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Local consumers induce resistance differentially between *Spartina* populations in the field

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Abstract. Intraspecific variation in the strength of inducible plant defenses plays a central role in the interactions between plants and herbivores. Studies of this variation are typically conducted in the greenhouse or laboratory rather than the field. We simultaneously manipulated densities of local consumers in the field within Maine and South Carolina populations of the smooth cordgrass *Spartina alterniflora*. South Carolina, but not Maine, plants induced resistance when grazed by local consumers. South Carolina populations of *Littoraria* snails and planthoppers colonized control more than previously grazed South Carolina plants, and *Littoraria* snails consumed more control than previously grazed plants. The inducible feeding deterrents in South Carolina plants appear to be water soluble, but not phenolic based. In contrast, grazed and control plants from Maine populations did not differ in attractiveness or palatability to Maine consumers. Thus, inducible plant responses by South Carolina plants had a strong effect on the South Carolina consumer community, but no analogous effect occurred in Maine. Field experiments are a powerful approach to detecting the strength of inducible plant resistance and its impacts on local consumers, which in this case were shown to vary with location.

Key words: inducible defense; Littoraria irrorata; *plant–herbivore interaction; salt marsh; spatial variation.*

Introduction

Many plants produce greater chemical resistance when previously grazed by herbivores. Such induced responses are common within marine, aquatic, and terrestrial biomes, and across a diversity of seaweed and plant taxa (Hessen and van Donk 1993, Karban and Baldwin 1997, Toth and Pavia 2007). Their ecological impacts cascade through herbivore populations (Agrawal 1998) and communities (Denno et al. 1995, Van Zandt and Agrawal 2004), and the interactions between herbivores and higher trophic levels (De Moraes et al. 2001). As with other ecologically important traits, there is abundant evidence that the strength of plant induced defenses varies among individuals, populations and species (Karban and Baldwin 1997), and some of this variation reflects evolutionary interactions among plants and their consumers that diverge spatially (Thompson 2005).

Most evidence of how induced responses may alter herbivore behavior or population dynamics comes from laboratory or greenhouse experiments or field observations, but not manipulative field experiments. For example, variation in the ability to induce resistance has been detected among plant clones and populations exposed to the same herbivore or damage in greenhouse and laboratory experiments but not in the field (Parejko and Dodson 1991, Underwood et al. 2000, Kishida et al. 2007). While these experiments identified the potential for intraspecific variation in inducible resistance and tested important evolutionary theory (e.g., trade-offs between constitutive and inducible defenses, correlations between defenses and consumer pressure), few studies examined whether inducible resistance varied between populations in the field. The distinction between potential and realized variation of inducible resistance could be critical given the importance of local abiotic and biotic factors on consumer–prey interactions (Cronin and Hay 1996, Karban and Baldwin 1997, Van Zandt 2007).

One model system to examine spatial variation of inducible plant defenses is the smooth cordgrass, *Spartina alterniflora* Loisel (Pennings et al. 2001, Pennings and Silliman 2005, Salgado and Pennings 2005). Along the east coast of the United States, this species ranges from the Gulf of Mexico to Maine, but several genetic loci indicate historical isolation between populations from southeastern Atlantic vs. New England shores (O'Brien and Freshwater 1999, Blum et al. 2007). Consumer intensity within these salt marshes increases with decreasing latitude, as measured by leaf damage and per capita grazing rates (Pennings and

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Silliman 2005, Pennings et al. 2009). In response to these latitudinal patterns in attack, low-latitude *Spartina* evolved higher levels of constitutive defenses (Salgado and Pennings 2005). Similarly, latitudinal gradients in consumer pressure may also have determined the relative strength of inducible *Spartin*a defenses. Although *Spartina* from New Jersey are able to induce resistance (Denno et al. 2000), there is no information on whether populations of *Spartina* vary in the strength of inducible resistance.

We examined spatial variation in the inducible defenses of *Spartina alterniflora* by simultaneously enclosing Maine and South Carolina *Spartina* plants in the field with and without a natural assemblage of local arthropod and gastropod consumers for 28 d. Then, we assessed plant quality by comparing, across populations, (1) the number of consumers moving onto plants, (2) the palatability of grazed and control plants, and (3) the palatability of artificial foods containing plant extracts.

Materials and Methods

We tested *Spartina alterniflora* for inducible responses at two populations: Maine and South Carolina (hereafter, ME and SC sites, respectively). Within both populations, we examined three sites separated by 0.85 to at least 1.74 km. ME sites were located in the Rachel Carson National Wildlife Refuge (two sites, 43°19′8.33″ N, 70°34′16.34″ W and 43°18′12.46″ N, 70°34′39.39″ W) and the Wells National Estuarine Research Reserve (43°20′7.39″ N, 70°32′35.78″ W). SC sites (33°20′0.0″ N, 79°11′52.5″ W; 33°19′41.0″ N, 79°12′18.0″ W; 33°20′19.1″ N, 79°11′28.2″ W) were located in the North Inlet-Winyah Bay (Georgetown, SC) adjacent to the Baruch Marine Field Laboratory. Our study was designed to compare the consequences of the responses of the two populations rather than latitudinal variation specifically. Because of the limited distance between sites within populations, our results can not be used to distinguish variation in inducibility. However, we have adopted terminology such as ME or SC sites as a tool for readers. We deployed 40 cages at each site: 20 consumer inclusion and 20 consumer exclusion cages.

Inducible defenses of *Spartina alterniflora* were manipulated during the induction phase and consumer responses to these defenses were measured in the bioassay phase. During the induction phase, *S. alterniflora* culms were enclosed with or without a natural suite of consumers (hereafter, grazed and control cages, respectively) for 28 d starting on 12 June 2006 (ME sites) and 20 June 2006 (SC sites). Cages consisted of thin-walled PVC pipe (30.5 cm [height] × 10.2 cm [diameter]; modified from Denno et al. 2000). Similar-sized cages have been used previously to examine the influence of consumers on marsh communities (Denno et al. 2000) and are useful because a manageable number of abundant planthoppers must be added to these cages. To maximize air and light passage, two opposing rectangular openings (11 × 29 cm each) were cut into cages and covered with mesh (0.065 mm). We placed cages 7 cm into the ground ($N = 20$ per treatment per site). Installation severed all rhizomes beneath cage walls. All cages were separated by 1 m and treatment (either grazed or control) was determined by coin toss (see Plate 1). Snails were removed by hand, and remaining consumers were removed by vacuum using a leaf blower.

After consumer removal, a natural suite of local invertebrate consumers was added to consumer cages. Our goal was to add known *Spartina* herbivores (e.g., planthoppers) as well as other consumers that might indirectly influence plant defenses. For example, planthoppers induced responses in New Jersey *Spartina* populations (Denno et al. 2000). Additionally, omnivorous *Littoraria irrorata* may radulate plants primarily to feed upon fungi that subsequently colonize these radulations (Silliman and Newell 2003). However, these snails may elicit inducible responses in *Spartina* because their activities damage plants and make them susceptible to additional attack by natural enemies. *L. irrorata* was used in SC only because it does not occur in ME marshes. Similarly, we added talitrid amphipods that may have no direct influence on inducible responses of living *Spartina* because they feed primarily on *Spartina* detritus (Parker et al. 2008). Although indirect interactions between these amphipods and the other consumers we used are unknown, allowing for this possibility adds ecological realism to our study because they naturally co-occur, and indirect interactions are common in a variety of ecosystems (Werner and Peacor 2003). At ME sites, we included the snail *Littorina littorea* because it is reported to consume the shoots and rhizomes of *Spartina* (Bertness 1984). Natural consumer densities per area of marsh surface were determined just prior to cage deployment in 50 haphazardly placed 32 × 32 cm quadrats (for snails), 24 1 × 5 m band transects (for katydids and grasshoppers), and 20 vacuum samples each collected from 519 cm^2 of marsh surface (for all other consumers).

We attempted to standardize grazing damage between our populations by using the same absolute numbers of consumers (e.g., one snail, two amphipods, four planthoppers) in ME and SC. This approach prevented us from standardizing grazing relative to ambient conditions because of differences in ambient consumer densities. At ME sites, we added one snail (*Littorina littorea*, natural density per marsh surface), two amphipods (Family Talitridae, natural density), four macropterous planthoppers (*Prokelisia* sp., 13× natural density), and one stink bug (Family Pentatomidae, 10× natural density). At SC sites, we added one snail (*Littoraria irrorata*, 2× natural density), two amphipods (Family Talitridae, natural density), four macropterous planthoppers (*Prokelisia* sp., 70× natural density), and one meadow katydid (Subfamily Conocephalinae, 169× natural density). Although these densities were sometimes elevated compared to the mean ambient densities,

they were typically within the range of densities encountered or reported. For example, we observed snail densities as great as 270 snail/m^2 within individual quadrats in SC (compared to 127 snail/m^2 in our cages). Although mean planthopper densities can reach 300 individuals/m^2 (Gustafson and Silliman 2006), we added 509 individuals/m^2 (four per cage) to attempt to account for their shorter life span and higher mortality. In the case of the stink bugs, *Littoraria* snails, and katydids, adding a single individual resulted in higher densities relative to ambient levels because of the cage size. A limitation of adding a single individual for some consumer species is that conspecific interactions are necessarily absent. However, we adopted this approach to avoid using even more elevated densities of some consumers relative to ambient levels.

To minimize damage to fragile consumers, planthoppers and amphipods were aspirated from plants and added to cages. Snails, katydids, and stink bugs were added to cages by hand. No consumers were added to control cages. Nylon mesh was clamped over all cages. After removing the cages 28 d later, half of the previously caged plants were flagged and monitored in field bioassays (10 grazer and 10 control plants from each site). The remaining plants were clipped (10 consumer and 10 control plants from each site) for laboratory bioassays.

We measured the palatability of control plants from ME versus SC to SC *Littoraria irrorata*. Laboratory experiments used plant tissues that were dried on kitty litter, and stored at −20°C for less than four months. We assayed feeding on dried plant material incorporated into agar foods because of rapid degradation of *Spartina* leaves after they were clipped. Foods were created by grinding plant tissue from three randomly selected control plants within a population in a Wiley Mill and then incorporating 0.5 g of this material into 50 mL of a 2% agar solution. Approximately 2 mL of this mixture was then added into a Petri dish lid (height × diameter = 4 × 50 mm) to yield 20–25 replicate dishes. After cooling, foods made from ME and SC control plants were paired and offered to four *L. irrorata* individuals collected less than 24 h earlier ($N = 20$). *Littorina littorea* did not graze *Spartina* during laboratory feeding assays so these were conducted with *L. irrorata* only. Assays were stopped when half of the food within one disc was consumed or after three days, whichever came first. We photographed the transparent Petri dish over window screen, and used ImageJ to quantify the number of window-screen squares that were visible because of snail consumption (software *available online*).[5]

To examine the effects of previous exposure to consumers (i.e., inducible resistance) on habitat preference of snails in the field, we monitored the number of colonizing snails on experimental plants for up to four consecutive days ($N = 10$ plants per treatment per site). Each site was sampled each day at SC sites. Due to logistical constraints, sampling of ME sites was site-specific. One ME site was sampled on days 2–4 and the second and third sites were sampled on days 1–2. Snail counts occurred within two hours of the morning low tide, and we recorded the number of snails on each plant. Snails were removed during each daily count. After four days, we measured the aboveground biomass of experimental plants from one of our SC sites to assess whether differences in snail densities were generated by available biomass. We found no difference in plant biomass between treatments (mean ± SE = 13.6 ± 1.2 and 11.2 ± 1.0 for control and consumer plants, respectively; $P = 0.14$) and did not notice striking differences in plant height between treatments. At least one plant within each SC consumer cage displayed chewed or clipped damage on the upper edge of its leaves, and few to no signs of grazing were apparent on plants in other caging treatments (i.e., control cages at both populations and consumer cages in ME). Because these data were not normally distributed (e.g., many plants had 0 snails), we analyzed differences in snail densities using a nonparametric Mann Whitney U test after pooling sites by day within both populations. Sites were pooled only after we failed to observe a significant effect of within-population variation (see Appendix A) using a series of two-way ANOVAs. During days 1–2, we sampled at least two sites per population and so pursued an ANOVA that nested sites within both population and plant type (grazed or control). During days 3–4, we only sampled a single site in ME populations, and pursued a two-way ANOVA using plant as the replicate. These ANOVAs were used to justify the pooling of data within populations. All statistical analyses were performed with the software R (R Development Core Team 2010).

To examine the effects of previous exposure to consumers on planthopper habitat preference, we offered previously grazed and control plants (paired per population) as choices to local *Prokelisia* sp. in the laboratory. Plants for laboratory bioassays were clipped at the base of the leaves (i.e., the base of the shoot at the marsh surface), immediately transported to the laboratory, and placed in vials with water. We determined planthopper habitat preference on paired 10 cm lengths of plants ($N = 10$ per treatment per site; four planthoppers per replicate). Planthopper containers (1000-mL glass jars) were kept on a shaded, outdoor porch where container position was randomized. For three days, we recorded the number of planthoppers on each plant. We pooled the data from all sites within each population. We analyzed planthopper habitat preference using a two-tailed Wilcoxon signed-ranks test because these data were not normally distributed (e.g., many plants had 0 planthoppers). The remaining plant material was pooled (within population and within treatments), dried in kitty litter, and frozen for snail feeding bioassays.

[5] ⟨http://rsbweb.nih.gov/ij/⟩

Given that snails colonized control SC plants more than previously grazed plants in the field (see *Results*), we conducted laboratory-based feeding choice assays to determine if control plants were more palatable. An initial choice experiment with fresh tissue suggested that *Littoraria irrorata* preferred control to grazed SC plants (mean ± SE of amount consumed = 0.012 ± 0.004 g and 0.004 ± 0.003 g, respectively; $P = 0.001$; $N = 26$). However, these grazing rates were low and thus sensitive to measurement error, so we conducted feeding experiments with dried, ground *Spartina* tissues. Dried foods were created as described above except that foods were made from previously grazed and control plants and these were paired within a population ($N = 13$ or 18; ME and SC plants, respectively). For SC and ME plants, we initially pooled plant material before creating foods. To examine within-population variation in SC, we repeated this experiment but pooled plants by site and by treatment.

SC plants that were previously grazed were less palatable than control plants to *Littoraria irrorata* (see *Results*). To determine if chemical differences could account for palatability patterns, we extracted additional samples of the control and grazed *Spartina* tissue from SC (using either dichloromethane : methanol or 80% methanol as solvents) and offered these extracts in a paired feeding choice assay to *L. irrorata*. Lipid-soluble extracts were created by extracting 2 g of dried, ground tissue in 40 mL of dichloromethane : methanol (2:1) for ~1 h. Solvent was removed with rotary evaporation. The remaining extract was solubilized in ether and added to 2 g of freeze-dried, ground *Ulva intestinalis* Linnaeus collected from Grice Cove, South Carolina. *U. intestinalis* is a palatable food that *L. irrorata* will feed on when incorporated into artificial foods (H. Giddens and E. Sotka, *personal observation*). Water-soluble extracts were created by extracting 2 g of dried, ground tissue in 40 mL of 80% methanol for 3 h (following Barlocher and Newell 1994). Solvent was removed with rotary evaporation and extracts were dried with nitrogen gas. The remaining extract was solubilized and added to 2 g of dried control tissue. We conducted sets of water-soluble extract assays by incorporating extracts into two types of foods: dried ME *Spartina* control tissue and dried *U. intestinalis* because these are both palatable food items that are readily fed upon by *L. irrorata* in the lab. Given that there was no statistical difference in the results between assays that incorporated extracts into foods containing dried *Spartina* vs. dried *Ulva* (data not shown), these data were pooled to increase replication. During all assays, four *L. irrorata* snails were offered a paired feeding choice between plant tissues with extracts from grazed *Spartina* and plant tissues with extracts from control *Spartina* (paired within populations). Dried foods were created as described above. To examine within-population variation in extract palatability in SC, we repeated this experiment but pooled plants by site and by treatment and applied extracts only to dried tissue from ME *Spartina* controls. Assays were stopped when half of the food was consumed or after three days, whichever came first. All snail grazing data were analyzed with paired *t* tests.

We also measured three *Spartina* traits that may underlie snail feeding choices: organic, protein, and phenolic content (Valiela and Rietsma 1984, Barlocher and Newell 1994). Organic content of approximately 0.5 g of lyophilized plant tissue was assessed for grazed and control plants from ME ($N = 6$) and SC ($N = 12$). Samples were combusted at 500°C for 20 h. Mass was recorded before and after combustion, and we calculated ash-free dry mass (AFDM) per dry mass. Protein of approximately 10 mg of lyophilized tissue was measured for five replicate plants for grazed and control plants from ME and SC. Plant material was extracted for approximately 24 h in 1 mol/L NaOH. Absorbance was measured via a spectrophotometer at 5- and 10-minute intervals within a 1:1 solution of extract to Bradford reagent. Absorbance values were compared to a bovine serum standard curve. Phenolics were measured with the Folin-Dennis assay using ferrulic acid as the standard for grazed and control plants from ME and SC ($N = 15$). For each trait, we compared the levels for all four treatments with ANOVA.

Results

Cage treatments were effective in increasing densities of some consumers throughout the induction phase. Immediately after removing cages from SC plants, we found one of nine control cages held a snail, while nine consumer treatment cages each held one snail. Amphipod (2.2 ± 1.1, 1.2 ± 0.3 amphipods/cage, control and consumer cages, respectively; mean ± SE; $P = 0.28$) and planthopper densities (3.4 ± 2.2, 4.8 ± 2.6 planthoppers/cage, control and consumer cages, respectively; $P = 0.70$) were similar. No katydids or stinkbugs were found in any cages in SC or ME. At ME, a single *L. littorea* was found in all grazed cages and none were found in controls. We did not record the final densities of any other consumers in ME cages. Given the consistent densities of all consumers but *L. irrorata* snails across treatments, the induction response observed in SC was likely the result of snail grazing, but we can not exclude the possibility that initial differences in other consumers generated this response.

Immediately following the induction phase, the relative number of local snails colonizing grazed vs. control *Spartina* varied across population. We analyzed snail density on the first day after cage removal using a two-way nested ANOVA and observed a significant effect of population ($P < 0.001$), treatment ($P < 0.001$), and their interaction ($P = 0.001$; see Appendix). Because the effect of site nested within-population was not significant on Day 1 ($P = 0.999$) or Day 2 ($P = 0.397$), we pooled all replicates within each population for post hoc analysis. At ME sites, there was no significant difference in the number of *Littorina littorea* found on grazed

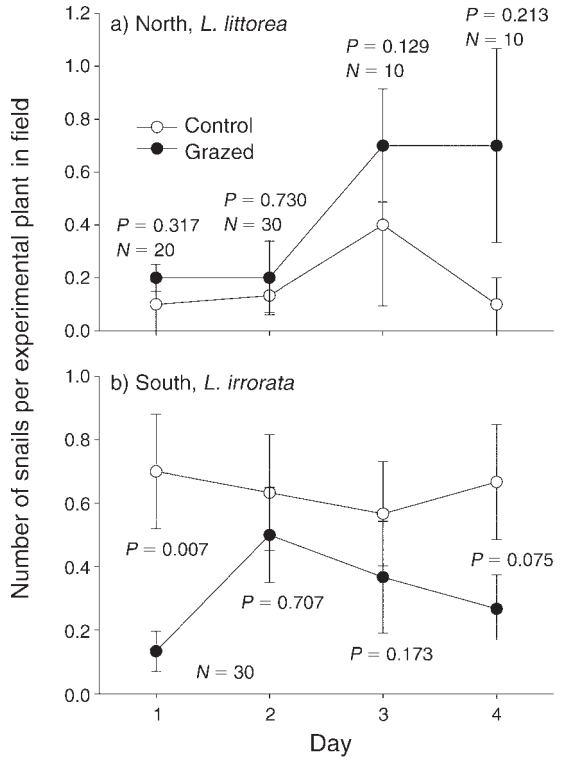

FIG. 1. Field densities of snails (mean ± SE) per *Spartina alterniflora* plant that was previously grazed by consumers for 28 d or not (consumer-free controls). We repeated this experiment at (a) Maine (ME) sites with ME snails (*Littorina littorea*) and (b) South Carolina (SC) sites with SC snails (*Littoraria irrorata*). Differences between densities on grazed and control plants were calculated using a Mann Whitney *U* test.

versus control *Spartina* (Fig. 1a). In contrast, SC control plants were colonized by almost four times as many *Littoraria irrorata* as grazed *Spartina* on the first day (0.70 vs. 0.18 snails/plant; Mann-Whitney U, $P < 0.007$; $\alpha = 0.013$; Fig. 1b). On days 2 and 3, there were no significant differences in population, treatment and their interaction. On day 4, there was a significant interaction between population and treatment (see Appendix). Over the four days, more snails were found on control plants compared to previously grazed plants on 9 out of 12 site-days in SC and 0 out of 7 site-days in ME.

We detected a similar population difference in the response of local planthoppers during laboratory habitat-choice assays. The number of ME planthoppers moving onto ME control versus grazed *Spartina* never differed significantly (Fig. 2a). However, more SC planthoppers were found on SC control than grazed *Spartina* during the course of the assay; this difference was significant on the second day and nearly significant on the third day ($P = 0.035$ and $P = 0.058$, respectively; Fig. 2b).

There was no significant difference in the palatability of dried tissues from ME control versus grazed *Spartina* to *Littorina littorea* (Fig. 3a), as was suggested by the similar abundances of *L. littorea* on these plant types (grazed or control; Fig. 1a). In contrast, dried foods from SC grazed *Spartina* were 48% less palatable than SC controls (Fig. 3b), as was suggested by the lowered colonization of SC grazed *Spartina* in the field (Fig. 1b). SC grazed *Spartina* was less palatable than SC controls when sites were pooled (Fig. 3b) and for two of three sites when sites were not pooled (Fig. 3c).

Thus, dried tissues from SC grazed *Spartina* were less palatable to *Littoraria irrorata* than tissues from SC controls (Fig. 3b), but previous exposure to consumers had no influence on the palatability of ME *Spartina* (Fig. 3a). We did not observe a clear relationship between these preferences and plant traits. Although AFDM increased after attack at both populations (by 4% and 10% at SC and ME, respectively), these changes did not correlate well with feeding preferences. For example, we observed no difference in palatability of grazed and control ME tissues (Fig. 3a) despite the higher ash-free dry mass of ME grazed tissue (Table 1). Thus, a 10% increase in AFDM of ME plants did not change plant palatability so it is unlikely that a 4% increase in AFDM of SC plants was responsible for the

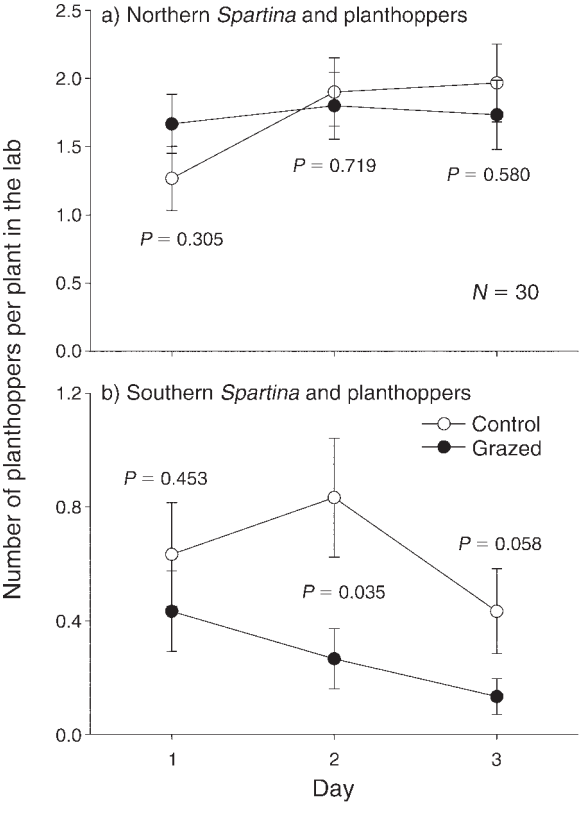

FIG. 2. Densities of a guild of planthoppers (mean ± SE) per *Spartina alterniflora* plant that was previously grazed by consumers for 28 d or not (consumer-free controls). We repeated this laboratory habitat choice assay at (a) ME sites or (b) SC sites, using local planthoppers in both cases. Differences between densities on grazed and control plants were calculated using a two-tailed Wilcoxon signed-ranks test.

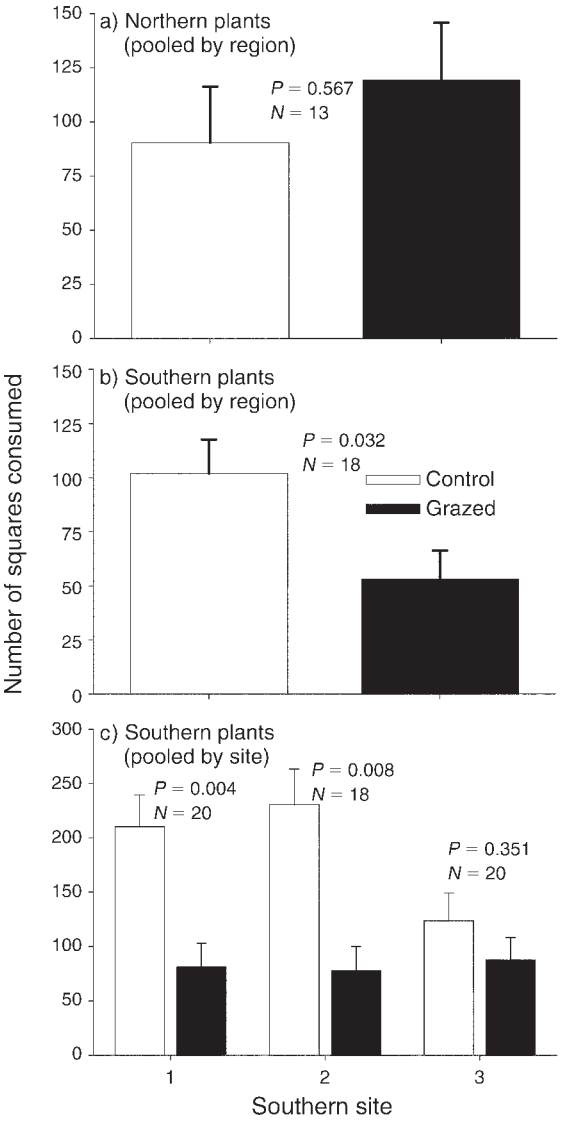

FIG. 3. Consumption (mean and SE) of dried tissue from grazed vs. control *Spartina* when offered to SC *Littoraria irrorata* in a paired feeding-choice assays. Plants were from (a) ME sites or (b, c) SC sites. Differences in consumption rate were calculated using paired *t* tests.

induced responses in SC. There were no significant differences in the protein content (Table 1), and phenolic content was equivalent between SC control and grazed tissues (Table 1).

There was no significant difference in the palatability of a lipophilic extract of SC control versus grazed *Spartina* to *Littoraria irrorata* (Fig. 4a). However, we found a significant deterrent effect of the water-soluble extract from SC grazed *Spartina* on *Littoraria* consumption. Foods containing these extracts were consumed 50% less than foods containing water-soluble extracts of SC controls (Fig. 4b). This pattern was evident when plants were pooled by site instead of by population but was significant for only two of the three sites (Fig. 4c). Although phenolics would have been present in the water-soluble extracts, the concentration of total phenolics was equal between SC control and grazed plants (Table 1). Similarly, although consumer attack may reduce amino acid concentration, and therefore protein concentration, in *Spartina* (Bacheller and Romeo 1992), our finding of equal protein concentrations in SC control and grazed plants suggests that induction in SC plants was unrelated to protein. Thus, non-protein, water-soluble compounds (that were not evident in measurements of total phenolics) of SC grazed *Spartina* deterred *Littoraria* feeding.

DISCUSSION

South Carolina (SC) but not Maine (ME) *Spartina alterniflora* populations displayed inducible resistance in response to local consumers in the field. As a consequence, previously grazed SC plants were less likely to be colonized or consumed by snails (*Littoraria irrorata*) and planthoppers (*Prokelisia*), but previous attack of ME plants had no similar effect on consumers. Given the important roles of planthoppers and snails in structuring salt marsh communities (Denno et al. 2000, Silliman and Bertness 2002), this realized variation in plant-consumer interactions likely has cascading effects on consumer population dynamics.

If these phenotypic patterns are genetically mediated, then the stronger inducible resistance we observed in SC than ME reflects evolution of greater ability to induce within SC than within ME plants. A chloroplast DNA locus and nuclear RAPDs indicate that these populations are genetically isolated (O'Brien and Freshwater 1999, Blum et al. 2007) which indicates that geographic differentiation in *Spartina* traits may evolve via local selection and without the homogenizing effects of gene flow. It is also clear that southern plants are more likely to experience short, intense and spatiotemporally variable bouts of attack relative to northern plants.

TABLE 1. Plant traits of *Spartina alterniflora* from South Carolina (SC) or Maine (ME) populations that were previously grazed by a natural suite of consumers or from consumer-free controls.

Trait	SC controls	SC grazed	ME controls	ME grazed
AFDM (g/g DM)	11.4^{A} (0.3; 12)	11.9^{BC} (0.3; 12)	12.1^{B} (0.1; 6)	13.3^{C} (0.4; 6)
Protein (g/g DM)	2.4 (0.1; 5)	2.4 (0.1; 5)	2.5 (0.1; 5)	2.2 (0.5; 5)
Phenolics (mg/g DM)	0.5 (0.1; 15)	0.5 (0.1; 15)	0.4 (0.1; 15)	0.4 (0.1; 15)

Notes: Standard errors and sample size are in parentheses. Tukey-Kramer post hoc analyses for ash-free dry mass (AFDM; overall ANOVA $F_{3,32} = 6.356$, $P = 0.002$) are indicated. Groups that share a letter are statistically indistinguishable.

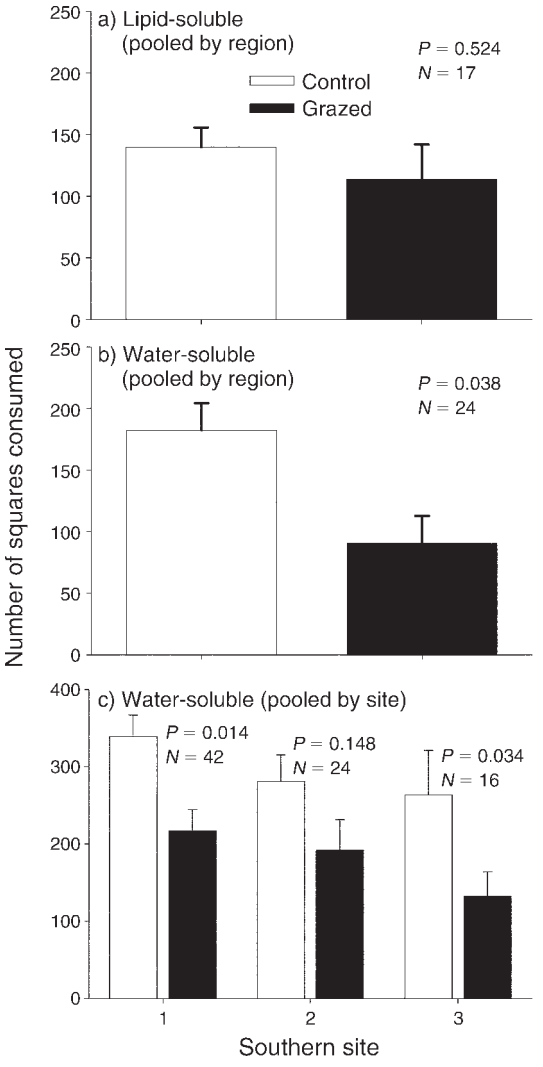

FIG. 4. Consumption (mean and SE) of extract-coated tissues from SC control and grazed *Spartina* when offered to SC *Littoraria irrorata* in a paired feeding-choice assay. We tested palatability of (a) lipid-soluble and (b, c) water-soluble *Spartina alterniflora* extracts. Differences in consumption were calculated using paired *t* tests.

For example, *L. irrorata* sometimes aggregate into fronts whose densities can reach 10× the densities of snails outside these areas, but are spatially and temporally heterogeneous (Silliman et al. 2005), and therefore unpredictable. Snail fronts occur in the southern marsh where we conducted our experiments (Silliman et al. 2005; J. D. Long, *personal observation*) but are unknown in northern marshes. In theory, unpredictable and intense bouts of herbivory favor the evolution of greater inducible defenses (Karban and Baldwin 1997).

However, local evolution of inducibility is not the only mechanism that can explain the pattern of inducible resistance in SC and ME plants. It is also possible that inducible responses were present in both populations but were only apparent in SC because SC consumers are more sensitive to induced plant responses. This hypothesis seems unlikely given that SC consumers were sensitive to differences between grazed and control plants from SC but not ME (Fig. 3). A second possibility is that the strength of the signal eliciting inducible plant responses in our consumer inclusion cages was stronger in SC because of higher grazing rates of SC consumers. Although we never quantified grazing rates within our cages, we attempted to standardize grazing rates between populations by using similar absolute numbers of consumers. Unfortunately, this approach required that we used higher consumer densities compared to ambient levels, especially in SC. Thus, our result of higher induction in SC compared to ME could be caused by greater plant sensitivity to damage, different degrees of damage by consumers in the two sites, or a combination of these factors. Although we do not currently have data to address these alternative mechanisms, neither hypothesis changes the interpretation of our most important finding: that the activities of local consumers at ambient or elevated densities alter the subsequent feeding and recruitment behavior of local consumers in SC but not ME.

Water-soluble compounds mediate induced resistance in SC *Spartina*. Despite previous reports that low-latitude plants contain higher phenolic concentrations than high-latitude populations (Siska et al. 2002) which may influence the palatability of *Spartina* (Barlocher and Newell 1994, Pennings et al. 1998, Zimmer et al. 2004), we did not detect latitudinal differences in total phenolics (Table 1). Our results suggest either that (1) induced SC plants produce higher concentrations of non-phenolic, water-soluble compounds that deter *Littoraria* grazing or (2) grazing induces a response in SC plants that replaces one phenolic compound for another. Other factors related to feeding preferences in salt marsh consumers (e.g., protein content and AFDM; Pennings et al. 1998) appear unrelated to inducible resistance despite a positive effect of grazing on AFDM (Table 1).

There is emerging evidence that the plant induced resistance we describe here has cascading effects throughout the speciose guild of *Spartina* consumers in southern and mid-Atlantic marshes (Denno et al. 1995, Denno and Kaplan 2007). Previous exposure of southern *Spartina* to a natural suite of consumers lowered the attractiveness of the plants to *Littoraria irrorata* in the field, and reduced planthopper habitat preference and *L. irrorata* feeding preference in the lab (this study). Similarly, previous feeding by a planthopper, *Prokelisia dolus*, on New Jersey *Spartina* negatively affected development time, body size, and survival of the congener, *P. marginata* (Denno et al. 2000). Two additional studies found evidence of interspecific competition between consumers feeding on southern *Spartina*, though the authors did not describe the underlying

PLATE 1. To manipulate consumer access to *Spartina alterniflora*, we used cages made of four-inch (1 inch = 2.54 cm) PVC and mesh windows. The cages shown here are from the induction phase at site 3 in South Carolina, USA. Photo credit: J. D. Long.

mechanism. Removal of *Littoraria irrorata* from Georgia plants increased the density and growth rate of the snail, *Melampus bidentatus* (Lee and Silliman 2006), and removal of *L. irrorata* from South Carolina plants resulted in a 50% increase in planthopper density (Gustafson et al. 2006). Neither study distinguished between the indirect effects of *L. irrorata* (mediated via inducible plant responses) from either exploitative or interference competition. Nevertheless, there is increasing evidence to suggest that competition between consumers is common in some *Spartina* marshes (from Georgia to New Jersey) and other ecosystems (Long et al. 2007), and that these interactions may be mediated via inducible plant responses.

The interactions between grazed *Spartina* and consumers may depend largely on the timing and duration of previous attack. Initially, consumer-damaged plants may become unattractive to consumers as plants display inducible resistance (this study). After longer periods, however, this effect may be moderated by fungal colonization of grazed tissues and subsequent feeding on fungi by *Littoraria* snails (Silliman and Newell 2003). Although these studies differed in research focus, together they provide insight into a further level of complexity in salt marshes that warrants additional study using standardized methodology.

Although previous studies documenting between-population variation of inducible resistance shed important light on the evolution of plant defenses, they may fail to predict the ecological consequences of such variation because they were conducted in the laboratory with a single inducing agent (either mechanical damage or a single herbivore species). Using local plants and consumers, we show that (1) inducible resistance varies naturally between plant populations after exposure to local consumers in the field and (2) this variation differentially influences the surrounding consumer communities. Given that the consumers most sensitive to these inducible plant defenses (e.g., *Littoraria* snails and *Prokelisia* planthoppers) exert strong top-down control of salt marshes, the impacts of these responses likely extend beyond the attacking and attacked individuals. Clearly, realized variation in plant–herbivore interactions influences the ecology and evolution of plant communities (Thompson 2005) and understanding these ecological and evolutionary consequences will be impossible without field studies where multiple plant populations are exposed to local consumers.

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APPENDIX

ANOVA testing the effect of region (northern vs. southern) and treatment (grazed vs. control) on the field densities of snails per *Spartina alterniflora* plants that were previously grazed by herbivores for 28 days or not (grazer-free controls) (*Ecological Archives* E092-014-A1).