This article is from the
October 2014 issue of

Phytopathology

published by
The American Phytopathological Society

APS

Healthy Plants • Healthy World

A Tripartite Interaction Between *Spartina alterniflora*, *Fusarium palustre*, and the Purple Marsh Crab (*Sesarma reticulatum*) Contributes to Sudden Vegetation Dieback of Salt Marshes in New England

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Accepted for publication 17 March 2014.

ABSTRACT

Elmer, W. H. 2014. A tripartite interaction between *Spartina alterniflora, Fusarium palustre*, and the purple marsh crab (*Sesarma reticulatum*) contributes to sudden vegetation dieback of salt marshes in New England. Phytopathology 104:1070-1077.

Tripartite interactions are common and occur when one agent (an arthropod or pathogen) changes the host plant in a manner that alters the attack of the challenging agent. We examined herbivory from the purple marsh crab (*Sesarma reticulatum*) on *Spartina alterniflora* following exposure to drought or inoculation with *Fusarium palustre* in mecocosms in the greenhouse and in crab-infested creek banks along intertidal salt marshes. Initially, drought stress on *S. alterniflora* and disease from *F. palustre* were examined in the greenhouse. Then, a second challenger, the purple marsh crab, was introduced to determine how drought and disease from *F. palustre* affected the attraction and consumption of *S. alterniflora*. Plant height and shoot and root weights were reduced in plants subjected to severe drought treatment when compared with normally irrigated plants. When the drought treatment was combined with inoculation with *F. palustre*, plants were significantly more stunted and symptomatic, had less fresh weight, more diseased roots, and a greater number of *Fusarium* colonies growing from the roots ($P < 0.001$) than noninoculated plants. The effects were additive, and statistical interactions were not detected between drought and inoculation. Estimates of herbivory (number of grass blades cut or biomass consumption) by the purple marsh crab were significantly greater on drought-stressed, diseased plants than on healthy plants irrigated normally. Drought increased attraction to the purple marsh crab more than inoculation with *F. palustre*. However, when only mild drought conditions were imposed, plant consumption was greater on inoculated plants. Healthy, nonstressed transplants set into plots in crab-infested intertidal creek banks were grazed less each year than inoculated plants or plants that were exposed to drought. Several hypotheses relating to nutrition, chemotaxis, and visual attraction are presented to explain how stress from drought or disease might favor herbivory.

A tripartite interaction occurs when arthropod infestation or pathogen infection changes the host plant in a manner that alters the attack of the challenging agent (39). Both positive and negative interactions occur, with one agent increasing or decreasing susceptibility to a second attacker. The role of environment can also complicate tripartite interactions and can dramatically shift the ecological costs of the host plant (25). Tripartite interactions are well documented for many insect–disease–plant systems (4), where the ecological cost to the insect or pathogen is enhanced or reduced (6,7,19,22,39). Multilayer interactions in natural ecosystems can collectively affect the structure and composition of natural plant communities; however, experimentally demonstrating the individual contribution of each agent is difficult.

In the late 1990s, sudden vegetation dieback (SVD) of smooth cordgrass (*Spartina alterniflora* Loisel.) occurred in salt marshes on the Atlantic and Gulf Coasts, leaving only remnant peat (2,5, 18,33,38). The patterns associated with SVD vary from marsh to marsh and from region to region but usually begin as specific foci of dieback that radiate outward and coalesce to create large, irregular areas within the marsh system, leading scientists to suspect a contagious agent. Surveys in Louisiana found that species in the *Gibberella fujikuroi* species complex (34) were able to infect *S. alterniflora* (18,42). Regional surveys from Maine to

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Louisiana discovered another pathogenic species called *Fusarium palustre* (W. H. Elmer and R. E. Marra) (16). Fungal incidence was higher in plants in SVD sites than in healthy marshes (16% in SVD sites and <1% in healthy sites) (15). Root-knot nematodes were also discovered but their role in SVD remains elusive (31). *F. palustre* was not implicated as a primary agent of SVD because pathogenicity tests on healthy plants rarely resulted in mortality. However, the close association between fungus and host in SVD sites sparked the hypothesis that, as an endophyte, *F. palustre* might function as a pathogen when its host was compromised by stress. Most marsh systems flood daily but tides can occasionally recede for extended periods of time, causing soil to be aerobic and acidic. However, extended drought events, as reported by Palmer Drought Indices, in the northern marshes have not been observed. Short-term droughts (less than 1 week) do occur but their effect on SVD is not clear. Scientists in Louisiana observed that plants inoculated with species in the *G. fujikuroi* species complex were significantly more diseased when plants were drought stressed (42). The relationship between *F. palustre* and drought was not known.

Another interacting stressor found in SVD sites in New England are high densities of the herbivorous purple marsh crab (*Sesarma reticulatum* Say) (27) (Fig. 1), and considerable loss of marsh biomass can be attributed to this invertebrate. The degree of herbivory in these marshes is variable and certain marshes are grazed more heavily than others. Although release from predation by blue crabs, fin fish, and birds can influence crab densities (3), creek banks previously denuded by SVD may also provide a better burrowing habitat for marsh crabs than more vegetated creek banks. Second, *Spartina alterniflora* plants on the perimeter

of SVD sites are commonly more stressed and stunted than plants more distal to the SVD site. Marsh crabs may preferentially graze on stressed plants in SVD sites because of their proximity or because of improved palatability. It remained to be demonstrated whether or not drought stress or colonization by endophytic *F. palustre* would influence herbivory by purple marsh crab.

The aim of this study was to first examine interactions between pairwise treatments (drought stress on *S. alterniflora* and disease from *F. palustre*), then introduce a third challenger to the interaction to determine how drought and inoculation with *F. palustre* affect attraction and consumption of *S. alterniflora* by the purple marsh crab.

MATERIALS AND METHODS

Production of plants and inoculum. Flower spikes of *S. alterniflora* were collected in fall 2009 from a salt marsh in Madison, CT where SVD was absent. Seeds were surface disinfested in 10% bleach (0.53% Na hypochlorite) for 1 min, rinsed in tap water, spread into the top centimeter of sand in trays, and set on greenhouse benches. Individual seedlings were transplanted into 36-cell inserts containing a 1:1 mix of sand and peat that was limed to achieve a pH of 6.5 to 7.0. Seedlings were held for 6 to 9 months and fertilized with 20-20-20 NPK twice a month. Preliminary studies have shown that *S. alterniflora* plants were larger and healthier when exposed to a 50% marsh water irrigation (approximately 0.14 M NaCl) than continuous tap water; therefore, plants were exposed to 50% autoclaved marsh water every third or fourth irrigation to help maintain osmoregulation. For each experiment, plants of uniform size were selected and arbitrarily assigned to treatment.

Four isolates of *F. palustre* (NRRL 54065, NRRL 54059, NRRL 54054, and NRRL 54056) (16) were grown on 25% potato dextrose agar (PDA) plates for 7 to 10 days at 20 to 25°C under light for 12-h photoperiods. These isolates were from different marshes along the Long Island Sound and had been shown in previous tests to be pathogenic (15,16). Inoculum was prepared two ways, depending on the experiment. In the first set of greenhouse experiments, each isolate was seeded onto twice-autoclaved millet, allowed to grow for 2 weeks, then air dried, ground, and passed through a 0.5-mm sieve. Equal amounts of each inoculum were bulked together and mixed into soil (1:1 peat/sand) at 2 g of millet inoculum per liter of soil. In later experiments on herbivory, we wanted the above- and belowground tissue to be exposed to *F. palustre*; therefore, conidial spray or drenches were used. For conidial inoculations, colonized 25% PDA plates were washed with distilled water and spores enumerated to 5×10^4 macroconidia/ml. Using a spray bottle, 30 ml were applied to each plant.

Greenhouse drought and disease experiments. To study the effect between drought and inoculation with *F. palustre*, greenhouse experiments were conducted with *S. alterniflora* transplants. There were six treatments: severe drought-not inoculated, mild drought-not inoculated, normal irrigation-not inoculated, severe drought-inoculated, mild drought-inoculated, and normal irrigation-inoculated. Each treatment was replicated 10 times (one plant per replicate) and the experiment was repeated two times. Plants were planted into soil infested with the millet inoculum and subjected to a severe drought that was imposed by withholding water until at least half of the leaf blades began to curl inward. Plants were irrigated with tap water until leaf blades become turgid, which occurred within 3 days in the summer and within 5 to 6 days in the winter, when overcast skies and cooler

Fig. 1. Two adult purple marsh crabs (*Sesarma reticulatum*) grazing on *Spartina alterniflora*.

temperatures prevailed. When the leaves became turgid, the treatment was imposed again. Mild drought conditions were imposed by withholding irrigation once a week till pots and soil were dry, then irrigating daily. The normal irrigation treatment included keeping pots in a reservoir of water at all times.

After 3 months, plants were washed, weighed, and rated for disease and vigor based on a scale where 1 = green healthy; 2 = slightly stunted, slightly off color; 3 = stunted and chlorotic; 4 = very stunted and chlorotic, collapsed stems; and 5 = dead or near death. Roots were assayed for disease by two methods described before (17). Briefly, disease severity and colonization by *Fusarium* spp. were determined on roots that were surface disinfested in 4% household bleach (0.21% $NaHClO_2$) for 4 min, followed by 2× rinses in tap water, then blotted dry on paper towels. Feeder roots approximately 1.0 to 4.0 cm long were aseptically placed on peptone pentachloronitrobenzene agar. The dishes containing the roots were placed over a 1.0-cm grid and the total root length was estimated using the line-intersect method (40). The fraction of intersects where a reddish lesion occurred also was counted and used to estimate root disease severity (percent root lesions = [number of intersects with root lesions/total intersects] × 100). Plates were incubated at room temperature for 5 days, and the number of *Fusarium* colonies that grew from the roots were counted and used to estimate root infection (*Fusarium* colonies per centimeter of feeder root). Approximately 1.5 to 2.0 m of feeder roots were sampled from each root system.

Greenhouse herbivory experiments. To assess whether drought or *F. palustre* made *S. alterniflora* more susceptible to herbivory by the purple marsh crab, experiments were conducted in the greenhouse. Individual mecocosms were prepared in 76-liter plastic tubs (0.58 by 0.40 by 0.40 m) and filled with two or three pieces of marsh peat that averaged 10 by 10 by 5 cm in size. Adult marsh crabs were collected periodically from a salt marsh in Madison, CT and held in plastic bins filled with fresh marsh water in the greenhouse. Crabs were fed healthy *S. alterniflora* plants and the water was changed with fresh marsh water weekly. Crabs were held for up to 6 months in this environment with less than 5% mortality. Five adult marsh crabs were released in each bin and provided no plant material for 24 h before each experiment.

In the first set of herbivory experiments, only two treatments were examined. They were severe drought-stressed plants that were inoculated with *F. palustre* or normally irrigated plants that were not inoculated. Drought was imposed as described above. Plants in these experiments were inoculated by spraying a conidial suspension onto the leaves and allowing the run-off to enter and drench the soil. Each plant received approximately 30 ml of the conidial suspension of 5×10^4 macroconidia/ml. Following inoculation, leaf blades were allowed to dry and soil to drain before being returned to their irrigation regimes. Control plants received equal amounts of distilled water applied the same way. After 3 to 4 weeks, inoculated plants in the severe-drought inoculated treatments were smaller than normally irrigated, noninoculated controls but no mortality was ever observed. In these trials, isolations of *F. palustre* from the tissue were performed on occasion to confirm infection but were not done routinely out of need to maximize available tissue for the study. A treated and a control plant were removed from their pot, the leaf blades were counted, and the plants were set in the mecocosms in random pairwise arrangements approximately 7.5 cm apart. Although the drought and inoculation treatments reduced the mean plant size, we tried to pair plants that had approximately similar numbers of leaf stems so that the number of cut stems would be reflective of the treatment. There were six replicates and the experiment was repeated three times. The plants in each bin were photographed daily to assess the disappearance of plant tissue. Herbivory was estimated by counting the number of leaf blades grazed on at each day. These numbers were integrated over time to produce a herbivory progress curve (HPC) in analogy to the area under the disease progress curve using the formula $HPC = \Sigma[Y_i + Y_{(i+1)}]/2 \times (t_{(i+1)} - t_i)$, where Y_i = the number of grass blades cut at time t_i.

In the second herbivory study, the mild drought treatment described above was added so that there were six treatments. They were severe drought-not inoculated, mild drought-not inoculated, normal irrigation-not inoculated, severe drought-inoculated, mild drought-inoculated, and normal irrigation-inoculated. Plants were inoculated as described above and exposed to drought treatments as previously described. Each treatment was replicated three times, and the experiment was repeated once. In this study, a treated plant was set adjacent to its respective control (e.g., drought versus normal irrigation and inoculated versus noninoculated). Although the drought and inoculation treatments reduced the mean plant size, plants that were approximately similar in size were chosen so that the percent loss estimate would be reflective of the treatment. Plants in each bin were photographed at 0, 4, 16, 24, 40, 48, 72, and 96 h. It was reasoned that any consumption preference of the crab for one plant versus another would likely be greatest in the initial stage of the test; therefore, we opted to photograph more frequently in the first 24-h period. Digital photographs were used to visually rate plants for the percentage of plant tissue that disappeared between measurements. Herbivory was assessed by visually estimating disappearance of aboveground tissue from photographs compared with the time 0 photo. Consumption over time was similarly integrated as an HPC, where $HPC = \Sigma(Y_i + Y_{(i+1)})/2 \times (t_{(i+1)} - t_i)$, and where Y_i = the percentage of biomass consumed at time t_i.

Field plots. Field plots were established 19 June 2011, 12 June 2012, 28 June 2012, and 17 June 2013 at a salt marsh in Madison, CT alongside an intertidal creek where SVD and crab herbivory were both severe (coordinates 41.271 N and –72.564 W). There were six treatments: severe drought-not inoculated, mild drought-not inoculated, normal irrigation-not inoculated, severe drought-inoculated, mild drought-inoculated, and normal irrigation-inoculated. Plants were inoculated as described above and exposed to drought treatments as previously described. Plots were arranged in a circle design with one plant from each treatment randomly placed in the center of a circle or spaced 30 to 45 cm away at the 2, 4, 6, 8, and 10 o'clock position, with the 12 position closest to the creek bank. The number of replicate plots varied depending on the year: 19 June 2011 ($n = 2$), 12 June 2012 ($n = 4$), 28 June 2012 ($n = 4$), and 17 June 2013 ($n = 6$). Plots were photographed at planting and every 24 h for 4 to 7 days, which allowed two tidal cycles between photographs. Herbivory was assessed by visually estimating disappearance of aboveground tissue from photographs. Ratings were made by two individuals, averaged, and HPCs were computed as described above.

Statistical analyses. Data from repetitions from greenhouse experiments were subjected to analysis of variance. After satisfying the requirement for a nonsignificant treatment–repetition interaction, data sets were pooled and analyzed for treatment interactions as randomized blocked factorial designs. All pairwise herbivory examinations done in mecocosms were analyzed using the paired t test at $P = 0.05$, where the HPC of the treated plant was statistically compared with the HPC of its respective control. Main effects (drought and inoculation) were analyzed using the Kruskal-Wallis test. The mecocosm studies were not designed to test for interactions. Field plots contained all six treatments, with their respective controls being randomly placed in situ; therefore, the data from plants in all six treatments were statistically compared using the Kruskal-Wallis test at $P = 0.05$.

RESULTS

Greenhouse drought and disease experiments. There were no significant experiment–drought treatment–inoculation interactions for the disease severity ratings, fresh weights, fraction of disease roots, or *Fusarium* spp. colonies per centimeter of root;

therefore, all three experiments were combined. In addition, there were no statistical interactions between the irrigation treatments and inoculation with *F. palustre* for any of the measured variables. However, the irrigation and inoculation treatment alone were both highly significant ($P < 0.001$) (Fig. 2). Averaging over the inoculation treatment, the severe drought treatment reduced fresh weight by 72% when compared with normal irrigation (control) and by 65% when compared with mild drought treatment. The 20% reduction in the mild drought treatment was not significantly different from normal irrigation (control). Plant height, root weight, and top dry weights were similarly affected (data not shown). Averaging over the drought treatments, inoculated plants were significantly more stunted and symptomatic ($P < 0.001$) and had less fresh weight ($P < 0.001$), more diseased roots ($P < 0.001$), and a greater number of *Fusarium* colonies growing from the roots ($P < 0.001$) than noninoculated control plants. A small number of root lesions were also observed on noninoculated plants but these lesions did not give rise to *Fusarium* colonies (Fig. 2). Mortality was only observed in the inoculated plants exposed to severe drought (31%) and in plants inoculated and exposed to mild drought (3%) (data not shown).

Greenhouse herbivory experiments. The first studies done in mecocosms with the purple marsh crab were designed to examine the number of grazed leaves from drought-stressed, diseased plants compared with healthy nonstressed plants. In all three repetitions of the studies, the purple marsh crab preferentially grazed on drought-stressed, diseased plants more than the healthy plants, as shown by the number of leaf blades cut (Fig. 3). Feeding in repetitions one and three lasted over 9 to 10 days whereas, in the second repetition, the crab consumed plants in the mecocosms

after 5 days. The integrated HPC was computed and found to be 5.2 cut blade days (healthy control) versus 20.6 cut blade days (stressed-diseased) ($P < 0.001$).

The second series of the experiment was similarly set up in mecocosms with purple marsh crabs. When plants in each drought treatment (severe, mild, or normally irrigated) were inoculated with *F. palustre* and compared with noninoculated plants exposed to the same treatment, only inoculated plants that received the mild drought were statistically more grazed than their noninoculated control (Table 1). Feeding by the purple marsh crab did not differ on inoculated or noninoculated plants exposed to severe drought or receiving normal irrigation. When irrigation was examined, analysis of the HPC was averaged over drought treatment to reveal that most grazing occurred on plants exposed to severe drought, then mild drought, and the least on normal irrigation. Plants inoculated with *F. palustre* had a greater level of grazing than plants that were not inoculated ($P = 0.017$). Interestingly, when the two drought treatments (mild and severe) were pairwise compared, there was no difference in grazing among noninoculated plants. However, plants exposed to a mild drought and then inoculated were grazed more heavily than noninoculated plants.

Field studies. When transplants were exposed to the six treatments and transplanted into purple marsh crab-infested salt marshes in Madison, CT in 2011 to 2013, the grazing was highly variable, depending on the plots and year. However, the rankings generated from Kruskal-Wallis revealed that the least amount of grazing was always observed on healthy plants that were not drought stressed and greatest on stressed or inoculated plants (Fig. 4). Because only two plots were examined in 2011, the variability prevented statistical separation. However, statistically
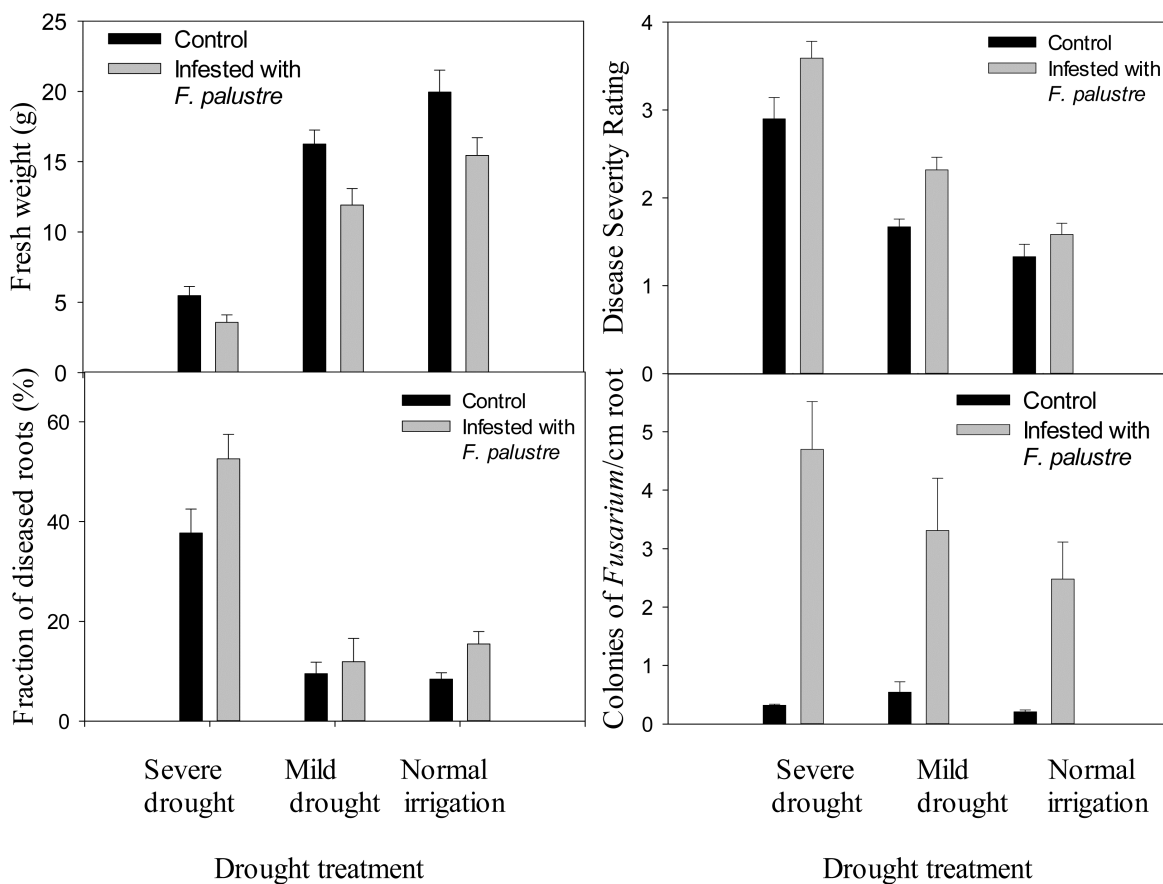

Fig. 2. Effect of different drought treatments with and without inoculation with *Fusarium palustre* on fresh weight (upper left: drought; $P < 0.001$; inoculation with *F. palustre*, $P < 0.001$), disease severity ratings (upper right: drought, $P < 0.001$; inoculation with *F. palustre*, $P < 0.001$), fraction of disease roots (lower left: drought, $P < 0.001$; inoculation with *F. palustre*, $P < 0.001$), and the number of colonies of *Fusarium* spp. per centimeter of root of *Spartina alterniflora* (lower right: drought, $P < 0.001$; inoculation with *F. palustre*, $P = 0.02$). Error bars represent the standard errors of selected means.

similar trends emerged in 2012, where both repetitions in time showed the least amount of grazing on healthy plants grown under normal irrigation. There was rarely any statistical difference in the degree of grazing done to plants that were drought stressed versus plants stressed by disease. In 2013, no statistical significance was observed in the HPC after the 4 days, probably due to excessive pressure from the crabs. However, in 2013, when the percent estimated biomass loss was analyzed after 24 h, the healthy plants grown under normal irrigation had significantly less grazing than the other treatments ($P = 0.032$) (data not shown).

DISCUSSION

SVD has continued to threaten Atlantic and Gulf coastlines with sporadic appearance since it was first recognized in Florida in the early 1990s (5). Many sites show no recovery after 10 years and new sites are reported every year. Mounting evidence has
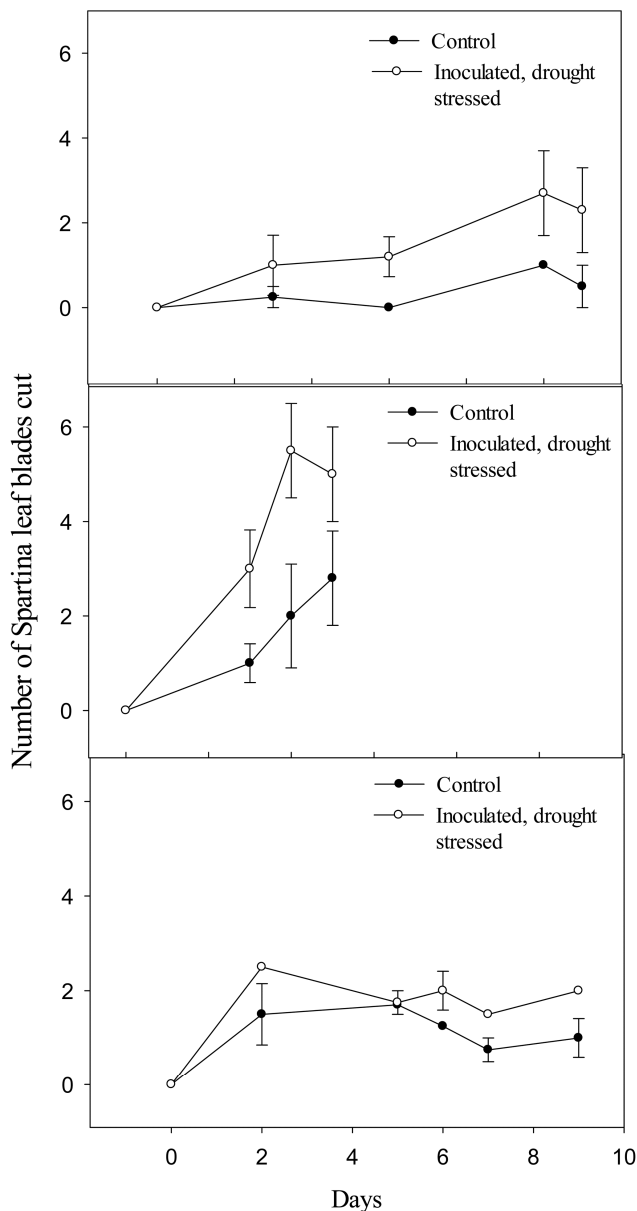
Fig. 3. Number of grass blades grazed and cut by the purple marsh crab (*Sesarma reticulatum*) on *Spartina alterniflora* that were drought stressed and inoculated with *Fusarium palustre*, or healthy plants that were normally irrigated. Error bars represent the standard errors of selected means. The integrated value under the averaged curves was 5.2 cut blade days (healthy control) versus 20.6 cut blade days (stressed and diseased) ($P < 0.001$).

implicated a combination of stressors that, in turn, lead to SVD (2,18). Although our findings are in agreement with the general hypothesis that an abiotic stressor, such as drought, initiates SVD (2,18,33), this report is the first demonstration of a tripartite interaction between a crab, pathogen, and plant that hinders recovery from SVD. Infected or drought-stressed plants were grazed more heavily by the purple marsh crab than noninfected plants in both mecocosms and in situ. Both biotic (fungal) and abiotic (drought) stressors altered the attractiveness or palatability in the plant, increasing the probability of being grazed by the purple marsh crab.

The tripartite interaction was studied utilizing a general protocol where an initial inducing attack on a plant by one agent (fungus) was followed by a second challenge by an organism of the other type (crab). In addition, drought was imposed as a separate treatment to examine its role in the interaction between plant, fungus, and crab.

The current study used visual estimates of biomass disappearance to measure herbivory. It was recognized that these estimates could be biased because the drought- or disease-stressed plants were smaller than control plants. To compensate for this potential bias, plants of relatively equal size were paired so that percent losses would be reflective of the treatment.

All agents—drought, fungus, and crab, and their combination—were deleterious to the host. Negative effects to the host have been observed in many systems where increased attraction to and increased fecundity of an herbivore occurs following host infection by a plant pathogen (21). Maize plants infected with stalk rot caused by *Colletotrichum graminicola* (Ces.) G. W. Wilson supported European corn borer larvae that developed approximately 20% faster than on healthy control tissue (6). Castle and Berger (7) observed increased growth rates of the green peach aphid on virus-infected potato plants. Many tripartite interactions also report a positive effect, where a challenging microorganism alters the plant to become more resistant to herbivory. Endophytes in the family Clavicipitaceae render their grass host more toxic to large herbivores (10). Plant infection with many microbes can often lead to activation of the systemic-acquired resistant pathway (13) which, in turn, decreases susceptibility to insect herbivores (39). Reduction of insect damage has been achieved following inoculation of plant roots with plant-growth-promoting rhizobacteria (43). Kruess (30) observed that infection of Canada Thistle with *Phoma destructiva* Plowr. resulted in reduced oviposition, survival, growth, and pupal biomass of the beetle *Cassida rubiginosa* O. F. Müller when compared with healthy plants. Alternatively, when the herbivore is the initial agent, plants frequently activate metabolic pathways that produce jasmonic acid that, in turn, cascade many metabolic signals involved in disease resistance (1,20,23,24). The current study did not examine the role of crab grazing on infection by *F. palustre*, because surveys show the fungus to be endophytic on healthy plants long before herbivory by crab is evident (18). It is unclear what physiological changes would occur on *S. alterniflora* following crab grazing.

An overriding factor that governed all interactions was drought. Herbivory was usually greatest on drought-stressed plants. Many times, inoculation of severe drought-stressed plants with *F. palustre* played no effect in the amount of biomass consumed by the purple marsh crab when compared with noninoculated plants. Both were consumed in equal proportions. The drought treatment alone was rarely fatal in these studies but, when combined with inoculation or herbivory, a greater percentage of plants died or was consumed by the crab, respectively. In situ studies were variable but consistent, in that plants that were healthy were grazed the least.

In southern marshes, drought was a primary stressor driving the dieback (2,33). Mortality was shown to be more common when *Fusarium* spp. that belonged to the *G. fujikuroi* species complex

were present (18,42). In those studies, *F. palustre* was not tested. In the current study, statistical interactions between drought stress and inoculation with *F. palustre* were not seen but the damaging effects were additive. Plants exposed to both treatments resulted in an averaged 31% mortality compared with 3% mortality with drought alone. However, the extended periods of drought that usually preceded SVD did not occur to the same degree in the northeastern marshes as observed in the southern marshes. Although the Palmer Hydrological Drought Index reports a few brief periods of drought (>2.0) in Connecticut during the 2000-to-2002 periods when SVD was noted, it is not clear whether short-term drought stress would have the same effect as prolonged periods (<3.0). On other crops, brief moisture deficits can predispose plants and allow increased ingress of *Fusarium* pathogens into plant roots (14,35).

There are at least three putative mechanisms, not mutually exclusive, that might explain the increased herbivory on stressed plants. One is the direct effect on the nutritional status of the plant, in that disease-stressed plants may be easier to mechanically harvest, consume, and digest. *Fusarium* spp. produce extracellular cellulytic enzymes that may affect the structural strength of *S. alterniflora* stems (9,41). Tensile tests of infected *S. alterniflora* stems need to be compared with healthy stems. However, if structural strength were a primary factor, it may be reasonable to expect lower levels of Si, an important structural element for grasses (12), in SVD sites. It was surprising that plants in dieback areas in Georgia had higher levels of Si than plants in healthy marshes (32), leading one to speculate that structural integrity may not be a governing factor in the crab's selection for consumption. On the other hand, fungal enzymatic digestion of the

TABLE 1. Effect of irrigation regimes and inoculation with *Fusarium palustre* on the integrated estimates of herbivory of *Spartina alterniflora* by purple marsh crabs (*Sesarma reticulatum*)

| Treatment | Herbivory progress curve (consumption days)[a] | | | | Paired *t* test | *P* value |
	Control	Inoculated[b]	Severe drought	Mild drought		
Irrigation treatment						
Severe drought	8,008	8,251	…	…	243	NS
Mild drought	5,819	6,679	…	…	860	0.012
Normal	4,240	5,425	…	…	1,185	NS
Kruskal-Wallis[c]	*P* = 0.001	*P* = 0.001	…	…	…	…
Average[d]	18,067	20,355	…	…	2,288	*P* = 0.017
Inoculation treatment						
Control	…	…	7,037	7,420	−383	NS
Inoculated	…	…	6,723	8,749	2,026	*P* = 0.001
Kruskal-Wallis	…	…	NS	*P* = 0.04	…	…

[a] Herbivory progress curve based on integrated measure of a visual estimate of plant consumption over time; NS indicates not significant.
[b] Inoculated with *F. palustre*.
[c] Kruskal-Wallis rank test.
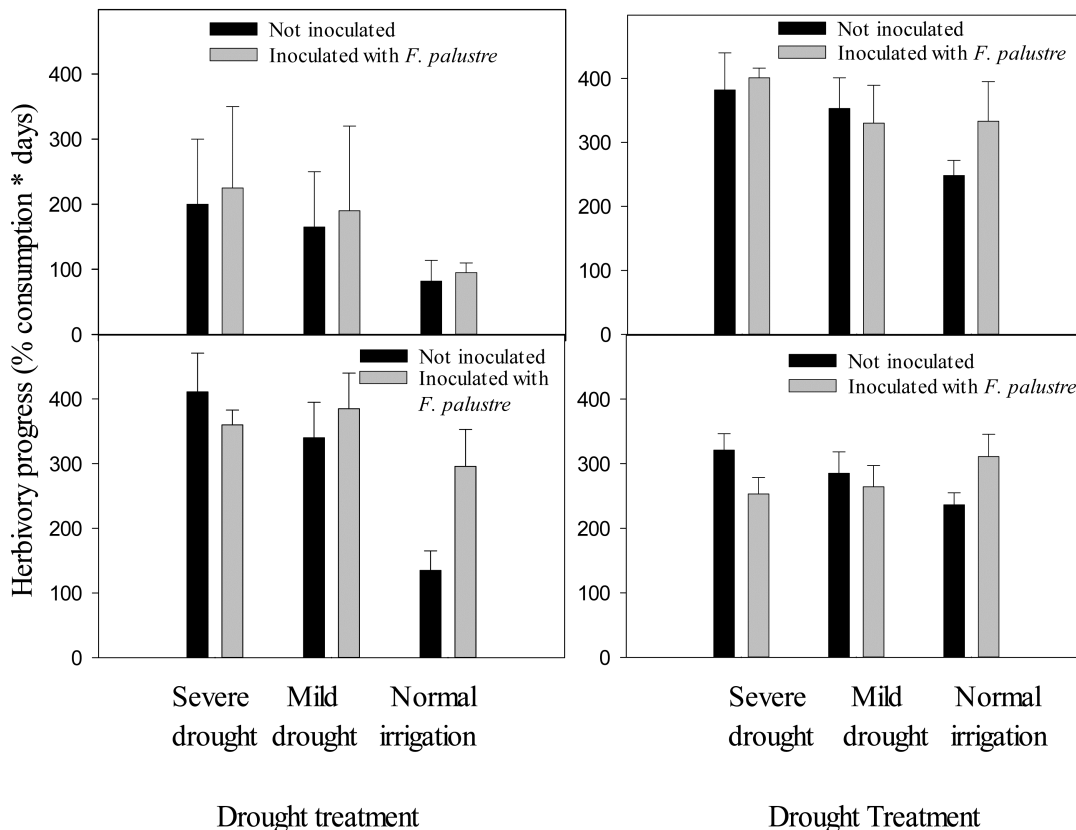[d] Average over irrigation regime.

Fig. 4. Effect of different drought treatments with and without inoculation with *Fusarium palustre* on the integrated value of herbivory progress consumption by the purple marsh crab (*Sesarma reticulatum*) in situ in a crab-infested intertidal creek bank. Error bars represent the standard errors of the means.

cellulose and hemicellulose components may also make the plant more digestible and, ultimately, more nutritious to the purple marsh crab. Feeding studies where the crab biomass is carefully monitored under diets of diseased and healthy *S. alterniflora* may lend support to this hypothesis. There is no indication that the purple marsh crab feeds directly on fungi, as was observed for the snail *Littoraria irrorata* Say in southern marshes. The snail climbs stressed *S. alterniflora* stems and scores the tissue to promote sporulation of *Phaeosphaeria* spp. and *Mycosphaerella* spp. in the wounds, then returns after a period of time to feed on the fungi (36,37). Because *Fusarium* colonists are more endophytic than epiphytic, and because the purple marsh crab consumes the entire plant tissue, it is more likely that some mechanism other than direct fungal feeding is operative.

Another possible explanation may involve chemotaxis, where stressed plants may emit volatiles that attract crabs. No such attractants have been identified. However, S. *alterniflora* is unique in that it contains dimethylsulfoniopropionate (DMSP), a naturally occurring putative osmolyte, that is oxidized to dimethylsulfoxide (DMSO) during periods of stress (28). Studies in Georgia found that the DMSO/DMSP ratio was a sensitive indicator of presymptomatic stress in *S. alterniflora* and was consistently greater in leaves and stems of plants in dieback sites (32). In preliminary trials, healthy *S. alterniflora* transplants sprayed with DMSO at 2.5 µmol/ml and set in mecocosms with purple marsh crabs were grazed significantly more in the first 24 h than untreated control plants ($P > 0.001$) (unpublished data). It is unclear whether these findings are biologically relevant because DMSO is found in lower concentrations (0.5 to 2.0 µmol/g fresh weight) in *S. alterniflora* (32), and the daily tidal flow may affect the release and dilution of certain volatiles. However, volatiles are an important attractant for insect arthropods to diseased plants. In studies with *Barley yellow dwarf virus* on wheat, significantly more aphids and extractable volatiles were found above headspaces of infected plants than above headspaces of noninfected plants (29).

A last possible explanation that needs validation may be a visual response, where the crab is attracted to certain wavelengths emitted from stressed plants. Infected stressed plants respire faster and radiate more in the infrared range than healthy plants (26). Given that the purple marsh crabs are nocturnal (8,11), it is speculated that they may see slight changes in infrared emissions from these plants that, in turn, may influence and attract the purple marsh crabs emerging from their burrows at night. Once again, the influence of tidal action and environmental variation may negate this effect (26).

The ecological implications of multiplayer interactions in SVD are staggering, given the destruction of New England coastline marshes. The complexities of interactions combined with environmental constraints such as drought make deciphering the etiology of SVD a daunting task. However, these findings may provide some insight into how the landscape of certain intertidal creeks in New England can remain denuded for decades whereas others recover over time and yet others remain unaffected. The current study demonstrates that drought, fungal infection, and herbivory can work in concert to hinder recovery from SVD.

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