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## Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing

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**Abstract** Tissues within individual plants can vary greatly in the strength of their constitutive (i.e., permanent) and induced resistance to herbivores. Optimal defense theory predicts that defenses should be allocated among tissues in proportion to the value of the tissues to the plant and the tissue-specific risk of attack by grazers. We examined the relationship between tissue value and defense in the highly-differentiated brown seaweed *Sargassum filipendula*. Tissues within *S. filipendula* varied widely in palatability to the herbivorous amphipod *Ampithoe longimana*, with younger tissues preferred over older tissues and blades preferred over stipes. Old stipes (at the base of the plant), which linked the other tissues to the seafloor and were thus the most valuable tissue to the plant, were defended constitutively and resisted amphipod grazing by virtue of their toughness rather than via deterrent chemistry. Induction of resistance as a result of amphipod grazing occurred only in the top stipes, which contain the meristematic tissue responsible for future growth. Induction in the top stipes was not due to toughness or other structural properties,

as the unpalatability persisted when top stipes were dried, ground to a fine powder, and reconstituted into an agar matrix. This suggests that the induced resistance to grazing resulted from an increase in chemical defenses. The demonstration of constitutive or induced defenses in only the more valuable tissues of the seaweed is consistent with predictions of optimal defense theory. Our finding of induction due to mesograzers (amphipod) feeding is also consistent with the notion that it is these small, more sedentary, herbivores that are most likely to induce defenses in seaweeds.

**Keywords** Algae · Chemical defense · Induced defense · *Sargassum filipendula* · Within-plant variation

### Introduction

Plants are defended against grazers via a variety of metabolites and structures (Hay and Fenical 1988; Hartley and Jones 1997; Hay 1997). Defensive traits can vary considerably among tissues within individual plants; and this variability often has a strong influence on herbivore preference for particular plant parts (McKey 1979; Denno and McClure 1983; Cronin and Hay 1996a; Van Alstyne et al. 2001). Because plant defenses can be expensive to produce and maintain (Zangerl et al. 1997; Baldwin 1998), constitutive (i.e., permanently expressed) defenses may be unnecessarily costly if they are not always needed. In such cases, it can be advantageous for plants to defend their tissues inductively in response to herbivory (Karban and Baldwin 1997; Harvell and Tollrian 1999).

Optimal defense theory holds that costly defenses should be allocated among tissues in proportion to the value of the tissues (in terms of replacement costs and their contribution to future plant fitness) and the likelihood of attack by grazers (Rhoades 1979). This theory thus predicts that valuable tissues at high risk to grazers will be protected by strong constitutive defenses, while tissues that are less valuable or less likely to be attacked

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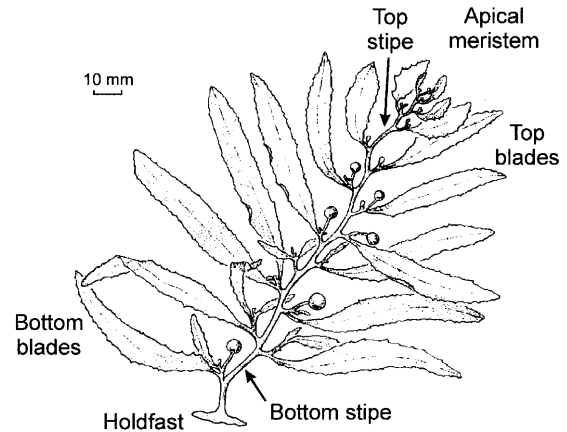
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will have lower levels of constitutive defenses and/or be capable of inducing defenses following attack (Zangerl and Rutledge 1996). There is some empirical support for these predictions, in that there is often a positive correlation between tissue value and the strength of constitutive defenses [e.g., see Krischik and Denno (1983) for terrestrial plants and Cronin (2001) for seaweeds] but the nature of the relationship for induced defenses is unclear (Karban and Baldwin 1997). We know of only one study that explicitly examines within-plant variation in both constitutive and inducible defenses. In the wild parsnip (*Pastinaca sativa*), the strength of constitutive defenses and the magnitude of an induced response to grazing were negatively correlated among tissues, with roots having weak constitutive defenses but a strong inductive response to simulated grazing damage, while fruits showed the opposite pattern and leaves were intermediate (Zangerl and Rutledge 1996).

Because individual plant tissues are often both valuable to the plant and subject to high risk of attack, it is difficult to estimate the relative influence of each factor on the allocation of defenses. This is particularly true for terrestrial vascular plants, where a significant proportion of biomass may be below ground and inaccessible to commonly investigated herbivores. In contrast, most seaweeds do not have a penetrative root system and the susceptibility of tissues to grazers is more uniform, thus potentially providing a less confounded view of the influence of tissue value on the allocation of defenses.

As with terrestrial plants, constitutive defenses against grazers are common in seaweeds (Hay and Fenical 1988; Paul et al. 2001) and often show considerable within-plant variation (Hay 1996; Van Alstyne et al. 2001), which strongly affects herbivore feeding preferences (e.g., Steinberg 1984; Hay et al. 1988; Paul and Van Alstyne 1988; Poore 1994; Cronin and Hay 1996a; Pennings et al. 1996). Several studies also have documented the induction of secondary metabolites, toughness, or resistance to herbivores in response to natural or simulated grazing (Lewis et al. 1987; Van Alstyne 1988; Lowell et al. 1991; Yates and Peckol 1993; Cronin and Hay 1996b; Peckol et al. 1996; Hammerstrom et al. 1998; Pavia and Toth 2000). In the only study on seaweeds that has examined within-plant variability in induction, Hammerstrom et al. (1998) measured changes in phlorotannin concentrations in various parts of five kelp species following artificial clipping. Stipes and holdfasts, the tissues that support the major photosynthetic and reproductive organs, had been ignored in previous studies of induction in seaweeds. However, along with the more frequently studied blades, they showed a rapidly induced response to damage in nearly all species surveyed.

In the present study, we examine within-plant variability in constitutive and induced resistance in the fucalean brown seaweed *Sargassum filipendula* C. Agardh, 1824. *S. filipendula* is more morphologically differentiated than most other seaweeds. Its small holdfast produces one to several cylindrical stipes, which are typically 0.2–0.3 cm in diameter and 10–30 cm long, with numer-



**Fig. 1** The fucalean brown seaweed *Sargassum filipendula* [Illustration modified from one by Julia S. Child for Schneider and Searles (1991). All rights reserved. Reprinted with permission]

ous narrow blades ~6 cm in length (Fig. 1). Pneumatocysts (air bladders that float tissues upright into the water column) and reproductive structures are also present. The meristem is apical, so that the youngest blade and stipe tissues are found at the tips of the plant and the oldest tissues are near the holdfast. We investigate differences in susceptibility to grazing among top blades, top stipes, bottom blades, and bottom stipes, and attempt to explain observed feeding patterns by reference to tissue traits. We also quantify the relative contributions of young and old tissues to plant growth. We then examine inductive responses to amphipod grazing for each of the four tissues and relate the results to the value and constitutive defense of each tissue. Finally, we discuss the results in the light of optimal defense theory.

## Materials and methods

### Collection sites

*S. filipendula* was collected from the shallow subtidal at Radio Island Jetty near Beaufort, N.C. (34° 42' N, 76° 41' W). Plants were transported to the laboratory in coolers filled with seawater and were kept in flowing seawater until used (within 24 h). Amphipod amphipods were taken from mixed-algal cultures maintained outdoors in flowing seawater at the Institute of Marine Sciences in Morehead City, N.C. The amphipod cultures were originally seeded with mixed collections of amphipods from local seaweed and seagrass beds. Seaweeds from the field were added to these cultures approximately biweekly, thus ensuring both fresh food and periodic addition of representative field-collected amphipods to these cultures. We chose to use the amphipod *Ampithoe longimana* Smith, 1873 as our assay herbivore because, in coastal North Carolina, it is one of the more common herbivores that can significantly impact large brown seaweeds like *S. filipendula* (Duffy and Hay 2000).

### Within-plant variation in susceptibility to amphipod grazing

A series of no-choice and choice assays were run to determine feeding preferences of *A. longimana* for each of the following tissues from *S. filipendula*: top blades, top stipes, bottom blades, and bottom stipes.

By isolating *A. longimana* with a single piece of tissue (a no-choice assay), one can assess feeding rates on that tissue when alternative foods are unavailable. These data can then be contrasted with data from choice assays (i.e., where multiple food types are simultaneously available to each consumer) to evaluate the importance of compensatory feeding and diet mixing (Slansky 1992; Cruz-Rivera and Hay 2000a, b, 2001). No-choice assays were run on 22 January 1999. For each of the four tissues, two paired pieces (~30 mg) were removed from each plant, blotted dry, weighed ( $\pm 1$  mg), and added to separate dishes ( $n=25$  pairs) containing seawater. Two *A. longimana* were added to one of the two paired dishes, while the other was left free of grazers to control for autogenic changes in plant mass. Plants were reweighed after 2 days. The amount of seaweed consumed in each replicate was calculated as:  $[(H_0 \times C_f / C_0) - H_f]$ , where  $H_0$  and  $H_f$  were pre-assay and post-assay wet masses of tissue exposed to the amphipod and  $C_0$  and  $C_f$  were pre-assay and post-assay wet masses of controls for autogenic changes in mass. Replicates were dropped from the subsequent analysis if the amphipods died during the assay.

In order to assess the relative preference of *A. longimana* for each tissue, amphipods were given pairwise choices of all six possible combinations of the four tissue types. Such choice assays were conducted similarly to the no-choice assays, except that: (1) two tissues were offered on opposite sides of a glass rod (to ensure they could later be identified by their position), (2) replicates were stopped earlier than 2 days if at least half of either piece of tissue had been eaten, and (3) replicates were dropped if the maximum mass eaten of either piece was  $\leq 3$  mg (approximating our maximal potential weighing error). These assays were run in December 1998 and January 1999.

To test whether differences in toughness or structure were responsible for the patterns of feeding preference observed in the fresh plant assays, we made a bulk collection of plants on 31 January 1999, separated the four tissues, and destroyed their morphological properties by freeze-drying and grinding them to a fine powder with mortar and pestle. We then reconstituted the separate tissue types in agar and gave amphipods all six possible pairwise choices. A mixture of 0.090 g of agar in 2.5 ml of distilled water was heated to boiling in a microwave oven, allowed to cool for a few seconds, and then added to a mixture of 0.25 g of powdered tissue and 2 ml of distilled water. The combined mixture was poured over fiberglass window screen and flattened between two layers of wax paper (method adapted from Hay et al. 1994, 1998). After cooling, the mesh was cut into squares of 6x6 cells ( $n=30$ ) and treatments were identified by clipping a corner off one tissue type. The reconstituted tissues were offered to 3–4 amphipods/replicate for 2 days or until at least half of either treatment had been eaten, whichever came first. For each replicate, we calculated the number of cells consumed (i.e., cells cleared of food) from each square as a percentage of the total number of cells consumed from both squares, i.e., a value of 50% indicated equal consumption of both squares (following Bernays and Wege 1987).

To examine the potential role of chemical defenses in determining the patterns of feeding preference observed in the fresh plant assays, we assayed the palatability of crude water-soluble (polar) and lipophilic (nonpolar) extracts from each of the four tissue types. We collected plants on 17 February 1999, separated the four tissues, and recorded their blotted weights (5.1–26.1 g). We then used dry mass:blotted mass ratios from the "Nutritional traits" procedures (see below) to estimate the dry mass of each tissue. Tissues were then ground in methanol in a blender, extracted four times in 2:1 dichloromethane:methanol, and then three times in 70:30 methanol:water. The organic solvents were removed by rotary evaporation and the remaining extracts were partitioned between dichloromethane and water. Extracts were incorporated at natural concentrations by dry mass into 0.25 g of freeze-dried powdered *Enteromorpha* sp. (a green seaweed palatable to *A. longimana*), which was then used to make reconstituted food squares. Lipophilic extracts were dissolved in diethyl ether and added to powdered *Enteromorpha*, which was then subjected to rotary evaporation to remove the solvent. Water-soluble extracts were dissolved in 2 ml of distilled water and added to powdered

*Enteromorpha* in place of the 2 ml of distilled water used in the recipe in the previous section. The rest of the procedure for making up the reconstituted food and the assays, were as described above for the powdered *S. filipendula*.

#### Nutritional traits

Toughness and crude organic and inorganic composition were measured for plants collected on 21 January 1999. Single samples of each of the four tissue types were taken from each of ten individual plants. Tissue toughness was quantified as follows, using the method of Duffy and Hay (1991). An insect pin with a small cup glued to the head was vertically mounted over a tissue sample. Sand was then added to the cup until the tip of the insect pin just penetrated the tissue. The combined mass of the sand and cup was used to determine the force required to pierce the thallus.

To quantify crude organic and inorganic composition, tissue samples of ~100–200 mg were blotted, dried to constant mass at 60 °C, and then combusted at 500 °C for 19 h, with the mass recorded at every step ( $\pm 0.1$  mg). Water content was calculated as the difference between the blotted and the dry masses, ash (inorganic content) was measured directly after combustion, and ash-free dry mass (organic content) was calculated as the difference between the masses of the dry matter and the ash.

Soluble protein was measured on plants collected on 31 January 1999. For each of the four tissues sampled, we collected a tissue type from numerous plants, pooled these collections, freeze-dried them, and then ground the sample to a powder using a mortar and pestle. Samples of ~10 mg were weighed ( $\pm 0.1$  mg), extracted for 20 h in 0.5 ml of 1 M NaOH, and analyzed using the Sigma colorimetric method (based on Bradford 1976), with bovine serum albumin as a standard. Blanks and standards included NaOH. Note that the errors on the means for protein represent measurement error only, as variation among individual plants was removed by pooling individual samples prior to grinding.

#### Relative contribution of top versus bottom blades to plant growth

The relative contribution of top and bottom blades to the growth of *S. filipendula* was assessed by removing top or bottom blades from genetically identical ramets and quantifying net growth over 17 days in the field. Thirty plants with multiple stipes sprouting from the same holdfast were collected on 7 November 1997. Two stipes were removed from each plant, spun dry for 10 s in a salad spinner, and weighed ( $\pm 0.01$  g). Blades weighing 30% of the total mass of each stipe were removed from the top half of the first stipe ("top blades removed" treatment) and the bottom half of the second stipe ("bottom blades removed" treatment). The next day, the stipes were returned to Radio Island Jetty in coolers. Each was strung through a 10-cm long three-stranded polypropylene rope and individually numbered. This rope was attached to a reinforcing-bar driven into the sand within 1 m of the jetty, at a depth of approximately 2.5 m below mean low water. Plants were harvested on 25 November 1997 (day 17), returned to the lab, and reweighed in order to quantify net growth.

#### Within-plant variation in response to amphipod grazing

An experiment examining within-plant variation in response to amphipod grazing was run outdoors at the Institute of Marine Sciences. The setup comprised 60 tanks (11 l), which each received fresh filtered seawater from nearby Bogue Sound at ~0.06l/s. All tanks were covered with a layer of burlap to mimic natural light levels that subtidal *S. filipendula* would receive. At noon on a cloudless day, photosynthetically active radiation beneath this burlap equalled levels at a depth of ~1.2 m at the site from which plants for the experiment were collected (Li-1000, measuring  $4\pi$  irradiance). *S. filipendula* plants at the site occupied depths ~0.1–2.0 m below low tide.



On 8 June 1999, *S. filipendula* plants were collected from Radio Island Jetty and held overnight in water tables with flowing seawater. On 9 June 1999, all plants were dunked for 20–30 s in a ~2 ppm solution of the insecticide Sevin (carbaryl) in seawater to remove crustaceans (Duffy and Hay 2000). Two individual ramets (20–30 cm long) were cable-tied at their bases to a short length of PVC pipe and placed in each tank. To reduce variability in the subsequent bioassays, ramets in adjacent treatment and control tanks came from the same individual *S. filipendula* plant (i.e., they were paired). The blotted weights of the paired plants were recorded ( $\pm 0.1$  g). A second plant in each tank was not paired with any other: its purpose was to contribute tissues for bulk collections to use in chemical assays designed to identify the tissue properties responsible for any induced changes in palatability observed in the paired plants. To half of the tanks ( $n=30$ ; the “grazed” treatment) we added a mixture of the gammarid amphipods *A. longimana*, *A. marcuzzii* Ruffo, 1954, and *A. valida* Smith, 1873, all of which are known consumers of *S. filipendula* (Duffy 1990; Duffy and Hay 1991, 2000). No amphipods were added to the remaining 30 tanks, which acted as controls. Adult amphipods ( $n=20$ ) were added to each “grazed” treatment tank on 11 June 1999 (day 0). On day 3, 10–20 more *A. valida* were added to 17 replicate tanks that contained larger plants. All tanks were stirred briefly every day to reduce the buildup of sediment on the plants.

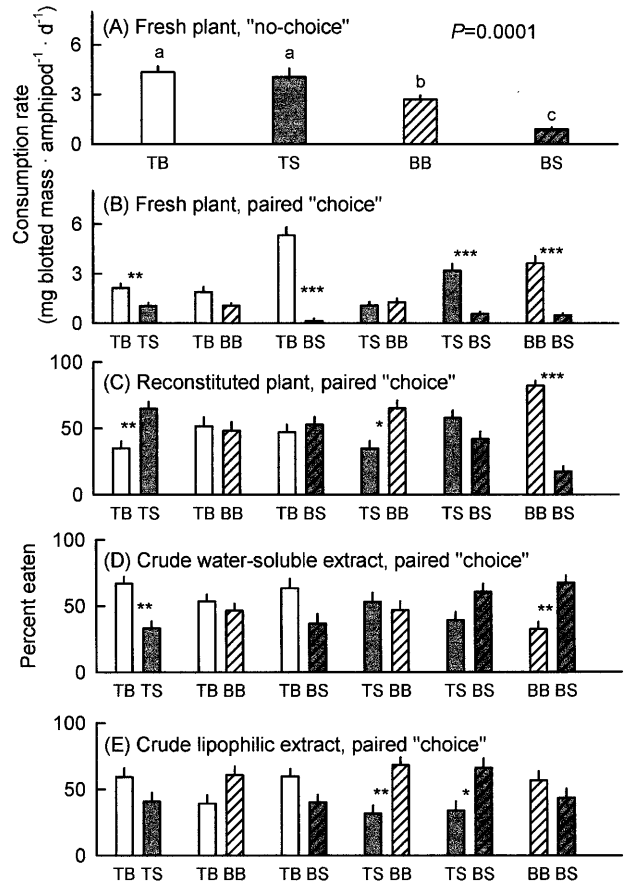
To determine whether previous attack by amphipods resulted in plant portions becoming less palatable, on 4 July (day 23) we compared the palatability, to *A. longimana*, of similar tissue types from plants that had been in “grazed” treatment tanks with amphipods for the previous 23 days versus paired control plants from tanks without amphipods. Short lengths of top and bottom stipes (with attached blades) were removed from all healthy paired plants, which were then reattached to their pipes with cable ties. Blotted masses of removed sections were recorded ( $\pm 0.1$  g). For each tissue type, an individual amphipod was offered a pre-weighed piece (~30 mg blotted mass) of tissue taken from paired grazed and control plants. These pieces were placed on either side of a glass rod in individual dishes filled with seawater. The glass rod allowed amphipods to move freely between plant portions, but kept each plant piece in its initial position, so it could be identified at the end of the assay. These choice assays were run as described earlier. A few replicate plants were not used for feeding assays due to their unhealthy or fouled appearance.

On 8 July 1999 (day 27), all remaining healthy unpaired plants from the main outdoors experiment were combined by treatment, tissues of the four types were removed and frozen separately, and these tissues were investigated in an effort to determine the factor(s) responsible for any observed induction. To determine how amphipod grazing had affected plant growth during the experiment, all remaining healthy paired plants were weighed after being twice dunked in freshwater for 10 s and then beaten gently against the side of a bucket to dislodge amphipods and other herbivores. These herbivores were trapped on a 202- $\mu$ m mesh sieve and counted. The blotted mass ( $\pm 0.1$  g) of each paired plant was recorded and added to the mass of the sections removed on day 23 for the fresh plant bioassays to get the total mass of the plant on day 27.

The bioassays revealed that top stipes of the grazed plants were less palatable than those of the controls. To check whether the change in palatability of the top stipes was due to a change in toughness or some other attribute of the grazed plants, we freeze-dried top stipe tissue from unpaired plants in each treatment at the end of the experiment and separately ground these tissues finely with mortar and pestle. We then reconstituted them on an agar-window screen matrix (as described earlier) and offered *A. longimana* a choice between these agar-based foods made from grazed versus control plants.

## Results

The ranking of feeding rates of *A. longimana* on fresh *S. filipendula* tissues in the no-choice assays was: top blades = top stipes > bottom blades > bottom stipes

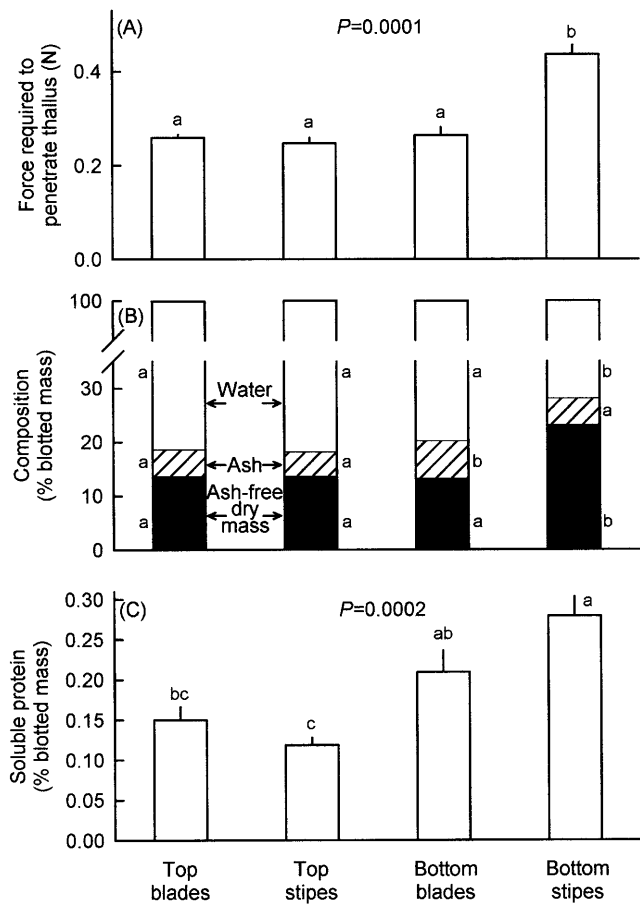


**Fig. 2A–E** Feeding rates of the amphipod *Ampithoe longimana* on tissues of the brown seaweed *S. filipendula*. Bars represent mean  $\pm 1$  SE ( $n=20$ –25 for A, B;  $n=26$ –30 for C–E). In A, statistic is for one-way ANOVA; bars labeled with the same lower case letter do not differ significantly ( $P>0.05$ ), according to Tukey’s HSD. In B–D, statistics are for two-tailed paired  $t$ -tests: \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ . *amphipod*<sup>-1</sup> Per amphipod, BB bottom blades, BS bottom stipes,  $d^{-1}$  per day, TB top blades, TS top stipes

(Fig. 2A). In the pairwise choice assays, the order of preference was crudely similar: top blades > top stipes = bottom blades > bottom stipes (Fig. 2B).

It was not possible to unambiguously rank amphipod preference for the four tissue types in pairwise choice assays of reconstituted *S. filipendula* (where the effects of morphological traits on feeding preference were eliminated) (Fig. 2C). For example, bottom blades were more palatable than top stipes, which in turn were more palatable than top blades, but bottom blades and top blades were of equal palatability in a direct contrast (Fig. 2C). Similar ambiguities hampered interpretation of the pairwise choice assays of crude water-soluble (Fig. 2D) and lipophilic extract (Fig. 2E).

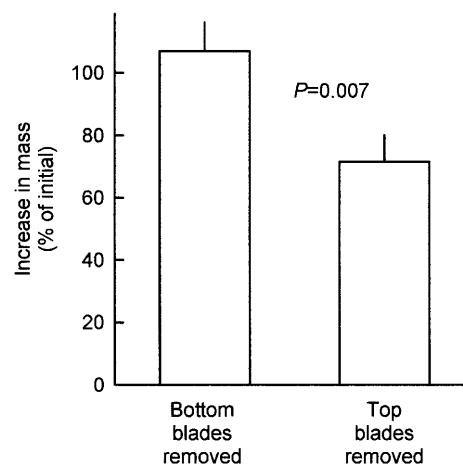
In the reconstituted plant assays, bottom stipes did not differ in palatability from top blades and top stipes (Fig. 2C). However, bottom stipes from fresh plants were highly deterrent relative to fresh top blades and top stipes (Fig. 2A, B), suggesting that bottom stipes were resistant due to their toughness, rather than due to chemical traits.



**Fig. 3A–C** Nutritional characteristics of tissues from the brown seaweed *S. filipendula*. **A** Toughness, **B** organic matter (ash-free dry mass), ash and water content, **C** protein content. Bars represent mean  $\pm 1$  SE ( $n=10$  in **A**, **B**;  $n=5$  in **C**). SEs are omitted for clarity in **B**, but were  $\leq 0.5\%$  of blotted mass for ash and  $\leq 1.7\%$  of blotted mass for ash-free dry mass and water. Statistics are for one-way ANOVAs (run on individual components in **B**).  $P=0.0001$  for all three tissue components in **B**. Bars labeled with the same lower case letter do not differ significantly ( $P>0.05$ ), according to Tukey's HSD

Toughness varied little among top blades, top stipes, and bottom blades (range of mean forces required to penetrate tissue = 0.247–0.264 N), but bottom stipes were  $\sim 1.7$  times tougher than all three other tissues (0.436 N; Fig. 3A). Top blades, top stipes, and bottom blades contained very similar percentages of water (range of means = 79.7–81.7%), ash (4.5–7.0%), and ash-free dry matter (13.4–13.7%), but bottom stipes contained  $\sim 1.7$  times more ash-free dry matter (23.3%), a similar amount of ash (5.0%), and less water (71.8%; Fig. 3B). Protein content was significantly higher in the bottom stipes (0.28% blotted mass) than in the top blades and top stipes (Fig. 3C).

Top blades contributed more to plant growth than did bottom blades. Plants from which top blades had been removed increased in mass by 72% over 17 days, whereas plants from which bottom blades had been removed increased in mass by 107% ( $P=0.007$ , Fig. 4).

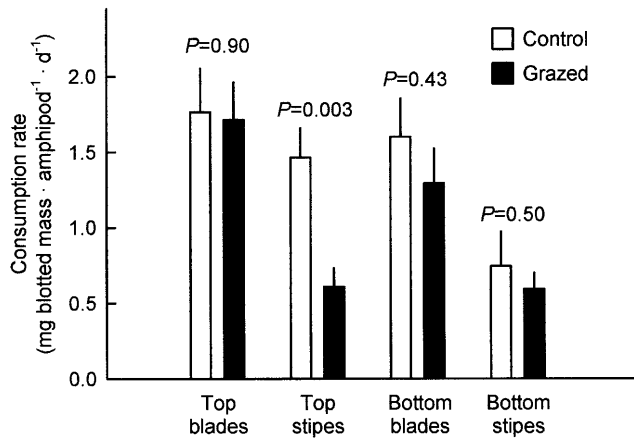


**Fig. 4** Percentage increase in mass of the brown seaweed *S. filipendula* 17 days after removal of blades comprising 30% of initial plant mass from either the top ( $n=23$ ) or bottom ( $n=26$ ) half of the stipe. Each pair of stipes was taken from the same individual plant. Bars represent mean  $\pm 1$  SE. Statistic is for two-tailed paired *t*-test

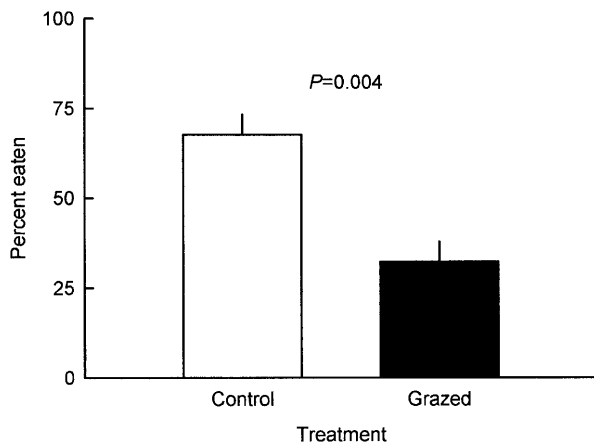
At the end of the experiment designed to assess within-plant variation in response to grazing, there were 26 times as many amphitoid amphipods on the plants in the “grazed” treatment as on the controls ( $3.64 \pm 0.39$  individuals/g blotted mass vs  $0.14 \pm 0.03$  individuals/g blotted mass, respectively; mean  $\pm 1$  SE,  $n=29$ ;  $P<0.0001$ , paired sample *t*-test). This is within the range of amphitoid densities found on *S. filipendula* at Radio Island Jetty (Duffy 1989). Other potential grazers (the polychaete *Platynereis dumerilii*, sphaeromatid isopods, small gastropods) were at low densities in both treatments (total of  $0.014 \pm 0.006$  individuals/g blotted mass on grazed plants,  $0.011 \pm 0.005$  individuals/g blotted mass on control plants;  $P=0.6$ , paired sample *t*-test). These other grazers may have survived the carbaryl treatment (isopods and molluscs appear to be more resistant to the insecticide than are amphipods; Duffy and Hay 2000), or may have colonized both treatments and controls at low numbers via movement through the seawater system.

During the experiment, the net increase in blotted mass of the control plants was  $19.2 \pm 2.6$  g, from an initial mass of  $8.6 \pm 0.9$  g (mean  $\pm 1$  SE,  $n=29$ ). However, the plants from the “grazed” treatment increased only  $2.4 \pm 1.7$  g, from an initial mass of  $9.7 \pm 1.1$  g ( $n=30$ ). Thus, the mean net growth in mass of the control plants was +223%, while that of the grazed plants was only +25% ( $P=0.002$ , paired *t*-test).

Induced resistance to herbivores occurred in only one of the four tissues tested. Only the top stipes showed a reduction in palatability due to previous grazing (Fig. 5). On average, amphipods consumed 2.2 times more mass of top stipes from control plants than from previously grazed plants. The palatability of top stipes from grazed plants dropped considerably and became comparable with the palatability of bottom stipes. Reconstituted top stipes from grazed plants were less palatable to *A. longi-*



**Fig. 5** Feeding rates of the amphipod *A. longimana* offered a choice between tissues from individuals of the brown seaweed *S. filipendula* that had either been grazed by amphipods or protected from amphipod grazing (Control). Bars represent mean +1 SE ( $n=23-28$ ). Statistics are for two-tailed paired *t*-tests



**Fig. 6** Feeding preference of the amphipod *A. longimana* offered a choice between reconstituted freeze-dried, powdered top stipes from individuals of the brown seaweed *S. filipendula* that had either been grazed by amphipods or protected from amphipod grazing (Control). Bars represent mean +1 SE ( $n=29$ ). Statistic is for two-tailed paired *t*-test

*mana* than top stipes from control plants, suggesting that the induced resistance was not mediated by morphological traits, but rather by nutritional or chemical traits (Fig. 6). The 2.1-fold difference was nearly identical to that for the fresh tissues.

## Discussion

Of the four tissue types examined, we reasoned that the bottom stipes were the most critical for future plant fitness because, if they were weakened by grazing, entire plants would be vulnerable to being ripped loose by turbulence (Koehl and Wainwright 1977; Padilla 1993). Despite having the highest organic and protein contents of the four tissues and thus presumably being the most

valuable nutritionally, the bottom stipes were by far the tissue type that was least preferred by *A. longimana* in assays using fresh tissue (Fig. 2A, B). The bottom stipes were also the most resistant tissue to penetration (Fig. 3A). This toughness appeared to be important for resistance to amphipod grazing because: (1) bottom stipes that were powdered and reconstituted lost their resistance when compared with top blade and top stipe tissue; but they retained their lesser preference compared to bottom blades (Fig. 2C contrasted to B), and (2) crude water-soluble and lipophilic extracts from bottom stipes were not deterrent when incorporated into a palatable seaweed (Fig. 2D, E). The lack of induced resistance in a tissue that already possesses strong constitutive resistance parallels results for fruits of the wild parsnip (Zangerl and Rutledge 1996).

We reasoned that top stipes should be more valuable to the plant than top blades, because the top stipes contain the meristematic tissues that produce the blades and reproductive structures. Top stipes were relatively palatable in plants freshly collected from the field (Fig. 2A, B), but were the only tissue to show an induced response to herbivory (Fig. 5); this result was replicated in an experiment designed to test for the transmission of waterborne grazing cues in *S. filipendula* (Sotka et al. 2002). Significantly, induction due to attack by herbivores made the top stipes as unpalatable as the bottom stipes (Fig. 5). The reduction in palatability was due to a change in chemical defenses, or some other non-structural plant property, as freeze-dried, powdered, and reconstituted top stipes from grazed plants retained their deterrence relative to controls (Fig. 6). Unfortunately, the experimental plants did not yield sufficient tissue for us to pursue the separation and identification of the potential chemical defenses suggested by this result. Because upper stipe tissue constitutes only a small proportion of each *S. filipendula* plant, obtaining adequate tissue mass to pursue chemical investigations would have required having many-fold more induced plants than were produced by our experiment; therefore the identity of the compounds induced by these herbivores remains unknown. Phlorotannins occur in *Sargassum* and some compounds in this class of metabolites deter feeding by some, but not all marine herbivores (Steinberg and van Altena 1992; Targett and Arnold 1998). However, *S. filipendula* plants in North Carolina tend to have relatively low (<0.6% dry mass) concentrations of phlorotannins (Cronin and Hay 1996c). Further, plants differing in phlorotannin concentrations did not differ in palatability to *A. longimana* (Cronin and Hay 1996c), suggesting that induction of phlorotannins is an unlikely explanation for the reduced palatability seen in this investigation.

The blades comprise the major photosynthetic surface of *S. filipendula* and top, young blades contributed more to whole plant growth under field conditions than did the bottom, older blades (Fig. 4), making these lower blades the least valuable tissues to *S. filipendula*. Although the bottom blades should be less valuable to the plant than top blades, they were slightly more resistant to grazing



by *A. longimana* (Fig. 2A, B). This is possibly attributable to the higher coverage of fouling organisms on bottom blades (this work, personal observation), which can sometimes deter or stimulate herbivores (Wahl and Hay 1995). It is also possible that, by only measuring their contribution to growth, we have underappreciated the value of the bottom blades, because they could potentially store photosynthate or other valuable products (McKey 1979) and thus warrant defending for that reason. Another possibility is that optimal defensive allocation to the blades is constrained by cellular differentiation processes, in that cell division and expansion in the younger, actively growing, top blades precedes the investment of resources into secondary metabolite production (Herms and Mattson 1992). Older tissues of the less differentiated dictyotalean brown seaweed *Dictyota ciliolata* were also less palatable to grazers, including *A. longimana*, apparently because they contained higher concentrations of secondary metabolites (Cronin and Hay 1996a). Neither top nor bottom blades changed in palatability following grazing, indicating that induction did not occur in these tissues.

As described above, we reasoned that the order of importance of the tissues to the survival, growth, and reproduction of *Sargassum filipendula* was bottom blades < top blades < top stipes < bottom stipes. In pairwise choice assays, the amphipod *A. longimana* preferred (in order from most to least preferred) top blades > top stipes = bottom blades > bottom stipes. The palatability of bottom blades was not well predicted by the optimal defenses theory, but the ranking of palatability for the other three tissues was as predicted, suggesting that reasoning based on optimal defense theory was useful for this species of morphologically differentiated seaweed.

Researchers have suggested that the “most important factor governing the degree of reliance on inducible defense in plants should be the likelihood of attack” (Zangerl and Rutledge 1996). Evidence for this is both theoretical and empirical: plants and tissues subject to intense and/or frequent attack likely benefit from strong constitutive defenses, while plants and tissues subject to low, intermediate or highly variable attack rates likely benefit from inducible defenses (see Karban and Baldwin 1997 for review). However, tissue value may also help explain within-plant variation in constitutive and induced resistance for plants that are not subject to continuous intense herbivory. Using examples consistent with results from the present study, highly valuable tissues (e.g., bottom stipes) may be worth defending strongly and constitutively, even if attack risk is low, given that even low levels of direct damage can cause large losses due to detachment during storms (Koehl and Wainwright 1977). Tissues of lower value (e.g., top and bottom blades) may be more economical to replace than to defend (whether constitutively or inductively). For tissues of intermediate value, replacement may be more expensive than inducible defenses (e.g., top stipes). It is probable of course that a combination of tissue value and attack risk influences the evolu-

tion of plant defensive strategies. There is a clear need for studies of other plant-herbivore systems in order to establish the circumstances under which each is important.

The combination of constitutive and induced defenses in *S. filipendula* has the potential to be highly effective. Our anecdotal observations suggest that *S. filipendula* plants can: (1) withstand intense herbivory, and (2) recover from such damage. In our culture tanks used to maintain amphipod populations for experiments, *S. filipendula* stipes always remained long after defoliation, even with herbivorous amphipods present at far higher densities than we have ever observed in the field (personal observation). The persistence of the top stipes under these conditions would appear to be due to their induction of resistance following grazing (Fig. 5), as they are initially palatable (Fig. 2). We have no direct proof that such defoliated stipes are capable of regeneration, but we have often noted stipes resprouting blades after they have been stripped bare during hurricane-generated storms or following seasonal loss of blades that may be due in part to amphipod grazing during times of low seaweed growth on offshore reefs (personal observation). Moreover, several other species of *Sargassum* are known to be capable of regeneration from a holdfast or primary axis (Schiel 1980; Vuki and Price 1994).

This study is the fourth clear example of induction of seaweed chemical or nutritional resistance due to herbivory and, in common with the other examples, i.e. *Fucus vesiculosus* (Van Alstyne 1988), *Dictyota menstrualis* (Cronin and Hay 1996b), and *Ascophyllum nodosum* (Pavia and Toth 2000), the herbivores responsible for the induction are mesograzers and the seaweed is a brown alga. Brown seaweeds are often resistant to consumption by larger herbivores, such as echinoids and fishes, and thus often serve as refuges for mesograzers, such as amphipods (e.g., Hay et al. 1990; Duffy and Hay 1994). The spatiotemporal patchiness of mesograzers (e.g., Cronin and Hay 1996b) and their modest removal of plant biomass relative to larger grazers make mesograzers prime candidates for inducing agents, because plants can induce resistance across temporal and spatial scales that are ecologically meaningful relative to scales of mesograzers feeding (Hay 1996). Additionally, because these herbivores can function on small spatial scales, they may selectively attack different tissues on an individual plant. This could result in tissue-specific induction of either reduced palatability (this study) or secondary metabolites that are thought to have a defensive function (Hammerstrom et al. 1998).

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