isolated plants at other sites (Figs 3 and 4). Secondly, mean seed set decreased with distance of transects from the meadow at SO. Thirdly, the very isolated plants from Gray's Harbor had extremely low seed set.

Pollen limitation appears to reduce seed production for *S. alterniflora* in its native range (Bertness & Shumway 1992), possibly via floral predation, rather than

plant density. However, *S. alterniflora* in Willapa Bay was not exposed to any specialist, or generalist, insect herbivores (Daehler & Strong 1997) until a recent biological control release (Grevstad *et al.* 2003). The only ovary/seed predator we observed on *S. alterniflora* was the fungus *Claviceps purpurea* (ergot), which prevents seed formation within colonized flowers, and this was very rare except at low densities in well-established meadows close to land. We saw no evidence of vertebrate grazing.

We are currently observing pollen traps and estimating stigma loads in addition to applying pollen addition and exclusion treatments to *S. alterniflora* in the field. We will use these data to derive a pollen dispersal density function to test how wind speed and direction, density and spatial arrangement of neighbours and floral elevation in the tidal gradient contribute to variation in seed production.

Pollen limitation over very short distances is a largely uninvestigated, although possibly common, feature of wind-pollinated plants (Koenig & Ashley 2003). Only a fraction of the ambient pollen may be viable as it is short-lived, particularly in grasses, and pollen quality differs between individual donors (see Kearns & Inouye 1993). Aquatic wind-pollinated species could be acutely vulnerable to pollen loss as pollen settles on water. Abiotic factors such as wind direction have a profound influence on the pollen deposition of grasses (e.g. Giddings *et al.* 1997), with a very limited upwind isolation restricting pollen availability (as occurs in grass crops, see Griffiths 1950).

Inbreeding depression could cause the lower germination rate of isolated plants in Willapa Bay and zero germination from Gray's Harbor. Even isolated plants had many inflorescences, and we infer that most of their seed set was due to geitonogamous self-pollination. In glasshouse trials, *S. alterniflora* plants from Willapa Bay were more self-compatible than were those from the native range (H. G. Davis, unpublished data), suggesting that the invasive populations may have a greater potential for selfing.

Seed of *S. alterniflora* disperses great distances by floating on water-borne wrack in winter within Willapa Bay. Some goes out to sea (Sayce *et al.* 1997) and the dominant current carries wrack northwards. Over the past decade seedlings have appeared hundreds of kilometres north of Willapa Bay, as far as the mouth of the Copalis River in Washington State (Sayce *et al.* 1997). At Willapa Bay, patches can grow as large as 8.5 m in diameter within a decade, reducing isolation, as do new recruits colonizing the open mud. Isolation and the open mud that separates recruits decrease as the plants grow together. Thus, spread of *S. alterniflora* is an example of stratified diffusion (Shigesada *et al.* 1995) with short-distance dispersal by vegetative growth and long-distance seed dispersal.

The result of incorporating an Allee effect into the process of stratified diffusion has not been studied, although in theory it could reduce the initial or overall invasion speed (Lewis & Kareiva 1993; Kot *et al.* 1996; Veit & Lewis 1996) and can halt invasion (Keitt *et al.* 2001). We are using the data from this field study to build analytical and simulation models describing the spread of *S. alterniflora* in Willapa Bay (Taylor *et al.*, in press). We will then explore if the Allee effect could be primarily responsible for the long establishment phase of this invasion and evaluate different control or management strategies.

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Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*)

Heather G. Davis*[††], Caz M. Taylor[§], John G. Lambrinos[¶], and Donald R. Strong*[†‖]

*Population Biology Graduate Group and [‖]Section of Evolution and Ecology, Storer Hall, and [§]Ecology Graduate Group and [¶]Department of Environmental Science and Policy, Wickson Hall, University of California, Davis, CA 95616; and [†]Bodega Marine Laboratory, Box 247, Bodega Bay, CA 94923-0247

It is usually assumed that pollen availability does not limit reproduction in wind-pollinated plants. Little evidence either supporting or contradicting this assumption exists, despite the importance of seed production to population persistence and growth. We investigated the role of pollen limitation in an invasive estuarine grass (*Spartina alterniflora*), with a manipulative pollen supplementation and exclusion experiment in areas of high population density and at the low-density leading edge of the invasion. We also quantified pollen deposition rates on stigmas and pollen traps along a windward to leeward gradient. We found pollen impoverishment at the low-density leading edge of a large invasion, causing an 8-fold reduction in seed set. We found 9-fold more pollen on stigmas of high-density plants than on those of low-density plants. Pollen deposition rates on stigmas and traps did not increase downwind of low-density plants but did increase downwind of high-density plants and dropped off precipitously across a gap that lacked pollen donors. The delay of appreciable numbers of seed caused by pollen limitation persists for decades until vegetative growth coalesces plants into continuous meadows, and this Allee effect has slowed the rate of spread of the invasion.

G rasses are among the most common, aggressive, and harmful invasive species (1). Almost all grasses are pollinated by wind. Many studies have demonstrated pollen limitation in animal-vectored plants (2, 3), but there is very little evidence on whether pollen availability limits reproduction in wind-pollinated plants. Wind-dispersed pollen has a leptokurtic dispersal distribution from point sources (4–7), and, therefore, the proportion of fertilized ovules and seed set in recipient plants decreases rapidly with distance from the pollen donor (8). Wind direction, speed, turbulence, and gravity also affect pollen deposition (9–11). The key to pollen availability is density of donor plants, again studied almost exclusively in animal-pollinated systems (12–18). The role of density in governing pollen limitation in wind-pollinated plants is only just beginning to be recognized (19–21) although some earlier work suggested its importance (22).

For nonindigenous plants, such investigations are crucial because pollen limitation can cause a depressed rate of seed production, and therefore of population growth, when individual density is low at the front of an invading population (23, 24). This is one mechanism causing an Allee effect (25, 26), a positive relationship between fitness and either numbers or density of conspecifics. A "strong" Allee effect results in negative per capita rate of growth when population density drops below a threshold. A "weak" Allee effect, as can be the case with long-lived adults, causes a depressed per capita rate of growth at low population density, but it never becomes negative (27, 28). A lack of mating opportunities among sparse or widely spaced individuals can result in an Allee effect and a slowing of the invasion (27–29).

In a large-scale, estuarine invasion of *Spartina alterniflora* (smooth cordgrass) in Willapa Bay, WA, colonists at the leading edge are isolated from one another (Fig. 1). Isolated colonists set <1/10th the seed of plants that have grown vegetatively and coalesced with their neighbors (30), and this has caused a weak

Allee effect (29). *S. alterniflora* spreads within Willapa Bay by seed that floats on the tide, with virtually no recruitment by rhizome fragments (30). This tall, dense salt marsh grass has spread to cover ≈60 of the 230 square kilometers of intertidal lands since introduction from the Atlantic coast a century ago. This area occupied is far less than would be expected without the Allee effect (29).

To test whether the distinct reduction in isolated plants' fecundity is due to insufficient availability of outcross pollen, we performed a manipulative experiment applying the treatments of pollen exclusion, pollen addition, and ambient control on closely adjacent isolated and meadow plants at the Palix River mudflats in Willapa Bay. This area was colonized about 1985, the plants began coalescing by 2000 (30) and continuous swards of *S. alterniflora* occupied most of the available substrate at the time of this experiment. To determine relative pollen loads between isolated and meadow plants, we collected stigmas from all experimental plants, as well as additional plants in the Palix River and two other areas within the bay. We set out pollen traps and collected adjacent stigmas across a gradient of plant density to measure the correlation between ambient pollen in the air and stigmatic pollen deposition rates and to assess the relative amounts of airborne pollen across a windward to leeward gradient.

Materials and Methods

Pollen Manipulations. Experimental manipulations occurred August 2003, beginning on August 11 (week 1) and August 25 (week 2), when daytime low tides coincided with early female phase flowering at the Palix River mudflats. On Monday and Tuesday of each week, we haphazardly chose equal numbers of isolated plants and meadow plants completely coalesced with their neighbors. We haphazardly assigned the treatments of pollen exclusion, pollen addition, and ambient unmanipulated control with 3, 5, and 5 inflorescences per plant respectively. On week 1, we applied all treatments to 8 plants and applied the pollen addition and ambient treatments to an additional 20 plants. On week 2, we applied all treatments to 16 plants. For the pollen exclusion treatment, we encased the inflorescences in 18-in-long × 2-in-diameter (1 in = 2.54 cm) clear plastic pollen tubes, affixed to poles, capped with bridal veil screening on top, and partially sealed at the bottom with duct tape. Complete sealing was not possible; the tide rose daily above the level of the tubes so they had to be allowed to drain. The tubes were removed 2 weeks later when stigmas were no longer receptive. For the pollen addition treatment, we collected anthers from at least ten plants each morning and pooled them in sterile centrifuge tubes. For three consecutive days (Wednesday, Thursday, and Friday) during the same weeks of the pollen exclusion treatment, we hand pollinated all plants by using a sable brush, haphazardly changing the order of pollination. The pollen was applied to the receptive stigmas of the bisexual florets, each of which has the
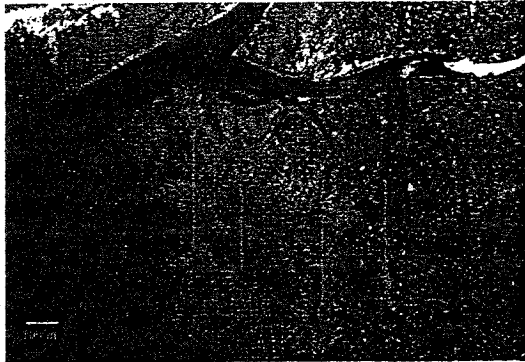
Fig. 1. Palix River study site. Shown is an aerial photograph of a low-density area on the left grading to dense meadow on the right. Locations of trap transects I–IV are represented by yellow lines. The image was prepared by J. C. Civille.

potential to form a single seed. Stigmas are receptive for ~3 days, and the inflorescence for ~10 days as the hundreds of florets ripen from top to bottom. We collected all treated inflorescences 4 weeks after manipulations. Seed set is expressed as the number of seeds per all florets per inflorescence.

Stigma Pollen Loads. To determine relative pollen loads of isolated and meadow plants, we collected inflorescences with receptive stigmas from all experimental plants, as well as additional plants in the Palix River and two other areas within the bay. For week 1's plants, we collected inflorescences on all 3 pollen-addition days from plants that had pollen-exclusion tubes and on 2 of the days from plants without exclusion tubes. For week 2, we collected inflorescences on 2 of the pollen-addition days. We collected inflorescences from 50 additional plants at the Palix

River, half isolated and half meadow on any given day, over 3 days the week between the manipulations. We also collected from two additional sites, 40 plants at Bone River and 20 from Cedar Creek, in the week after week 2's manipulations. The Bone site is ~1.5 km north of the Palix River mouth, also on the eastern shore of the bay. The Cedar Creek site is ~11.5 km northwest of the Palix on the northern shore of the bay. At the Palix and Bone sites, the isolated plants were windward of the meadow plants and leeward at the Cedar site. It rained only on the day, and previous 3 days, that we collected from the Cedar site. It did not rain on the 3 days before, or during, the day of any other stigma collections.

We removed half (one lobe) of three stigmas from each inflorescence of three inflorescences per collection and applied Calberla's pollen stain, which contains basic fuschin (Surveillance Data, Plymouth Meeting, PA). We squashed each stigma lobe and counted the total number of pollen grains. We observed only rarely one other type of pollen that was easily distinguishable from *Spartina* pollen. The lack of other species' pollen was presumably due to there being only water and mudflats upwind. The wind along the North American Pacific coast is typically onshore and laminar (horizontal and smooth) except during the winter. Inland sites will typically have much more vertical and other directional movement than coastal sites, due to thermal activity and turbulence caused by obstructions, affecting the pattern of pollen deposition (31, 32).

Pollen Traps and Stigmas. To determine the relationship between pollen borne on the wind and that reaching stigmas, we trapped pollen on an array of pollen traps and collected adjacent stigmas across a leeward to windward gradient of plant density, dissected by a 100-m-wide channel at the leeward end (Fig. 2b). The array consisted of six transects oriented to span the gradient in *Spartina* density and the direction of the prevailing wind: two in windward-isolated plants (I and II), one along the windward meadow edge (III), one in the windward meadow (IV), one in the leeward meadow (V), and one 100 m from the leeward side of the meadow across a channel (VI) (Fig. 2). Each transect was
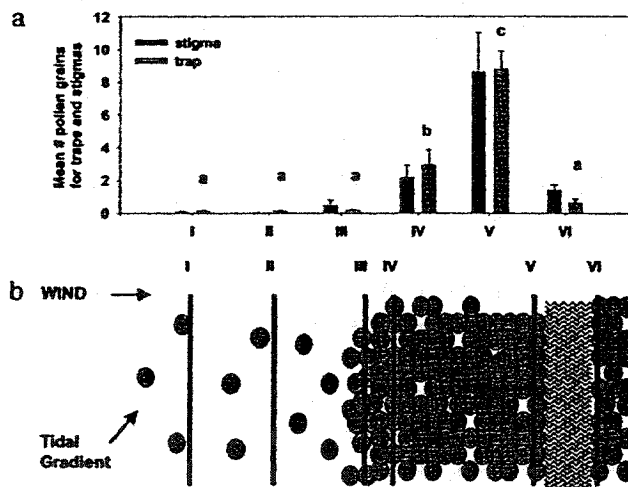

Fig. 2. Mean pollen loads (+1 SE) on pollen traps and stigma lobes (a) and schematic of trap transect setup (b). Lines on the schematic (b) correspond to trap transects on graph windward to leeward (I–VI). Small circles represent plants. The wavy lines represent an unvegetated channel. Stigma lobe pollen loads (black) on the graph (a) are provided for purposes of visual comparison. For each trap transect of pollen traps (gray), bars with different letters are statistically different at P < 0.05 according to Tukey pairwise comparisons. Close correspondence between trap field-of-view and stigma lobe pollen loads is coincidental.
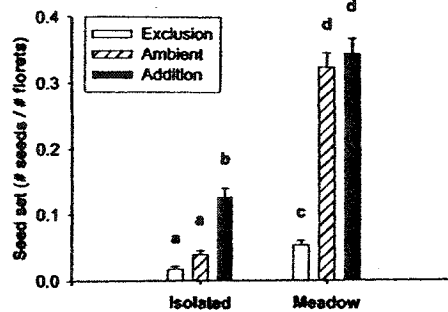
Fig. 3. Mean seed set (+1 SE) of pollen exclusion (open), ambient or open-pollinated (hatched), and pollen addition (black) treatments for isolated and meadow plants. Bars with different letters are statistically different at P < 0.01. All treatments were applied to 24 plants, and addition and ambient only to a further 20 plants.
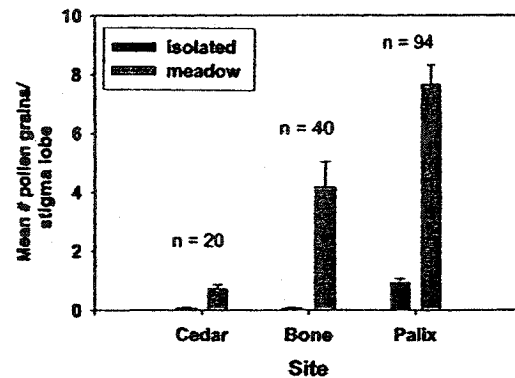

Fig. 4 Mean number of pollen grains per stigma lobe (+1 SE) for each inflorescence of isolated and meadow plants at three sites. Untransformed data were used to generate graph. Plant densities are different at P < 0.0001. No comparisons were attempted between sites.

composed of 10 traps spaced 30 m apart. Traps were 10-cm × 10-cm sheets of clear acetate rolled into cylindrical sleeves and attached to 50-ml centrifuge tubes. We coated traps marked with each cardinal direction with a thin layer of aerosol Tangle-Trap (Tanglefoot Co., Grand Rapids, MI) and fixed them atop lengths of electrical metallic tubing at a height of 2 m, just above surrounding inflorescences to prevent inflorescences banging against the traps.

We set traps on 3 days (August 29, September 3, and September 4). In addition, on September 4 we collected inflorescences for stigma pollen counts from eight plants along each of the trap transects. These inflorescences were collected and processed as above. On each day, we set traps at 0700 hours and collected them 7 h later. This time period corresponded to one low-tide cycle where all inflorescences were above water. All 60 traps were set within 30 min and collected in reverse order. We measured wind direction at a height of 2 m every half hour to assess the general wind direction over the duration of the 3 days that the traps were out (Fig. 2b). After 7 h, the exposed acetate sleeves were laid flat and covered with a clean piece of acetate. We cut the sheets into four 2-cm × 10-cm strips corresponding to the four cardinal directions. We counted stained pollen grains in three nonoverlapping haphazardly chosen fields of view

(19.635 mm^2) per strip under ×4 magnification and averaged the fields of view for a mean pollen load per trap.

Data Analyses. To determine whether the pollen-addition and pollen-exclusion treatments caused differences in seed set for isolated and meadow plants, we performed a 2-way unbalanced ANOVA with the factors of density, whether isolated or meadow, and treatment (exclusion, addition, ambient). The random variable "plant" was nested within density. Seed set was log-transformed and weighted with the total number of florets of each inflorescence. Differences between pollen treatments were explored by using post hoc Tukey pairwise comparisons. This and all other analyses were performed with SAS 8.02 (SAS Institute, Cary, NC).

To test whether isolated and meadow plants had different mean stigma lobe pollen loads per inflorescence, we used an unbalanced ANOVA with the factors of density, whether isolated or meadow, and site (Palix River, Cedar River, and Bone River), the interaction between the two and the random variable "plant," which is nested within the interaction. We log-transformed pollen loads. We did not attempt comparisons between sites because collections at the sites took place on different days under different conditions.

To test whether pollen loads on traps and stigmas among the six trap transects were correlated, we used a Pearson's correlation procedure. Then, to find whether there were windward to leeward differences in pollen loads among trap transects, we performed a one-way ANOVA with individual trap nested within trap line. We log-transformed pollen trap loads and compared pairwise differences between trap transects with a post hoc Tukey comparison.

Results and Discussion

In meadow plants, the pollen-exclusion treatment reduced seed set by >6-fold (P < 0.0001) but caused no reduction in the isolated plants (P = 0.89). The few seeds that were set within the exclusion tubes could have been due to pollen leaking in through the open bottom of the tubes and/or by geitonogamous pollen transfer within inflorescences. S. alterniflora is largely self-incompatible (33) although the Willapa Bay population exhibits a relatively greater capacity to set self-fertilized seed in greenhouse studies than do plants from the native range (H.G.D., unpublished data). The pollen addition treatment had no effect on seed set of meadow plants (P = 0.65) but did raise that of

Table 1. Statistical results

Analysis	Source	df	Type III MS	F	P
Pollen addition and exclusion	Density	1	0.45	26.31	<0.0001
	Treatment	2	0.04	49.43	<0.0001
	Density × treatment	2	0.02	21.74	<0.0001
	Plant (density)	42	0.02	28.24	<0.0001
	Error	427	0.0008		
	Total	474			
Stigma pollen	Density	1	15.32	95.29	<0.0001
	Site	2	4.87	30.04	<0.0001
	Density × site	2	1.21	7.46	0.0008
	Plant (density × site)	148	0.19	3.13	<0.0001
	Error	463	0.06		
	Total	616			
Pollen trap	Trap transect	5	2.51	23.19	<0.0001
	Trap (trap transect)	54	0.03	0.36	1.0000
	Error	101	0.10		
	Total	160			

isolated plants by >3-fold ($P < 0.0001$) (Fig. 3). We added pollen for only 3 of the potential 10 days of stigma receptivity per inflorescence, because we were restricted to the concurrent timing of low tides and anther dehiscence. That seed set of the isolated plants' addition treatment was not as high as that of the meadow plants' addition and control treatments could be explained by our inability to saturate inflorescences with pollen. However, the results indicate that colonists, isolated plants at low density, are extremely pollen-limited whereas seed set in the high-density meadow is not limited by pollen. A complete table of statistical results of this and following analyses is in Table 1.

Stigma lobes of meadow plants (overall mean ± SE, 6.31 ± 0.51, $n = 77$) captured more than nine times the pollen of isolated plants (overall mean ± SE, 0.69 ± 0.09, $n = 77$) ($P < 0.0001$) (see Fig. 4). Although sites differed in overall pollen receipt, meadow plants (Palix mean ± SE, 7.66 ± 0.66, $n = 47$; Bone mean ± SE, 4.18 ± 0.85, $n = 20$; Cedar mean ± SE, 0.72 ± 0.14, $n = 10$) always had relatively higher pollen loads than isolated plants, these having on average less than one pollen grain per stigma lobe (Palix mean ± SE, 0.94 ± 0.13, $n = 47$; Bone mean ± SE, 0.07 ± 0.03, $n = 20$; Cedar mean ± SE, 0.09 ± 0.03, $n = 10$). Our work suggests that the small amount of pollen reaching colonists is insufficient for seed set. A threshold of pollination intensity needed for successful seed production has been documented from many other species (32). The very low amount of pollen on Cedar River stigmas, collected after 3 days of rain, suggests that weather could have a profound effect on within- and among-year variation in seed set. Pollen flow is also likely to be inhibited when high tides cover inflorescences during warm and sunny times of day, or directly before.

We found a high correlation between pollen loads on the traps and on the stigmas ($r = 0.99$, $P = 0.0002$, $n = 6$). This finding supports the simplest hypothesis that the amount of pollen in the air determines that on stigmas. We also found that pollen loads on traps were highly influenced by transect location (Fig. 2). The

wind blew consistently from the same northwest direction (Fig. 2b) over the 3 days the pollen traps were out. There was very little airborne pollen anywhere in the field of isolated colonists, with no more at the leeward end than at the windward end. The pollen load at the meadow edge, where only isolated plants were upwind, was no higher than that of the colonists. Pollen loads increased sharply on the traps inside the meadow, more so further downwind, but dropped precipitously across the unvegetated channel to levels as low as those among isolated plants. This result suggests that effective pollination drops off rapidly with distance from pollen sources.

Our work raises the possibility of pollen limitation in other wind-pollinated plants and could have particular importance for invaders. It directs attention that Allee effects can slow rates of colonization at the fronts of invasions of such plants (29). An Allee effect could contribute to a lag time between introduction and rapid spread that could offer a window of opportunity for control and management. At the same time, such a lag might induce a false sense of security about introduced plants that might become aggressive invaders. Native wind-pollinated plants could also suffer from the effects of pollen limitation as populations fragment due to human-mediated habitat loss or when they compete for space with invaders. A final implication is that pollen limitation in wind-pollinated plants could be an important selective agent influencing the evolution of life history traits (19).

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r-Selected Traits in an Invasive Population

Heather G. Davis[1,2]*

Population Biology Graduate Group, 2320 Storer Hall, University of California,

Davis, California 95616, USA

Bodega Marine Laboratory, Box 247 Bodega Bay, CA 94923-0247

*To whom correspondence should be addressed. (530) 752-4399 Lab, (530) 752-1449 FAX; Email: hgdavis@ucdavis.edu.

Abstract

I compared life-history traits and self-fertilization rates in greenhouse culture of native *Spartina alterniflora* with an invasive population from a low-density Pacific estuary to see whether plants in the novel r-selective regime exhibit early reproduction, greater self-compatibility and high reproductive effort putting individuals at greater risk of death. Plants were grown from seed collected from the Atlantic and Gulf coasts of North America and a population introduced approximately 100 years ago to Willapa Bay, Washington, USA that has expanded to cover over ca. 6000 hectares. Pacific marshes have almost no native emergent vascular plants in the intertidal habitat, offering a virtually empty niche for invasive *S. alterniflora*. The low-density plants at the leading edge of this rapidly expanding population suffer a severe Allee effect of greatly reduced fecundity, caused by pollen limitation, compared to high-density areas. I found 99% of the invasive plants initiated reproduction in the first year of this study, while only 52% of the native range plants did so in the first year, followed by 34% in the second and 14% of native plants had not initiated reproduction after three years. The invasive plants had more than twice the reproductive effort of the native plants. Plants with the highest reproductive effort in the first year of growth died regardless of coastal site of origin, although nearly all of those that died over two years were invasive Pacific plants (27%). The invaders set two times the seed as the natives under forced selfing, suggesting greater self-compatibility or reproductive effort. These results suggest either a founding and bottleneck event and/or the invasive population has evolved

from the long-lived, predominantly self-incompatible, K-selected state of the probable

invasive propagules originating in dense, competitive native marshes.

Key words: r-traits, life-history evolution, self-compatibility, reproductive effort,

Spartina alterniflora

Introduction

The study of r- versus K-selection in life-history evolution theory has a

contentious history (Reznick *et al.* 2002). r-selection favors high reproductive output

in density independent settings. At the opposite extreme, K-selection favors long-

lived individuals with high competitive ability in density dependent settings

(MacArthur and Wilson 1967). An r-environment selects for a short prereproductive

stage, smaller body size, large reproductive effort, greater fecundity and semelparity,

with a continuum to the K-environment selecting for traits with the contrasting pattern

in each case (Pianka 1970, 1974). This simple paradigm has been lionized, criticized,

extensively revised, discarded and resuscitated (Boyce 1984; Mueller 1997; Reznick

et al. 2002). It has a more recent incarnation as a competition-colonization tradeoff

with dispersal ability as part of the colonizer syndrome (e.g. Tilman 1994). It has also

been extended by the inclusion of demographic theory (e.g. Gadgil and Bossert 1970)

that posits the direction of selection depends on relative risks of mortality at different

life stages and these risks are contingent on density dependence (e.g. Charlesworth

1980).

Vegetative growth, correlating with competitive ability or survivorship, can

trade off with reproductive output (Van Kleunen *et al.* 2002). The difference in

fitness conferred by the ability to sexually recruit offspring as opposed to asexually expand may depend on how much open space is available for colonization (Winkler and Fischer 1999). Measuring density dependence, though usually difficult to assess, is somewhat more tractable in plants (Antonovics and Levin 1980). Where there is much space open for colonization, individuals that contribute seeds early, even with earlier mortality, will have an advantage over individuals that postpone flowering in favor of vegetative growth (Cole 1954).

An Allee effect, where there is a positive relationship between number or density of individuals and some constituent of fitness (Allee 1931, Stephens *et al.* 1999), can cause isolated obligatory outcrossing plants to have reduced fecundity because of pollen limitation (Lloyd 1980). If no other mates are available, only self-compatible individuals can found a sexually reproducing population (Baker 1955). If there are multiple individuals in a colonizing population but mates are still limited for any of many reasons such as density or relatedness, the only reproductively successful individuals will be those that are self-compatible and so can ensure fertilization (Jain 1976; Uyenoyama 1986; Jarne and Charlesworth 1993). Populations that have passed through occasional bottlenecks, as invasive species often do, are more likely to be self compatible than large, outcrossing populations that are prone to possess mechanisms to prevent inbreeding (Lande and Schemske 1985).

In this comparative study among *Spartina alterniflora* Loisel. (smooth cordgrass) from sites in an invasive population and sites in native populations from the Gulf and Atlantic coasts, I ask the question: Does the invasive population exhibit *r*-traits relative to the native populations? The leading edge of the invasive population

has been at low density since establishment, is experiencing an Allee effect (Davis *et al.*, 2004a; Taylor *et al.*, in press) caused by pollen limitation (Davis *et al.* 2004b) and is rapidly expanding. Native marshes are typically at high density with very long generation intervals. I compared life-history traits from maternal families of field collected seeds in a common greenhouse environment to determine whether the invasive plants, relative to native plants, have a shorter pre-reproductive period, are smaller at the end of the growing season, have more reproductive effort, have a higher risk of death correlated with greater reproductive effort and have a higher rate of self-fertilization.

Methods

Study System

S. alterniflora is a perennial grass that grows up to two meters tall. Seedlings grow non-directionally by rhizomes forming circular plants that merge to form monotypic stands. This species is a hexaploid and is considered "almost completely" self-incompatible (Somers and Grant 1981). Pacific marshes have no or few other emergent plant species. When *S. alterniflora* invades Pacific estuarine mud flats, it causes channelization of intertidal zones, displaces native species in the high marsh and transforms open tidal mud flats to meadows (Daehler and Strong 1996). Floating seed disperses in the Fall and seedlings germinate the following Spring in unoccupied habitat (Mooring *et al.* 1971; Woodhouse 1979). In the native range, sufficiently large

enough openings for seedling recruitment become available when hurricanes, ice sheets or other infrequent catastrophic events or human disturbance kill plants. Rhizomes from adjacent established clones most often colonize small unoccupied sites (Hartman 1988).

S. alterniflora was introduced to Willapa Bay, Washington, on the Pacific coast of North America approximately 100 years ago and has spread explosively by seed (Davis *et al.*, 2004a). This is the only long colonized, large-scale invasion by *S. alterniflora* on the Pacific coast that has not hybridized with the California native cordgrass, *S. foliosa*. By 1997, ca. 6,000 of the 23,000 ha of Willapa Bay intertidal mudflats were colonized. This invading population probably started with very few initial colonizers (Stiller and Denton 1995). Isolated individuals on the leading edge of this invasion can set less than $1/10^{th}$ the viable seed than can plants in coalesced, high density meadows indicating the action of an Allee effect in recently colonized areas (Davis *et al.*, 2004a; Taylor *et al.*, in press) caused by pollen limitation (Davis *et al.* 2004b).

Experimental Design

This study was conducted under common greenhouse conditions at the University of California at Davis (UCD). With the assistance of the Washington Departments of Natural Resources and Fish and Wildlife, we collected *S. alterniflora* seed from six sites from the invasive population at Willapa Bay, Washington over five collection days in October, November and December of 1999. We harvested 5-10

inflorescences within a square meter per maternal genet with at least 10 meters between maternal genets to reduce the possibility of mistakenly pooling seeds across maternal families. Maternal genets were identified by morphological similarity. We collected from marshes at Bruceport County Park (BCP), Kaffee-Lewis (KL), LeadBetter Point south (LBS), North River (NoR), Naselle River (NR) and Palix River (PRSS) (Fig. 1, Table 1). Seed bearing inflorescences from the native range of *S. alterniflora* on the Atlantic and Gulf coasts of North America were collected and sent by six individual researchers in November of 1999. Collectors were instructed to harvest one inflorescence per maternal genet, with at least 10 meters between collections. I received inflorescences from six marshes, however the collection from Texas contained only a single viable seed. The five native marshes in this study are located on the Atlantic coast at Marsh Landing, Georgia (ML), Shell Hammock, GA (SH), South Jax, Florida (SJ); and the Gulf Coast at Dauphin Island, Alabama (DI) and Weeks Bayou, Mississippi (WB) (Fig. 1, Table 1).

Seed dormancy was broken by the method described in Davis *et al.* (2004a) in December 1999, excepting seeds were soaked in 1:2 sea to deionized water. Seedlings from each maternal genet were planted in a 2:1 mixture of vermiculite and autoclaved Yolo County sandy loam in five six-inch plastic pots with four seedlings per pot. Trays holding the pots were kept constantly filled with deionized irrigation water supplied with fertilizer (Dosatron) with N:P:K=2:1:2 (218:109:218 ppm), pH of between 5.0 - 5.5. Three times each week, the irrigation water was automatically set to overflow the trays for three minutes to flush microbial growth and replenish oxygen in the soil. Two weeks later, all but one seedling were removed from each

pot. Both pots and trays were re-randomized every two weeks until the first plant produced an inflorescence in May of 2000 after which they became too fragile to move. The number of viable seeds and survival rates determined the number of maternal families (Table 1). Nearly all maternal families were comprised of 4 siblings.

Seed set and inflorescence emergence

All inflorescences grown in the greenhouse were individually bagged prior to anther emergence by using a KimWipe™ (Kimberley-Clark) cut and stapled to the appropriate size and taped around the bottom. The plants were screened 2-3X per week for new inflorescences until all plants had senesced for the season. This data was collected over two years. The date of inflorescence emergence was calculated as the number of days to first stigma emergence per inflorescence since seedlings were potted. Stigmas extrude progressively from florets of an inflorescence from top to bottom, followed by the progressive extrusion of anthers. There is an overlap when stigmas are receptive and anthers are shedding pollen on a single inflorescence. On occasions when an inflorescence was missed and had extruded anthers before it was bagged, all possible pollen recipient inflorescences, those with receptive stigmas, were marked and excluded from the seed set dataset to prevent including unintentionally outcrossed seed. Bagged inflorescences were collected when inflorescence production had ceased, in February of 2001 and 2002. Seed set was measured on two inflorescences per year selected randomly from each plant. Not all

plants produced inflorescences, or more than one per plant and some plants died after the first year (see Results). Seed set was measured first by stripping and counting all florets from each inflorescence, then screened for the presence of seeds. Each floret contains either zero or one seed. Seed set is expressed as the number of seeds per florets for each inflorescence (subsample).

Size at reproduction

I recorded the size of plants at first inflorescence emergence in the second year of growth. This data was not taken in the first year due to the unexpectedness of the early flowering. Height and diameter of the three tallest stems and total number of stems were recorded the day each plant produced its first inflorescence for the year. Most of the plants extruding inflorescences the second year had produced inflorescences the previous year except for ca. one third of the plants from the native range.

Biomass, reproductive effort and death

Above ground biomass was harvested from all plants one month after inflorescence collection in 2000 and 2001. Plants were considered dead when new stems failed to emerge after harvest. All above-ground material was dried in a drying oven and weighed. It was necessary to remove all rhizomes emerging from the bottom of the pots because these invade other pots. Reproductive effort per plant for

each year was calculated as the total number of inflorescences produced / end of season above ground biomass. Above ground biomass was used to calculate reproductive effort instead of total biomass due to the multiple years of the study and the necessity of trimming rhizomes. To justify this, a random sample, excluding those that had not yet produced any inflorescences, of approximately half of the remaining living plants (24 invasive, 31 native) were harvested for above and below ground biomass and dried in April of 2002 to assess whether above ground biomass and total biomass are correlated. The below ground portion is underestimated in the plants that had rhizomes removed from outside the pot. The remaining plants (10 invasive, 36 native) were harvested and dried after the completion of flowering in February of 2003. Inflorescences and above and below ground biomass were weighed separately and number of inflorescences per plant was recorded.

Statistical Methods

All variables of plant growth, biomass and the number of inflorescences per plant, were log-transformed to improve the normality of the residuals. Lifetime reproductive value was not possible to assess, as it is not feasible to maintain these plants over their potentially very long life spans. To control the experiment wide error rate as some of the traits measured are possibly correlated, where applicable, the significance tests were adjusted for multiple comparisons using the sequential Bonferroni correction (Rice 1989).

To find whether age of first reproduction varied among coasts, I ran a Fisher's exact test. Age of first reproduction is categorical, whether year 1, year 2, or surviving but not producing inflorescences to the end of the experiment. I repeated the analysis with pairwise comparisons between coasts. It was necessary to analyze these data nonparametrically due to extreme departures from parametric assumptions. To account for the non-independence of the units, I ran a logistic model with backward, forward & stepwise selection, and found all variables dropped out of the model in all attempts except coast (see description of death analyses).

I used two nested mixed model ANOVAs as above to find whether there are differences in the timing of inflorescence emergence for the first (2000-2001) and second (2001-2002) growing seasons. Each inflorescence emerged is considered a subsample for the individual plant. There were between one and fourteen subsamples per plant for the first growing season and between one and twenty-seven subsamples per plant for the second growing season. I used a Tukey test for comparisons among coasts.

I explored differences for log transformed reproductive effort among all plants using a nested mixed model ANOVA with the explanatory variables of coast, site nested within coast, maternal family nested within the interaction of coast and site, year and the interaction of year and coast. Site and maternal family are considered random variables. I used a post hoc Tukey test to determine differences among coasts. To determine which component of reproductive effort could be responsible for differences among coasts, I performed the ANOVA above for each year separately, with log-transformed number of inflorescences or biomass as the dependent variable.

To find which variables predict age of death, I ran two logistic regression models, one for each year with the binary response variable of alive or dead. Each initial model contained the explanatory variables of reproductive effort in that year, coast, site nested within coast, maternal family nested within site and coast, the interaction of reproductive effort and coast and for year 2, the reproductive effort of year 1. I then submitted these models to a backward effect-selection method which removes an effect from the model if the chi-square statistic is over the 5% significance level (SAS 8.02) A forward and a stepwise selection method were also run to check for concordance of results among methods. These models necessitated all variables be processed as fixed effects making it more likely to detect effects, so variable rejection is conservative.

To test for differences in seed set under forced selfing, a nested mixed model ANOVA was run with each seed set observation weighted by the number of florets on the inflorescence. It was necessary to use the procedure "proc mixed" as it was the only one capable of analyzing the model. Seed set was arcsine transformed to normalize the residuals. The model consisted of the fixed explanatory variables of coast and year and the random explanatory variables of site nested within coast, maternal family nested within site, individual plant nested within maternal family and coast and year crossed with coast. To determine differences between coasts I used a post hoc Tukey test for all pairwise comparisons.

Results

Age of first reproduction

Plants from the Pacific invasive population were much younger when they initiated reproduction, all but one starting in the first year. In the first year, 62% of the Atlantic and 45% of the Gulf plants became reproductive. In the second year, the last Pacific plant, and 34% of both the Atlantic and Gulf coasts plants initiated reproduction. By the end of the experiment, 4% of the Atlantic plants and 21% of the Gulf plants had not produced inflorescences. (Fig. 2). The Fisher's exact test for all comparisons indicated differences among coasts ($X^2 = 47.59$; $p < 0.0001$). However, though the Pacific coast was different from the Atlantic and Gulf coasts ($X^2 = 42.34$; $p < 0.0001$ and $X^2 = 28.51$; $p < 0.0001$ respectively), the Atlantic and Gulf coasts were marginally not different from each other ($X^2 = 5.53$; $p = 0.075$).

Size at reproduction

The Pacific coast plants are smaller as well as younger when they initiate reproduction. I present in the text simple means and standard errors of the height of the three tallest stems per plant, their diameters at pot level and the number of stems in the pot. These statistics represent the second year of inflorescence production in all but one of the Pacific coast plants (51 plants), and is evenly distributed among first and second reproduction for the Atlantic (47 plants) and Gulf coast (30 plants) plants. Because this data was collected for both first and second reproduction, I did not consider a more sophisticated analysis was justified. For height the Pacific coast

plants were shorter at 39.28±1.97 cm, with the Atlantic coast at 60.86±2.64 cm and

Gulf coast 60.39±4.33 cm. For diameter the Pacific coast stems were narrower at

2.44±0.08 mm, the Atlantic coast at 6.45±0.18 mm and the Gulf coast at 6.8±0.41

mm. For number of stems the Pacific coast had fewer with 5.39±0.46, the Atlantic

coast at 23.2±1.42 and Gulf coast had 21.77±2.08.

Dates of inflorescence emergence

In both seasons, the plants from the Pacific coast extruded their inflorescences

earlier than either native coast (p <0.0001 for both comparisons) (Table 2, Fig. 3). In

the first season the two native coasts were not different from each other (p = 0.3305),

though the Atlantic coast distribution appears somewhat earlier (Fig. 3) but were

different in the second (p = 0.0002) with the Gulf coast distribution later than the

other coasts (Fig. 3). There were no between site differences, but there were maternal

family differences for the second season only (Table 2).

Reproductive effort

Plants from the Pacific coast had vastly more reproductive effort than those

from either the Atlantic or Gulf coasts regardless of year of growth (Table 2, Fig. 4).

The reproductive effort of the Pacific plants was different from both the other coasts

(p < 0.0001 for both comparisons), but the Atlantic and Gulf coasts were not different

from each other (p = 0.909).

The greater reproductive effort of the Pacific plants appears to be due both to the greater number of inflorescences they produce and their lower biomass at the end of the season, in all cases principally dependent on coast (Table 3). For both years, differences among inflorescence production were dependent upon coast, and maternal family in the second year only (Table 3). The Pacific plants (year1 mean = 4.55 ± 0.29(SE) n = 71; year 2 mean = 5.43 ± 0.59(SE) n = 51) produced more inflorescences in both years than those from the Gulf (year 1 mean = 1.72± 0.55(SE) n = 32, p = 0.003; year 2 mean = 4.00 ± 0.88(SE) n = 30, p = 0.009), though only the first year Atlantic (year 1 mean = 2.64 ± 0.47(SE) n = 47, p = 0.018; year 2 mean = 6.06 ± 0.70(SE) n = 47, p = 0.859). The Gulf and Atlantic coasts did not differ in the first year (p = 0.259), but did in the second (p = 0.001). For both years, differences among biomass were dependent upon coast and marginally for maternal family (Table 3). The Pacific plants had much less biomass (year1 mean = 8.62± 0.57(SE) n = 71; year 2 mean = 8.09 ± 0.79(SE) n = 51) than both other coasts in both years (p<0.0001), though the Gulf (year 1 mean = 70.13± 7.46(SE) n = 32; year 2 mean = 56.91± 8.97(SE) n = 30) and Atlantic (year 1 mean = 87.90± 0.6.49(SE) n = 47, p = 0.018; year 2 mean = 58.91± 4.72(SE) n = 47) coasts did not differ in either year (year 1 p = 0.431; year 2 p = 0.559).

Death and reproductive effort

Overall, Pacific coast plants died more frequently with a death rate of 43%. Plants from the Gulf coast died less frequently (16%) and no Atlantic coast plants

died over the course of this study. For the first year, individual plants were more likely to die if their reproductive effort in year 1 was high (Fig. 5). All other variables (coast, site, maternal family and coast*reproductive effort) were rejected (Table 4). The percent concordance for the final model is 81.5 and the deviance is 84.44 (df = 127, p = 0.99). For the second year, again the sole explanatory variable was reproductive effort in year 1, with all other variables (coast, site, maternal family, coast*reproductive effort and reproductive effort in year 2) rejected (Table 4). The percent concordance for the final model is 73.5 and the deviance is 83.4 (df = 125, p = 0.99). All model selection methods produced identical results for both years.

Self-fertilization

Pacific plants set nearly twice the seed (mean = 0.405 ± 0.016(SE) n = 71) as Atlantic plants (mean = 0.209 ± 0.020(SE) n = 47) and more than three times that of Gulf plants (mean = 0.105 ± 0.021(SE) n = 32) (Fig. 6). Seed set under forced selfing conditions was dependent on coast and year but not on the interaction of coast and year (Table 2). In the pairwise comparisons, the plants from the Pacific coast are different from both the Atlantic and Gulf coasts (t-value = -3.61 p = 0.007; t-value = -5.00 p = 0.001), but the Atlantic and Gulf coasts are marginally not different from each other (t-value 1.9 p = 0.094) (Fig. 6). Though the means were lower for the Native coast plants, there were two individuals from the Atlantic coast that could set seed at a (untransformed) rate of ca. 60%. The SAS command of "proc mixed" does not return p-values for random variables, so only the covariance parameter estimates

are reported here for individual plant, maternal family and site. These are: individual = 0.009, maternal family = 0.005, and site = 0.002.

Use of above-ground biomass

There were strong positive correlations between above and total biomass justifying the use of only above-ground biomass for calculation of reproductive effort. There was a strong correlation (Pearson correlation coefficient = 0.87, p < 0.0001) between above ground end-of-season Fall biomass in 2002, exclusive of inflorescences and Spring 2003 beginning-of-season total biomass. There was an even stronger correlation (Pearson correlation coefficient = 0.92, p < 0.0001) between above ground end-of-season Fall biomass in 2003, inclusive of inflorescences and Fall 2003 end-of-season total biomass.

Discussion

I found that for all the traits measured, the invasive Pacific population of *S. alterniflora* displayed relatively *r*-selected responses in the greenhouse common garden. The plants from the invasive Pacific population initiated reproduction in a unimodal pattern at a very young age, while the plants from both of the native coasts displayed a multimodal pattern (Fig. 2). The invasive plants had a much greater reproductive effort (Fig. 4), and these died at a high, disproportional rate (Fig. 5).

They also set many more seeds when they were restricted to self-fertilization (Fig. 6).

There were very few maternal or site effects for most of the traits studied (Tables 2,3,4), perhaps because there were few maternal families or sites per coast tested or that there is little genetic variation within a coast. While the invasive population has very little neutral genetic variation as evinced by chloroplast DNA & microsatellite data, populations from the native range do (Bando & Blum, unpublished data), suggesting the possibility that more maternal and site quantitative effects would become apparent with greater sample size.

Age of first reproduction and total inflorescence distributions

All but one of the invasive plants from the Pacific coast achieved reproductive maturity in the first year (Fig. 2) and were smaller in the second year when they initiated inflorescence production than those from the native areas. The lack of variance in timing is expected because of the inbred nature of the Pacific population, where the level of relatedness is very high, likely because of a bottleneck following colonization by few genets (Stiller and Denton 1995). The Atlantic and Gulf coast populations displayed a very dissimilar pattern to the Pacific coast pattern. These native range plants had a longer prereproductive period both within and between years.

Not only do the invasive Pacific plants initiate inflorescence production early, they also produced nearly all of their inflorescences in both seasons before the plants

from either of the native coasts had barely started (Fig. 3). The plants from the native

coasts both extruded their inflorescences at approximately the same time as their field

source populations. Not so for the invasive Pacific population, those plants produced

most of their inflorescences more than four months before the field population (pers.

obs.). This early flowering might indicate greater plasticity for the invasive

population in response to environmental cues; however, this study was not

specifically designed to test this hypothesis.

It is possible that the within year early flowering is an evolutionary response

to the more northerly climate (e.g. Reinartz 1984; Lacey 1988; Olsson and Ågren

2002) in Washington. The Willapa Bay invasive population flowers earlier within

season, as well as earlier in life, than do plants at higher latitudes from near the

northern extreme of the native range (unpublished data). However, adaptation to the

Pacific environment could still play a role, particularly considering the more northerly

native populations do flower earlier than the southern ones in the greenhouse

(unpublished data) and there are many other climatic differences among the Pacific

and the Atlantic and Gulf coasts not attributable to among coast latitudes. One strong

possible cause is that a shift to earlier flowering has occurred to avoid pollen

limitation on the Washington coast in the rainier autumn compared to the relatively

sunny mid-Summer. Pollen is nearly non-existent on *S. alterniflora* stigmas on rainy

days in Willapa Bay (Davis *et al.* 2004b).

One of the benefits for early lifetime first reproduction in a habitat where

density is not yet limiting, includes the production of precocious offspring (Cole

1954). In the scenario of a rapidly growing population with relatively low juvenile

mortality, the most fit individuals will be those that donate to recruitment early and so capitalize on population exponential growth. Those individuals that "play it safe," growing larger and older before reproductive maturity will have a much smaller genetic legacy.

Reproductive effort and death

The plants from the Pacific invasive population had much greater reproductive effort than those from the native populations (Fig. 4). Again, there were no differences between the Atlantic and Gulf coasts in either year.   Death of plants at the end of both the first and second year was dependent on reproductive effort in the first year of growth. It did not matter what the coast population was; if they had a high reproductive effort in the first year, they died. High reproductive effort when the plants were young appears to have weakened them to such an extent that they were more susceptible to death, even if they managed to survive through the second year. *S. alterniflora* could experience a trade-off between current and future, or residual (Williams 1966), reproductive value. This trade-off is often ascribed to an evolutionary response to limitation of resources (Roff 1992; Stearns 1992) and may be true for *S. alterniflora*, even in the benign, competition free environment of the greenhouse. In this environment, the invasive population is tending toward semelparity. If it were possible to follow these experimental plants through their lifetimes, doubtless the native plants would have a greater lifetime reproductive value. However, in the non-equilibrial, rapidly growing state of the invaded estuary's

population size, greater lifetime reproductive value would not necessarily confer greater lifetime fitness. A bet-hedging strategy of trading off early reproductive success for longer survival time and repeated reproductive events is likely more profitable where recruitment opportunities are few and unpredictable, but may not when establishment probabilities are relatively high and predictable (see Levins 1962, 1968; Seger and Brockman 1987; Phillippi and Seger 1989).

Reduced competitive ability in the field is a possible cost with increasing reproductive effort, particularly considering the small size of the surviving invasive plants at the end of each growing season. New recruits on the leading edge of *S. alterniflora* invasion in Willapa Bay are isolated from conspecifics (Davis *et al.*, 2004). Thus, the new recruits enjoy a hiatus from competitive pressure (see discussion in Proffitt *et al.* 2003). Eventually, the plants merge and coalesce into continuous meadows and thereafter would be liable to be at a competitive disadvantage. However, this hypothesis was not directly tested in this study.

Rapid evolution in invasive species can occur as the result of changes in interspecific interactions (Thompson 1998). In the context of a newly invaded area, where herbivory is lacking, individuals that do not allocate resources to defense will have a greater fitness than those that do, assuming such defense has a cost (Keane and Crawley 2002). *S. alterniflora* in Willapa Bay escaped all of its specialist herbivores (Daehler and Strong 1997) until a recent biological control release (Grevstad 2003). Willapa Bay *S. alterniflora* have a generally low, though variable tolerance to one specialist herbivore, the planthopper *Prokelisia marginata* (Daehler and Strong 1997; Garcia-Rossi *et al.* 2003) compared to plants from native areas. It is possible that this

population has experienced a genetically based trade-off between predator defenses and reproductive effort, rather than between defense and growth (Blossey and Notzold 1995). This trade-off could be in addition to or an alternative to a trade-off between competitive ability and reproductive effort.

Self-fertilization

The plants from the invasive Pacific population were able to set two- to three-times the seed in both years when restricted to their own pollen than were the native plants from both the Atlantic and Gulf coasts (Fig. 6). This increased seed set suggests greater self-compatibility in the exotic Pacific population. However, this study did not include an outcrossed control necessary to adequately determine relative rates of self-compatibility. The percent of viable seed is typically much higher for inflorescences without herbivore damage collected from native areas than from the Pacific population (Davis, unpublished data). Additionally to develop a cross-pollination technique, I performed 20 crosses among the native range plants with a resulting mean seed set of about 50% (Davis, unpublished data). These results argue against the odds that native plants are incapable of setting much seed when provided with outcross pollen. A final possibility is that the increased rate of selfed seed set for the invasive plants is due to their greater reproductive effort. However, when reproductive effort is included in the statistical model as a covariate, it is not significant ($p = 0.1837$).

Lloyd (1980) proposed that low density resulting in reduced fertility is likely to be the major selective force fostering self-compatibility in angiosperms. Self-compatibility and self-incompatibility are considered by Lande and Schemske (1985) theoretically to be alternative stable states. They reviewed this pattern in empirical studies (Schemske and Lande 1985) and found their predictions to be supported. However, Aide (1986) pointed out that this pattern was driven primarily by the wind pollinated Poaceae and Pinaceae and suggested the bimodal pattern is due to the greater predictability, whether for or against, of wind pollination. This bimodal pattern was later confirmed for wind-pollinated species, though animal pollinated species were shown to commonly evince intermediate rates of self-compatibility (reviewed in Vogler and Kalisz 2001). Populations at low local densities in species that do not maintain a seedbank, such as *S. alterniflora*, were selected for self-compatibility in Pannell and Barrett's metapopulation model (1998). While this result did not hold true for perennials, rapid population growth could ameliorate the effects of iteroparity on the inhibition of the evolution of self-fertilization. The compatibility rate of the invasive, low density, wind pollinated *S. alterniflora* population at Willapa Bay could be considered intermediate, possibly because it is not equilibrial.

Mechanisms

This study is not sufficient to distinguish the mechanism of the differences among the invasive population and the native populations. One possibility is that by mere chance, the founder or founders of the population possessed relatively *r*-selected

traits. If it were so, happenstance would have dictated whether the population survived and grew. The degree of self-compatibility would have been particularly important, or sexual reproduction could not have occurred. This hypothesis is plausible, for though the great majority of the plants from the native range had little or no self-compatibility, there were a very few with nearly equivalent seed set when restricted to their own pollen to those from the invasive population. However, the differences in the other traits measured were so profound, that it was possible to tell, in every case, whether a mature plant was from the invasive population. I might not have sampled the source population of the invasive plants, or the right genotypes within the correct population. It may be that population or those genotypes no longer exist, especially considering the heavy human-mediated impacts on coastlines, so it is impossible to unequivocally state all the pertinent comparisons were made. Another possible mechanism is that selection has acted upon the rapidly expanding invasive population. It is unlikely that the native populations have diverged in the time frame of ca.100 years, due to the relative stability of the populations and so their long generation time. Genetic drift after foundation is unlikely to have been a factor in the invasive population, as the time scale involved is small and generations are overlapping. Founder effect and selection are not mutually exclusive, so may both have operated possibly at different stages, in the invasion process of *S. alterniflora* at Willapa Bay.

Acknowledgements

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Table 1. Sites for each coast of collection of seeds, number of maternal families per

site, and percent seedling survival for those families assessed for life-history traits.

Coast	Site	Number maternal families	Percent seedling survival
Atlantic coast	Marsh Landing, GA (ML)	4	87
	Shell Hammock, GA (SH)	4	87
	South Jax, FL (SJ)	4	65
Gulf coast	Dauphin Island, AL (DI)	4	92
	Weeks Bayou, MS (WB)	4	92
Pacific coast	Bruceport Co. Park, WA (BCP)	2	85
	Kaffee-Lewis Marsh, WA (KL)	2	100
	Leadbetter Peninsula, WA (LBS)	4	69
	North River, WA (NoR)	4	91
	Naselle River, WA (NR)	1	90
	Palix River, WA (PRSS)	5	80

Table 2. Summary of the analyses for inflorescence emergence, reproductive effort, and self-fertilization. Covariance parameter estimates for the random variables in the self-fertilization mixed model analysis are reported in the text.

Analysis	Effect	df	F-value	*P*-value
Inflorescence emergence 2000-2001	Coast	2	287.87	**< 0.0001**
	Site	8	0.31	0.957
	Maternal family	26	1.66	0.023
Inflorescence emergence 2001-2002	Coast	2	256.43	**< 0.0001**
	Site	8	0.71	0.682
	Maternal family	27	2.38	**0.0001**
Reproductive effort	Coast	2	38.21	**0.0002**
	Site	8	2.13	0.062
	Maternal family	27	0.82	0.730
	Year	1	1.78	0.184
	Coast*year	2	0.06	0.938
Self-fertilization	Coast	2	14.35	**0.0023**
	year	1	17.01	**< 0.0001**
	Coast*year	2	0.16	0.85

Table 3. Summary of the analyses for inflorescence production and biomass.

Analysis	Effect	df	F-value	*P*-value
Inflorescence production	Coast	2	25.52	**< 0.0001**
2000-2001	Site	8	1.67	0.15
	Maternal family	27	1.04	0.43
Inflorescence production	Coast	2	3.54	**0.04**
2001-2002	Site	8	0.23	0.98
	Maternal family	27	2.03	**0.007**
Biomass year 1	Coast	2	157.21	**< 0.0001**
	Site	8	1.60	0.170
	Maternal family	27	1.61	0.045
Biomass year 2	Coast	2	44.00	**< 0.0001**
	Site	8	0.47	0.870
	Maternal family	27	1.64	0.045

Table 4. Summary of the logistic regressions for year 1 and 2 for death. Backward

model selection dictated rejected variables to have a probability of > 0.05. RE $=$

reproductive effort.

Year	Accepted variables	Rejected variables	df	Wald chi-square	p-value
1	RE in year 1		1	23.27	**< 0.0001**
		Coast	2	0.05	0.98
		Site	10	12.59	0.25
		Maternal family	37	10.86	1.0
		Coast*RE	2	0.1	0.95
2	RE in year 1		1	14.04	**0.0002**
		RE in year 2	1	2.13	0.15
		Coast	2	0.311	0.86
		Site	10	3.12	0.98
		Maternal family	37	3.66	1.0
		Coast*RE	2	0.01	0.95

Figure 1. Map of seed collection sites. Pacific coast sites at Willapa Bay, WA are

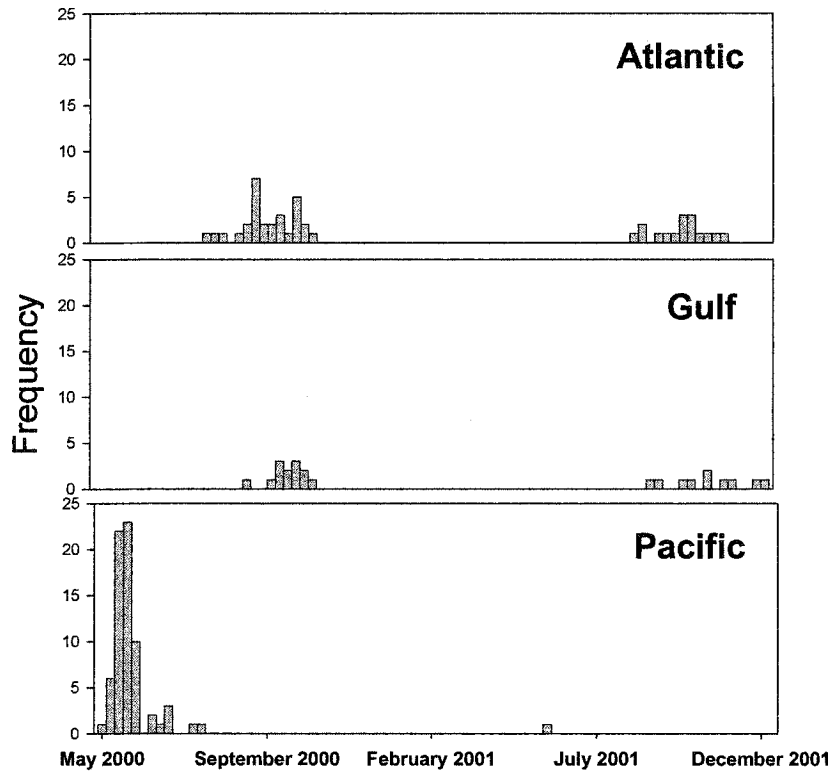represented by stars, clockwise from the top: NoR, PRSS, BCP, NR, KL, LBS.

Figure 2. Distributions of age of first reproduction for the native Atlantic and Gulf

coast populations and the invasive Pacific population. Nine Gulf and two Atlantic

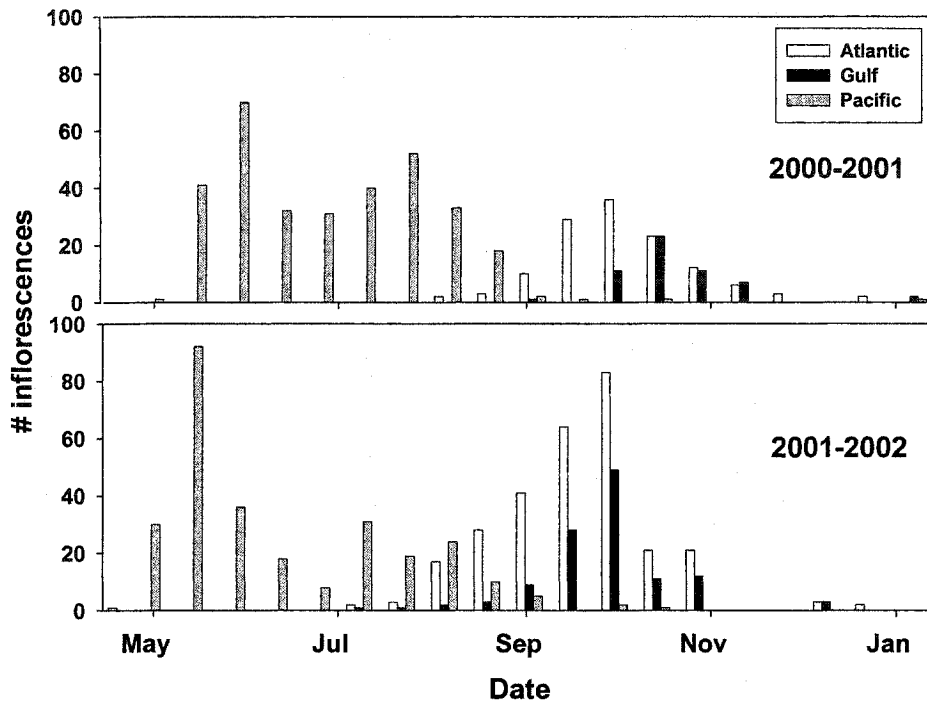coast individuals did not produce inflorescences through 2003.

Figure 3. Distributions of inflorescence emergence for the growing seasons of 2000-2001 (top) and 2001-2002 (bottom) for native Atlantic (white) and Gulf (black) coast populations and the invasive Pacific (grey) population. Each bar represents the number of inflorescences extruded for that coast in a two-week period. The number of plants with inflorescence emergence for 2000-2001/2001-2002 are: Atlantic = 30/44; Gulf = 13/21; Pacific 70/49.
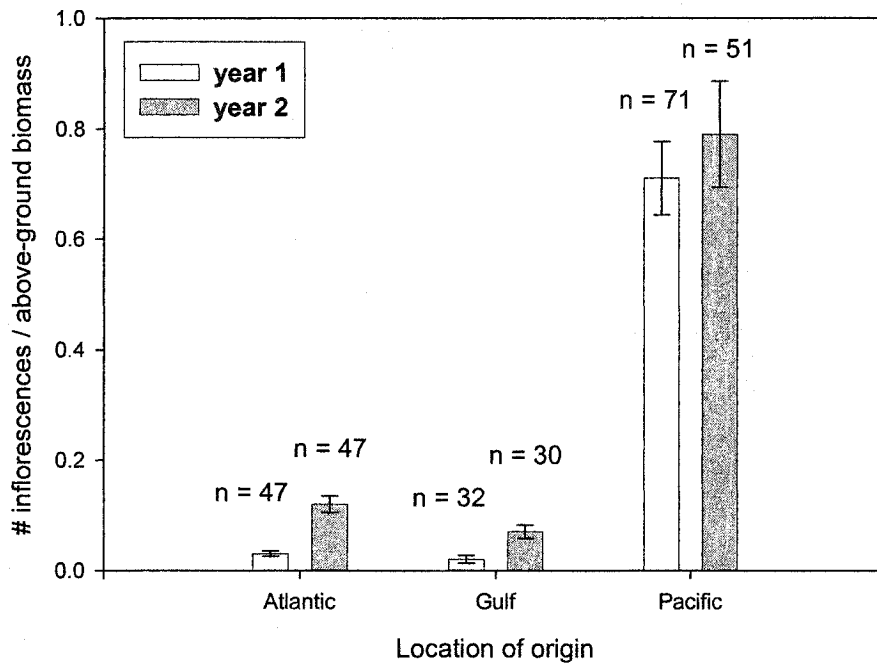
Figure 4. Reproductive effort (raw means ± 1 SE) for plants from the native Atlantic and Gulf populations and the invasive Pacific population for the first and second years of growth.
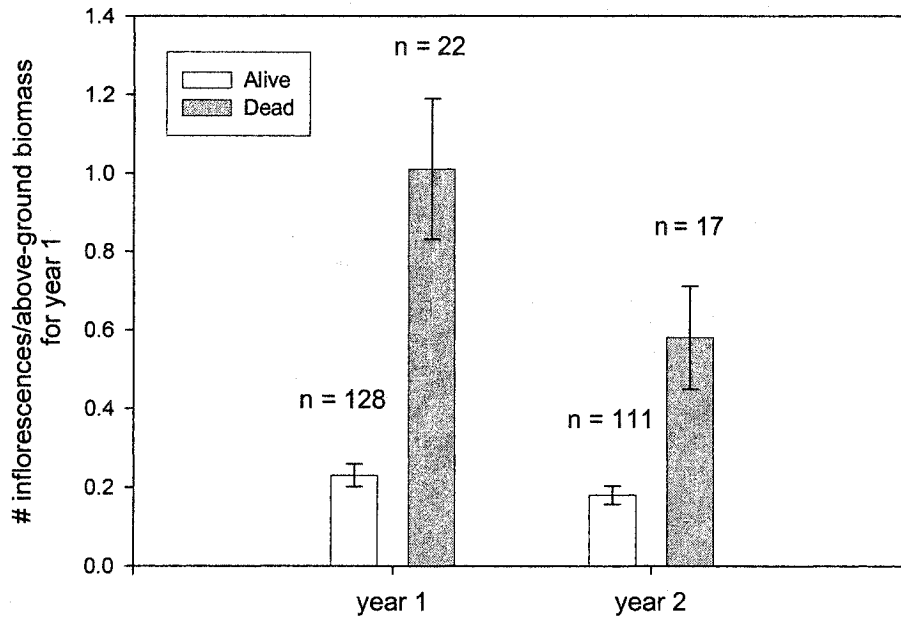
Figure 5. Reproductive effort (raw means ± 1 SE) in the first year of growth for the

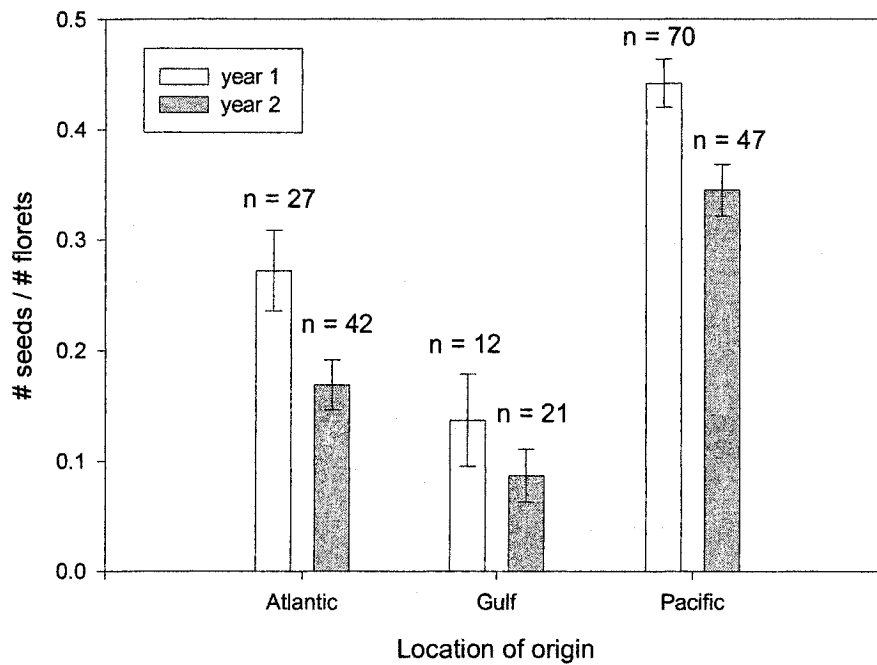plants that lived or died after the first or second year.

Figure 6. Seed set using only self pollen (raw means ± 1 SE) for plants from the native Atlantic and Gulf populations and the invasive Pacific population for the first and second years of growth.

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