Salt Marsh *Claviceps purpurea* in Native and Invaded *Spartina* Marshes in Northern California

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ABSTRACT

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The fungal pathogen *Claviceps purpurea* (subgroup G3) has a worldwide distribution on salt marsh *Spartina* spp. In Northern California (United States), native *Spartina foliosa* sustains high rates of infection by G3 *C. purpurea* in marshes north of the San Francisco Estuary. Invasive populations of *S. alterniflora* and *S. alterniflora* × *foliosa* hybrids are virtually disease free in the same estuary, although *S. alterniflora* is host to G3 *C. purpurea* in its native range (Atlantic Coast of the United States). Greenhouse inoculation experiments showed no differences in susceptibility among *S. foliosa*, *S. alterniflora,* and *Spartina* hybrids. Under field conditions, *S. foliosa* sustained a higher incidence of disease in coastal marshes than in marshes within the bay. This geographic effect may be attributable to environmental differences between the coast and the bay proper, with the former being more conducive to infection by *C. purpurea*. Seed set of *S. foliosa* spikelets was 40 to 70% lower on infected than on uninfected inflorescences, but seed germination was not affected. The *C. purpurea* epidemic on *S. foliosa* on the coast north of the San Francisco Estuary further reduces the meager competitive ability of this declining native plant species.

Additional keywords: biological invasions, ergot, nonsystemic floral pathogen, plant disease epidemics, San Francisco Bay

Epidemics caused by plant-pathogenic fungi are relatively rare in wild plant populations (7). Relatively constant, low disease incidence is expected in natural plant populations because environmental heterogeneity, species richness (26,30), host density (8), genetic diversity (2,37), age structure (11), and host–pathogen coevolution (36) each militate against range-wide epidemics. However, plant-pathogenic fungi can reach high levels of infection in natural plant populations when low species diversity is combined with high plant densities under favorable environmental conditions (8,22,34). For example, *Spartina anglica* on the coast of England is host to yearly epidemics of ergot, caused by the fungal pathogen *Claviceps purpurea*, which have been attributed to the high density and genetic uniformity of host plant populations (23,34). Similar conditions prevail in conventional agriculture where disease outbreaks are more common.

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When plant-pathogenic fungi are successful in parasitizing host plants in wild systems, they can have major impacts on plant survival, growth, fecundity, and competitive interactions (21). For example, Chestnut blight, caused by *Cryphonectria parasitica*, has essentially eliminated overstory chestnut from eastern U.S. forests (3), and Dutch elm disease, caused by *Ophiostoma ulmi* and *O. novo-ulmi*, has decimated both planted and native stands of American elms (20,27). Chestnut blight and Dutch elm disease are illustrative of pathosystems in which high rates of infection by exotic pathogens occur in temperate forests, where species diversity is often low.

Salt marshes along the Atlantic and Pacific Coasts of North America are also habitats typically dominated by single species: cordgrasses in the genus *Spartina*. This includes smooth cordgrass, *S. alterniflora*, on the Atlantic Coast of the United States and California cordgrass, *S. foliosa*, on the Pacific Coast from Baja, Mexico to Bodega Bay, CA. These species dominate lower intertidal marshes in their respective ranges. In England, the allopolyploid species *S. anglica* (*S. alterniflora* × *S. maritima*), which originated in the last century (35), fills a role in the biotic community similar to that of its U.S. congeners. Similar to *S. anglica*, *S. alterniflora* and *S. foliosa* host ergot outbreaks in their respective native ranges (14,19). Ergot appears to be a natural component of the *S. alterniflora* marsh community, having first been identified on this species on the Atlantic Coast of the United States in 1895 (15).

S. alterniflora was introduced intentionally to the San Francisco Estuary in the 1970s (16) and subsequently hybridized with native *S. foliosa,* which is now being displaced by its hybrid progeny and their affiliated introgressants (5,6). Many *Spartina* hybrid genotypes, such as *S. alterniflora* (9), are competitively superior to *S. foliosa*, showing a wider intertidal distribution, greater reproductive fitness as measured by seed and pollen production, and greater above- and belowground biomass (6). Spreading from the sites where *S. alterniflora* was introduced in central San Francisco Bay, hybrids are expanding rapidly throughout San Pablo and San Francisco Bays. *Spartina* hybrids are now the most common exotic *Spartina* spp. in the estuary (5).

Overlaid on competition between plant species is an ongoing epidemic of ergot on *S. foliosa* in the San Francisco Estuary. The origin of the causal pathogen, *Claviceps purpurea*, is unknown; however, its occurrence on the Pacific Coast dates at least to 1888, when it was collected near San Diego from *S. stricta* (synonym of *S. foliosa*) (Michigan State University Herbarium). The pathogen was documented in San Francisco Bay in 1952 (17) but may have been present for much longer. The extent to which ergot influences the outcome of competition between native, exotic, and hybrid *Spartina* spp. will be determined by the relative impact of this disease on its local host species. To better understand the possibilities in this regard, the present study was undertaken to (i) characterize temporal and spatial variation in ergot incidence on native and invasive *Spartina* spp. within the San Francisco Estuary and on the Pacific Coast north of the estuary, (ii) determine whether *Spartina* spp. and hybrids in the San Francisco Estuary differ in their inherent susceptibility to ergot, and (iii) quantify the impacts of ergot on *S. foliosa* seed quantity and quality.

MATERIALS AND METHODS

Disease incidence. Salt marsh *Spartina* spp. are found in the San Francisco Estuary, including San Francisco and San

Pablo Bays, and in coastal marshes north of the San Francisco Estuary. Seven native *S. foliosa* marshes along the coast and within San Francisco Estuary and six marshes dominated by *Spartina* hybrids (all within San Francisco Bay) were surveyed for the presence of ergot during the fall of 2000, 2001, and 2002. Sampling dates ranged from late November to early January due to differences in flowering phenology between years. Native (dominated by *S. foliosa*) and hybrid (dominated by *S. alterniflora* × *S. foliosa* hybrids) marshes were identified previously using randomly amplified polymorphic DNA (RAPD) by Ayres et al. (4,5). Native marshes included coastal marshes north of San Francisco Estuary at Drakes Estero, Point Reyes National Seashore (Point Reyes), Tomales Bay, and Bolinas Lagoon, and marshes within San Francisco Estuary at Palo Alto, Mountain View, China Camp State Park (China Camp), and Petaluma River (Fig. 1). Marshes dominated by *Spartina* hybrids were all within San Francisco Bay and included sites at San Bruno, San Mateo, Alameda Island, San Leandro, Hayward, and Tiburon Marsh (Fig. 1). Currently, there are no hybrid marshes along the outer coast.

Spartina plants in tidal marshes grow in monoculture and fuse with adjacent plants at their margins. Consequently, it is not possible to identify individual plant boundaries and, for this reason, inflorescences were treated as individuals as described by Raybould et al. (34). To sample for ergot, 0.25-m^2 quadrats were placed every 10 paces along two 100-m transects for a total of 20 quadrats per site. Disease incidence was measured at each marsh as the proportion of the total number of inflorescences per quadrat with ergot. If no ergot was observed while sampling, marshes were inspected visually by walking transects through the center of the marsh and along the marsh edges to determine whether the pathogen was present at the site.

Using the proportion of inflorescences with ergot per quadrat as the response variable, we used two-way analysis of variance (ANOVA) to evaluate differences in disease incidence among *Spartina* populations as implemented in the Proc Mixed procedure in SAS (SAS System, version 8.2; SAS Institute, Cary, NC). In this analysis, site was treated as a fixed factor and year and interaction terms were treated as random factors. To determine the significance of the random factors, we used a χ^2 test to compare residual log likelihood values with and without the random factor included in the analysis. The Student-Newman-Keuls test was used for mean separation after the ANOVA. We transformed the proportion of inflorescences with ergot by the natural log $(x + 1)$ to achieve homogeneity of variances, as indicated by Levene's test (28).

Because plant-to-plant contact facilitates movement of *C. purpurea* in a host population, inflorescence density may contribute to infection rates in the field (8). For data taken in native *S. foliosa* marshes, multiple regression was used to test for a correlation between the incidence of ergot and inflorescence density per quadrat; year, site, and a year–site interaction were included as categorical variables. There were too few infected inflorescences to conduct this test using data from hybrid marshes.

Unlike *S. alterniflora* and *Spartina* hybrids, native *S. foliosa* is found in marshes both on the outer coast and within the San Francisco Estuary. To test for differences in infection rates between coastal and bay *S. foliosa* marshes, a two-way ANOVA was performed using the Proc Mixed procedure in SAS with infection rate as the response variable, region (coast or bay) as a fixed factor, and year as a random factor.

Genetic analysis was used to confirm whether infected plants in invaded marshes were *S. foliosa*, *S. alterniflora,* or *Spartina* hybrids. Native and invasive *Spartina* plants share many morphological characteristics and can be difficult to differentiate in the field (5). In 2003, leaf material was collected from infected inflorescences at sites invaded by *S. alterniflora* and *Spartina* hybrids. DNA was extracted from leaves according to Ayres et al. (4). RAPD analysis was used to determine the identity of infected plants as a pure *Spartina* sp. or a hybrid as described by Ayres et al. (4).

Variation in susceptibility. *Preparation of plants.* Seed and cuttings of California cordgrass (*S. foliosa*) were collected in fall 2000 from four native populations (Bolinas Lagoon, Mountain View, Palo Alto, and San Mateo) (Fig. 1). Seed of smooth cordgrass (*S. alterniflora*) were collected from its native range on the Atlantic Coast of the United States at two sites on Sapelo Island, Georgia (University of Georgia Marine Institute) and two sites in Florida (Levy County and Saint Augustine). Seed

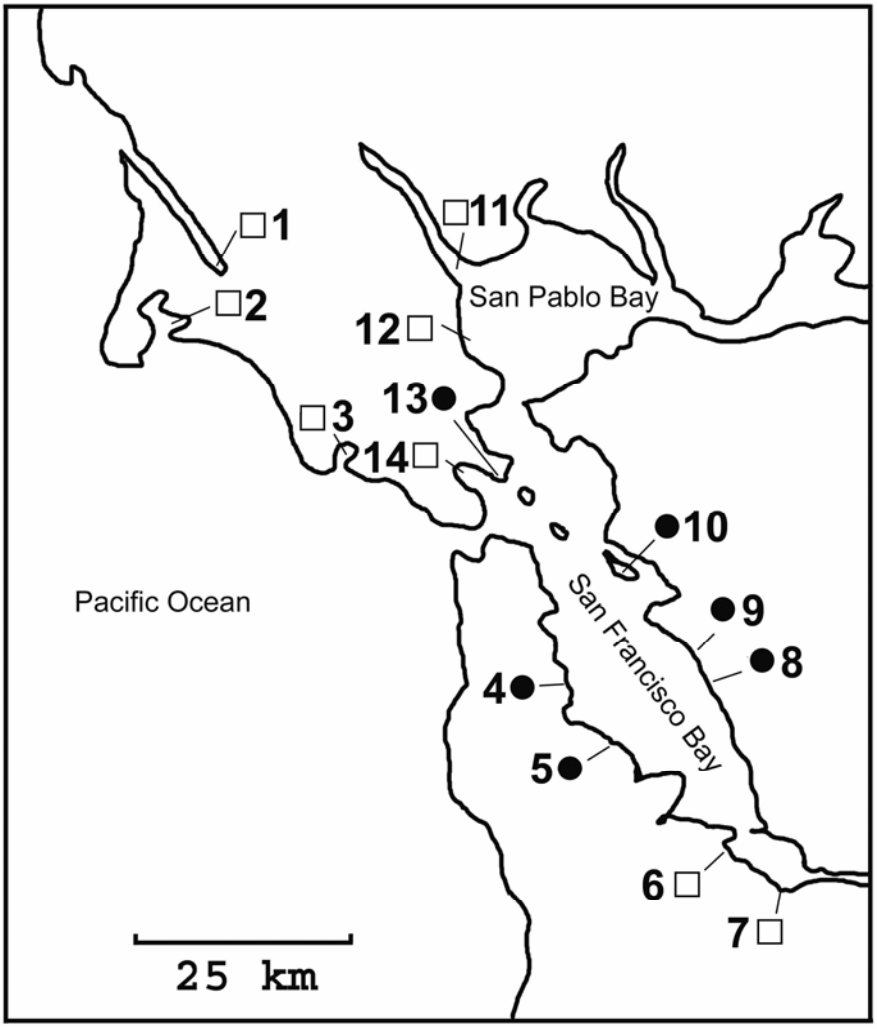

Fig. 1. *Spartina* marshes sampled for ergot in the San Francisco Estuary and Pacific Coast. Squares (□) signify native marshes and circles (●) denote marshes invaded by either *S. alterniflora, Spartina* hybrids, or both. Numbers correspond to the following locations: 1 = Tomales Bay, 2 = Point Reyes, 3 = Bolinas Lagoon, 4 = San Bruno, 5 = San Mateo, 6 = Palo Alto, 7 = Mountain View, 8 = Hayward, 9 = San Leandro, 10 = Alameda Island, 11 = Petaluma River, 12 = China Camp, 13 = Tiburon, and 14 = Mill Valley.

were collected from marked hybrid plants identified by Zaremba (38) in Cogswell Marsh, Hayward, CA. Prior to planting, seed were stratified in 25% sea water for 6 weeks, germinated in the spring of 2001 and grown in a greenhouse at the University of California, Davis (UC Davis). From May to November 2002, plants were inoculated with *C. purpurea* isolate CDE-1 collected from *S. foliosa* in Point Reyes, CA (NRRL 37645, Agricultural Research Service Culture Collection, United States) using the technique described below. Isolate CDE-1 is associated with the G3 subgroup of *C. purpurea*, as defined by Pažoutová et al. (32). The G3 subgroup is equivalent to *C. purpurea* var. *spartinae* (12). CDE-1 was considered representative of this subgroup because population genetic studies based on RAPD and amplified fragment length polymorphism markers show that, unlike ergot populations elsewhere, there is very low genetic diversity in ergot samples collected from *Spartina* spp. in the San Francisco Estuary (18,19). After inoculations, half the plants were maintained in the greenhouse at UC Davis in central California and half were taken to a greenhouse at the Bodega Marine Laboratory, on the coast north of the San Francisco Estuary. Temperature and humidity measurements were not taken in 2002; in 2003, the average daily greenhouse temperature and relative humidity for Davis were 24ºC and 75%, respectively; average daily greenhouse temperature and humidity for Bodega were 16ºC and 75%, respectively.

To increase the number of genotypes represented in our tests, additional plants were collected in 2002. Cuttings of *S. foliosa* were collected randomly from three new marshes (China Camp, Petaluma River, and Bodega Bay) (Fig. 1). Additional cuttings were used from plants originally collected on the Atlantic Coast of the United States at two sites in New York (Long Island) and one site in North Carolina (Carteret County). Plants were inoculated from April to September 2003 and maintained in a greenhouse at UC Davis to monitor for infection.

Spartina hybrids include a range of genotypes from close *S. alterniflora* affiliates to close *S. foliosa* affiliates (4). To ensure that our sample represented a broad range of these diverse hybrid genotypes, divisions from 19 additional hybrid plants (38) were collected in the winter of 2002 and grown in the greenhouse at UC Davis. In 2003, these plants were inoculated with CDE-1 using the methods described below.

Inoculation protocol. Colonized, dried filter paper was used to establish potato dextrose agar cultures of isolate CDE-1 (19). Cultures were maintained at 24ºC for 14 days before conidia were collected in 0.5% KCl for a final concentration of 10^6 spores/ml. Inoculations were accomplished by dipping *S. foliosa*, *S. alterniflora*, and hybrid *Spartina* inflorescences into the spore suspension just prior to anthesis. Control plants were dipped into a solution that contained only 0.5% KCl. Following inoculation, inflorescences were wrapped in polyvinyl chloride (plastic wrap) and placed in a growth chamber for 24 h (day and night temperatures, 17 and 14ºC, respectively; photoperiod day length, 15 h; light level, Max PAR 1,100 μE/m^2/s; relative humidity 80%). After 24 h, the plastic wrap was removed and plants were returned to the greenhouse (see above). Inflorescences were checked weekly for signs of infection. Inflorescences with spikelets producing honeydew, the first visible sign of infection, were scored as positive for disease (33).

The proportion of inoculations resulting in at least one infected spikelet per inflorescence were compared between *S. foliosa*, *S. alterniflora*, and hybrids. The null hypothesis of no association, or independence, was tested by computing the χ^2 statistic.

Effects on seed production and seed quality. *Seed production. S. foliosa* inflorescences were collected in the fall of 2001 and 2002 at four native marshes in the San Francisco Estuary (Palo Alto, Mill Valley, Mountain View, and the native portion of San Mateo) (Fig. 1). *S. foliosa* has variable seed production and these sites were chosen because plants located there had a history of producing seed (D. Ayres, *unpublished observations*). To obtain inflorescences ranging from disease free to severely infected, we selected a third of the inflorescences with no ergot at each site, a third that had 1 to 10 visible ergots, and a third that had more than 10 ergots in both years. In 2001, 60 inflorescences were collected from each marsh for a total of 240 inflorescences. In 2002, 50 inflorescences were collected from Palo Alto (where infection levels were too low to obtain the desired 60), 61 from Mill Valley, 81 from Mountain View, and 73 from San Mateo), for a total of 265 inflorescences. In the lab, spikelets were removed and the number of ergots, number of seed, and total number of spikelets were recorded.

Disease severity was defined as the proportion of spikelets in an inflorescence containing ergot, and fecundity was defined as the proportion of spikelets containing seed. The proportions were calculated by dividing either ergot spikelets or seed-filled spikelets by the sum of ergot spikelets, empty spikelets, and seed-filled spikelets. A multiple regression was used to characterize the relationship between fecundity and disease severity, with year and site included as additional explanatory variables. We transformed disease severity measures and fecundity measures by the natural log (x + 0.5) to improve normality and achieve homogeneity of variances. We estimated the percent reduction in seed production due to *C. purpurea* infection at each site based on a 2-year sampling period and assessed the significance of the reduction using a t test. Inflorescences also were divided into severity classes (0, >0 to 10, >10 to 20, >20 to 30, >30 to 50, and >50%) based on the proportion of spikelets

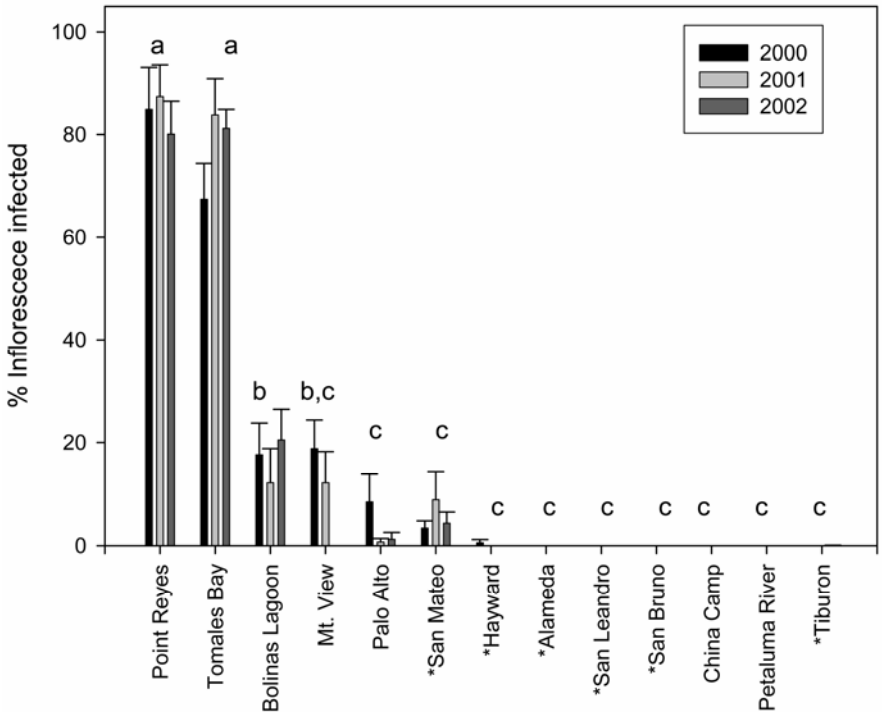

Fig. 2. Comparison of percent infected inflorescences per quadrat at hybrid and native marshes in the San Francisco Estuary. The Student-Newman-Keuls test was used for mean separation after analysis of variance. Error bars represent 1 standard error. Marshes dominated by *Spartina* hybrids are marked with an asterisk (*).

infected. To determine the effect of ergot on seed production, we compared mean seed number and proportion of spikelets containing seed among severity classes in a one-way ANOVA using Dunnett's multiple comparison test (13,34). To determine if there was an effect of ergot beyond the direct loss of spikelets to infection, we repeated the ANOVA above using the proportion of noninfected spikelets that produced seed rather than the proportion of total spikelets that produced seed. This proportion was calculated by dividing the number of seed-filled spikelets by the sum of empty spikelets and seed-filled spikelets.

Seed quality. Seed were placed in separate, perforated ziplock bags after being counted in the previous experiment and the collection was divided randomly into two groups, keeping seed bags and ergot bags from the same inflorescence together. One group was used to examine the relationship between disease severity and seed weight; the second was used to evaluate the relationship between disease severity and seed germination. Disease severity was defined as the percentage of infected spikelets per inflorescence. The number of infected spikelets per inflorescence also was recorded so that our results could be compared with Raybould et al. (34). Protocols were the same in 2001 and 2002.

Seed weight. Seed and ergot were transferred to small manila envelopes. Samples were oven dried at 86ºC for 2 h prior to weighing. To obtain an average seed weight for each inflorescence, the total seed weight was divided by the number of seed. In 2001 and 2002, seed from 55 and 93 inflorescences, respectively, were weighed. Of the 148 inflorescences used in this experiment, 75 had been infected with ergot and 73 were ergot free.

A two-way ANOVA was used to test for effects of site and disease severity on mean seed weight per inflorescence (34), using the Proc GLM procedure in SAS. The result of the previous analysis could be confounded by a relationship between seed number and seed weight; therefore, a regression analysis was used to test for this relationship.

Seed germination. In 2001, seed were stratified as described above. At the end of the 6-week period, seed were removed from bags and treated with 1.2% sodium hypochlorite (diluted commercial bleach) for 5 min, rinsed in deionized water, and placed on wet filter paper inside a petri plate. Petri plates were sealed with parafilm and placed in indirect sunlight. Seed were monitored for germination every 6 to 7 days. Seedlings greater than 1.25 cm in length were scored as positive for germination. A regression was used to characterize the relationship between disease severity (the proportion of infected spikelets) and the proportion of seed that germinated on an inflorescence. We also performed a two-way ANOVA with mean number of seed germinated per inflorescence as the response variable and ergot class and site as explanatory variables using the Proc GLM procedure in SAS.

RESULTS

Disease incidence. Infection rates varied widely between sites ($P < 0.01$) (Fig. 2), ranging from 0 to 87.5% in native marshes and 0 to 8.8% in hybrid marshes. Infection rates were not significantly different between years ($\chi^2 = 0$, df = 1, $P = 1$) and there was no interaction between site and year ($\chi^2 = 1.8$, df = 1, $P = 0.18$). Two native marshes along the outer coast, Point Reyes and Tomales Bay, had the highest rates of infection with means of 84% (±4.0 standard error [SE]) and 77% (±3.7 SE), respectively (Fig. 2), whereas four of six hybrid marshes sampled in the interior of the bay had no infected plants.

The effect of region (coast or bay) on infection rate for *S. foliosa* was significant ($P < 0.01$), being 10-fold higher in coastal marshes compared with bay marshes, and there was no interaction between region and year ($\chi^2 = 0.1$, df = 1, $P = 0.75$). There was a significant correlation between density of flowering stems and disease incidence ($R^2 = 0.71$, $P = 0.01$). This correlation was consistent between years ($P = 0.21$) but not between sites ($P = 0.01$) and there was no year–site interaction ($P = 0.09$).

Based on results from three RAPD primers, one of the four infected inflorescences collected at the hybrid San Mateo marsh in 2002 was identified as *S. foliosa*, whereas the other three were identified as hybrids (*data not shown*). All infected plants sampled at Tiburon Marsh were identified as *S. foliosa*. Infected inflorescences were observed during a visual inspection at Hayward Marsh in 2000 and in San Leandro Marsh in 2001. Both Hayward and San Leandro marshes are dominated by hybrids; however, it is unknown whether these observed, infected plants were *S. foliosa*, *S. alterniflora,* or hybrids.

Susceptibility of *Spartina* spp. and hybrids. Greenhouse location (Davis or Bodega) did not affect the proportion of inflorescences that became infected for *S. foliosa* ($\chi^2 = 0.72$, df = 1, $P = 0.40$), *S. alterniflora* ($\chi^2 = 1.87$, df = 1, $P = 0.17$), and hybrids ($\chi^2 = 0.34$, df = 1, $P = 0.56$); therefore, data from the two locations in 2002 were pooled (Table 1). Differences between species and hybrids were not statistically significant in either 2002 ($\chi^2 = 3.91$, df = 2, $P = 0.14$) or 2003 ($\chi^2 = 1.14$, df = 2, $P = 0.57$) (Table 1). Little or no infection was observed on control plants for both years, except for *S. alterniflora* in 2003. During that year, control plants sustained a level of infection that was not significantly different from inoculated plants ($\chi^2 = 0.53$, df = 1, $P = 0.47$), apparently due to cross-contamination.

Effects of *C. purpurea* on reproductive fitness in *S. foliosa*. *Seed quantity.* Ergot infection resulted in lower seed production in both years. Seed set in the lowest infection class (10% infected spikelets) was reduced by 40% in 2001 and by more than 70% in 2002, whereas no seed were produced in infection classes greater than 50% in either year (Fig. 3). The results were the same when the comparison was based on the proportion of noninfected spikelets that produced seed (*data not shown*). A two-way ANOVA revealed a significant effect of year ($P < 0.01$) and site ($P < 0.01$), and the interaction of year–site also was significant ($P < 0.01$). Consequently, years and sites were analyzed separately to examine the range of correlations between infection level and seed set. In most cases, the effect of ergot on seed production was significant: Mill Valley in 2001 ($R^2 = 0.31$, $P < 0.01$), Mountain View in 2001 ($R^2 = 0.34$, $P < 0.01$), Palo Alto in 2001 ($R^2 = 0.31$, $P < 0.01$), San Mateo in 2002 ($R^2 = 0.41$, $P < 0.01$), Mountain View in 2002 ($R^2 = 0.18$, $P < 0.01$), and Palo Alto in 2002 ($R^2 = 0.19$, $P < 0.01$). Disease effects were marginally significant in Mill Valley in 2002 ($R^2 = 0.06$, $P = 0.05$) and not significant at San Mateo Marsh in 2001 ($R^2 = 0.14$, $P = 0.21$). Infection reduced both seed number and percentage of spikelets in an inflorescence containing seed. The proportion of spikelets containing seed was lower in all infection classes compared with uninfected inflorescences in both years (Fig. 3). Seed number was consistently lower on plants with more than 10 ergots in 2001 and lower in all infection classes in 2002 compared with uninfected inflorescences (Fig. 3). In 2002, no seed was produced on inflorescences with greater than 20% ergot spikelets per inflorescence. Seed production among our samples in 2001 was twice that of 2002.

Seed quality. Infection by *C. purpurea* reduced *S. foliosa* individual seed weight

Table 1. Taxa-specific infection rates after experimental inoculations with G3 *Claviceps purpurea*

Species	Year	Percent infection (no. of plants)[a]
Spartina foliosa	2002	83 (15/18)
S. foliosa	2003	71 (27/38)
S. alterniflora	2002	60 (3/5)
S. alterniflora	2003	56 (9/16)
S. alterniflora × *S. foliosa* hybrids	2002	50 (6/12)
S. alterniflora × *S. foliosa* hybrids	2003	63 (7/11)

[a] Data are percentages of inoculated (raw data) plants infected. Differences in infection frequency among taxa were not significant.

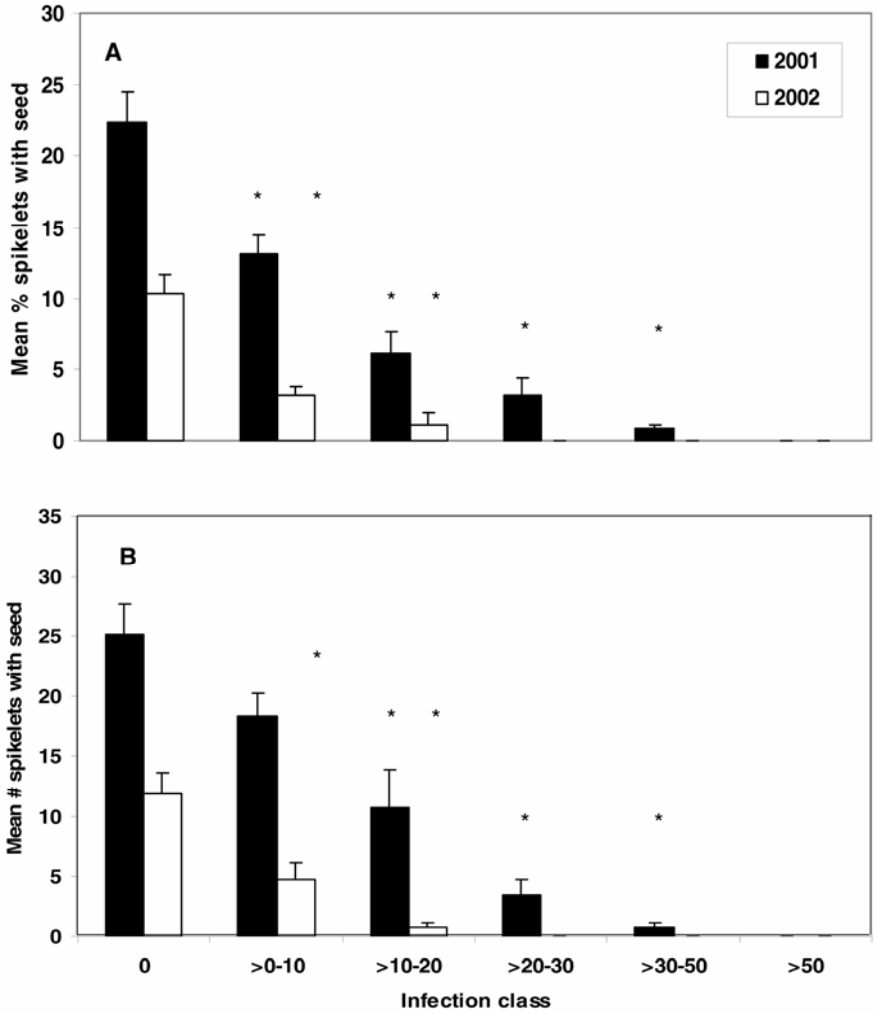

Fig. 3. Effects of *Claviceps purpurea* on seed production in *Spartina foliosa.* Dunnett's test (following a one-way analysis of variance) was used to compare infected (>0% ergot classes) with uninfected (0% infected) inflorescences ± standard error. For each year, infection classes (0, >0 to 10, >10 to 20, >20 to 30, >30 to 50%, and >50% of spikelets infected) with significantly lower seed than controls ($P < 0.05$) are marked with an asterisk (*).

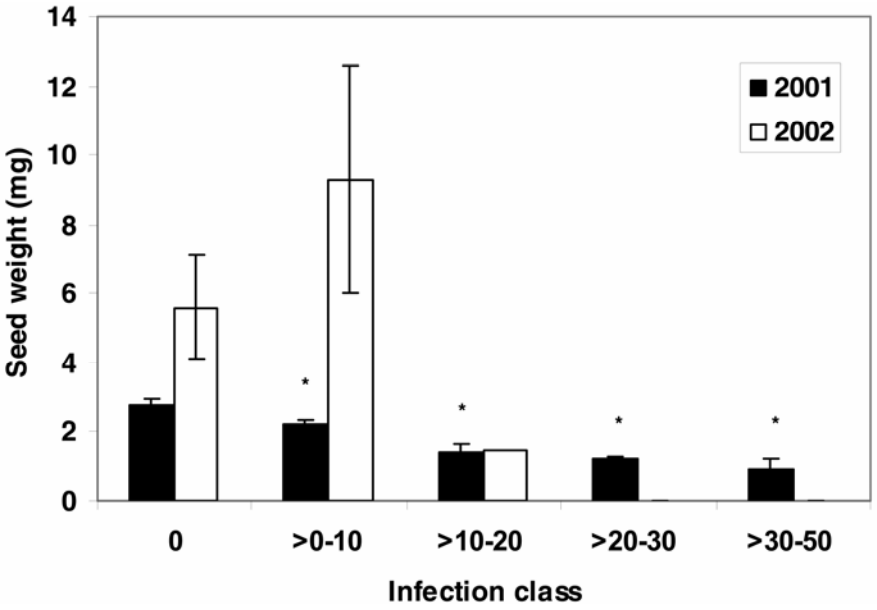

Fig. 4. Effects of *Claviceps purpurea* on seed weight on inflorescences of *Spartina foliosa*. Dunnett's test (following a one-way analysis of variance) was used to compare infected (>0% ergot classes) with uninfected (0% infected) inflorescences. Ergot classes with significantly lower seed than controls ($P < 0.05$) are marked with an asterisk (*).

in 2001 but not 2002. In 2001, individual seed weights were lower in every infection category compared with controls (Fig. 4). These results were consistent across sites ($P = 0.46$) and there was no interaction between site and ergot class ($P = 0.87$). There was no relationship between the total number of seed on an inflorescence and the weight of individual seed ($P = 0.34$).

Overall, seed from infected inflorescences germinated at the same rate as seed from uninfected inflorescences ($P = 0.16$; *data not shown*). Germination rates were highly variable, ranging from 0 to 69%. Inflorescences in higher infection classes produced too few seed to obtain estimates of germination rates.

DISCUSSION

The *C. purpurea–Spartina* spp. pathosystem offers examples of many different interaction outcomes resulting from the introduction and proliferation of susceptible exotic *Spartina* spp. Variation in outcomes would be expected because novel interactions often follow the establishment of exotic plant and microbial species in new environments (31). In southern England, the rapid expansion of the non-native hybrid *S. anglica* coupled with the wet and cool climate conducive to infection created the opportunity for a sustained *C. purpurea* epidemic (34). In Willapa Bay, WA, where invasive *S. alterniflora* already has colonized more than 20% of available mudflats, G3 *C. purpurea*, though present in the estuary, is rare (18). In the San Francisco Estuary, where native and invasive *Spartina* spp. coexist, the native is parasitized, with significant impacts on fecundity, whereas the invasive escapes infection almost completely.

Our study documents an epidemic of ergot on *S. foliosa* in coastal marshes north of the San Francisco Estuary, where more than 65% of inflorescences were infected with the pathogen every year. Within the estuary, *S. foliosa* inflorescences along the Petaluma River and China Camp State Park in the San Pablo Bay had almost no observable ergot; and fewer than 15% infected inflorescences generally were seen in native marshes in the San Francisco Bay. Marshes dominated by invasive *Spartina* hybrids were virtually ergot free. It was not possible to compare rates of infection on native and invasive *Spartina* spp. in northern coast marshes because invasive *Spartina* spp. only rarely invade these areas and individual colonizers have been eradicated.

S. foliosa, *S. alterniflora*, and *Spartina* hybrids are equally susceptible to G3 *C. purpurea* when inoculated under controlled conditions. These findings, as well as infection rates in *S. alterniflora* along the East Coast (15), argue against resistance in *S. alterniflora* or hybrid *Spartina* as an explanation for their low rates of

infection in San Francisco marshes. An alternative explanation would be a lack of inoculum in hybrid-dominated sites; however, this seems unlikely because infected inflorescences were observed on *Spartina* spp. next to marshes where hybrid populations were surveyed in 2000 (Hayward) and 2001 (San Leandro). It seems more likely that *S. alterniflora* and *Spartina* hybrids sustain low infection rates because of incongruities in host and parasite phenologies. In the San Francisco Estuary, *S. alterniflora* flowers several weeks after *S. foliosa* in late summer, and *Spartina* hybrids have an intermediate flowering time overlapping both pure species (D. Ayres and D. Strong, *personal communication*). This difference in flowering phenology may favor infection of *S. foliosa* because its period of susceptibility (flowering prior to anthesis) is more likely to coincide with conditions conducive to spore production and infection (i.e., wetter weather) that occur earlier in the season. It is not known whether flowers are open for the same length of time in the pure species and hybrids.

Weather differences also may account for greater severity of the ergot epidemic on *S. foliosa* in coastal marshes than in bay marshes. The climate on the outer coast, when *S. foliosa* inflorescences reach anthesis, is characterized by fog and cool temperatures, whereas relatively drier conditions prevail within the bay during this period. This regional difference is illustrated by comparative precipitation totals at representative sites during June and July when *S. foliosa* is flowering. On the coast at Point Reyes National Seashore, precipitation totals during June and July (averaged over 3 years, 2000 to 2002) were 11.7 and 0.00 mm, respectively. Precipitation totals recorded within the bay at San Francisco International Airport, in San Bruno, in June and July (2000 to 2002), were 2.03 and 0.00 mm, respectively. When hybrid *Spartina* is flowering in August and September, average precipitation was lower at both sites: 0.59 and 2.2 mm, respectively, in Point Reyes and 0.08 and 1.52 mm, respectively, in San Bruno. More abundant moisture should facilitate release of ascospores from sclerotia (1), infection, and vegetative growth of *C. purpurea* (29). Thus ambient conditions on the coast (cool and moist) are expected to be conducive to ergot outbreaks more often than in marshes within the San Francisco Estuary. Likewise, *S. foliosa* is more likely than *Spartina* hybrids to be flowering during periods when environmental conditions support disease development.

One way of quantifying the impact of ergot on *S. foliosa* is to assess fecundity, a key measure of fitness. Fecundity is a function of the quantity and quality of seed produced (24), and both were reduced by *C. purpurea* infection of *S. foliosa*. Infected inflorescences produced fewer seed and seed of lower weight than uninfected inflorescences. These findings are consistent with a previous report showing that seed developing on ergot-infected rye inflorescences was only half the weight of normal seed (10). In general, the presence of the pathogen during seed development did not reduce *S. foliosa* germination rates.

Detrimental effects of ergot on seed production also have been reported for other salt marsh cordgrasses. For example, Eleuterius and Meyers (15) monitored a *C. purpurea* epidemic in *S. alterniflora* Gulf and Atlantic Coast marshes over a 6-year period and estimated that the pathogen reduced seed production in Mississippi by 68.5% (14). In Poole Harbour, England, where rates of infection also were consistently high, *S. anglica* inflorescences with greater than 10% of spikelets infected produced fewer seed (34). Inflorescences with less than 10% of spikelets infected produced more seed than uninfected inflorescences. In contrast, any infection reduced seed production in *S. foliosa*. Low rates of infection in *S. anglica* produced higher individual seed weights, whereas infection reduced seed weight in *S. foliosa* (23). Thus, it appears that *S. anglica* can compensate for a loss of productive florets, whereas our data suggest that *S. foliosa* lacks this ability.

In addition to producing seed, *Spartina* spp. reproduce by clonal expansion and vegetative fragmentation. Based on evidence of low reliance on seedling recruitment, Gray et al. (23) concluded that ergot infection was not likely to heavily impact *Spartina* spp. given the lack of a competitor at lower salt marsh elevations. Similarly, Raybould et al. (34) speculated that a sustained ergot epidemic on *S. anglica* would have little effect on population growth because seedling recruitment is unsuccessful in the unstable marsh substrate. Due to the importance of vegetative reproduction via clonal expansion in cordgrass species, Jarosz and Davelos (25) concluded that the effect of ergot on plant fitness is likely to be minimal. However, in the San Francisco Estuary, seedling recruitment may be important for *S. foliosa* in areas where exotic cordgrasses are being removed for native marsh restoration and in areas with uncolonized open mudflats. For example, seedling recruitment has resulted in the restoration of tidal marshes in San Pablo Bay (6) and the relatively recent colonization of Tomales Bay on the outer coast (D. R. Ayres, *unpublished observations*). An important difference between San Francisco Estuary marshes and those in Poole Harbour, England dominated by *S. anglica* is the availability of mud flat for seed colonization in the former location. Threatened in its current range by invasive *Spartina* spp., *S. foliosa* may have to increasingly rely on seedling recruitment for maintaining populations into the future. Although ergot may impose a critical limitation on seedling recruitment under some circumstances, the disease did not prevent successful colonization by *S. foliosa* in the southern part of Tomales Bay.

In summary, marsh region (coast or bay) appears to be an important factor influencing the incidence of *C. purpurea* in the San Francisco Estuary, but differential host susceptibility does not; *Spartina* spp. and hybrids were equally susceptible when tested under controlled conditions. The results of this study show how an understanding of pathogen virulence and host susceptibility may not be sufficient to predict the outcome of pathogen–plant interactions under field conditions. Rather, it appears that the interplay of climate and the life histories of host and pathogen may be driving the dynamics of the ergot epidemic in the San Francisco Estuary.

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