Lack of short-term induction of phlorotannins in the Australasian brown algae *Ecklonia radiata* and *Sargassum vestitum*

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ABSTRACT: I measured changes in phlorotannin (polyphenolic) levels in the Australian brown sea-weeds *Ecklonia radiata* (Order Laminariales) and *Sargassum vestitum* (Fucales) following simulated herbivory. No significant changes in phlorotannin levels following damage were observed for either species over periods varying between 4 d and 8 wk. While there are few studies of inducible chemical defenses in seaweeds on which to base general conclusions, I suggest that because of predictable variation in the intensity of herbivory in benthic marine systems, inducible defenses in seaweeds may be relatively less important than persistent, constitutive defenses.

KEY WORDS: Inducible defenses · Phlorotannins · Brown algae · Marine herbivory

INTRODUCTION

Herbivores have a major impact on plants in both ter-restrial and marine systems (Lubchenco & Gaines 1981, Crawley 1983, John et al. 1992). One of the ways plants minimize the impact of herbivory is by the production of toxic, inhibitory, or deterrent compounds which function as chemical defenses against herbivores (Rosenthal & Janzen 1979, Hay & Fenical 1988, Paul 1992, Rosenthal & Berenbaum 1992). Chemical defenses in plants can be categorized by a number of criteria, but one important distinction between different kinds of defenses is the lability of their production (e.g. Rhoades 1983). 'Consti-tutive defenses' are maintained in the plant over time at some high level (with respect to relevant herbivores) and do not change in response to increases in herbivory in the short to medium term. Conversely, metabolites which function as 'inducible' defenses (Karban & Myers 1989, Raupp & Tallamy 1990) change (qualitatively or quantitatively) in response to herbivory on the plant, and subsequently decrease herbivore preference or performance while increasing the fitness of the plant (Karban & Myers 1989). Inducible responses to her-bivory may occur in a matter of hours or less (Carroll & Hoffman 1980), or be longer term (Haukioja et al. 1985).

Inducible responses to herbivores are well known in terrestrial plants for a variety of compounds and taxa (Karban & Myers 1989, Raupp & Tallamy 1990), as are inducible morphological defenses in aquatic inverte-brates (Adler & Harvell 1990). However, inducible chemical responses in marine macroalgae are known for only 1 genus, *Fucus* (Van Alstyne 1988, Yates & Peckol 1993), and for only 1 group of compounds, phlorotannins (polyphenolics). Because predictions about when plants should invest in inducible vs consti-tutive defenses are a crucial component of modern the-ories for the evolution of defenses against consumers (Rhoades 1983, Adler & Harvell 1990), the generality of different defense mechanisms in seaweeds is impor-tant for an understanding of the applicability of these models.

Possible induction of chemical defenses in seaweeds is also particularly relevant for phlorotannins in tem-perate Australasian brown algae because herbivores in these systems show a high degree of tolerance to the levels of phlorotannins typically present in the plants (Steinberg & van Altena 1992). However, Australasian algae which are damaged by herbivores may respond by further increasing their levels of phlorotannins, which may then deter herbivory. Here I describe ex-

periments in which I simulated herbivory on the brown seaweeds *Ecklonia radiata* and *Sargassum vestitum*, and measured subsequent changes in concentrations of phlorotannins in the plants.

MATERIALS AND METHODS

Study site and organisms. All experiments were done in the sublittoral zone at the Cape Banks Marine Research Reserve near Sydney, Australia. The sublittoral seaweed flora at Cape Banks, as elsewhere in temperate Australasia (Kirkman 1984), is dominated by the kelp *Ecklonia radiata* (Andrew & Jones 1990). Several species in the genus *Sargassum* (Order Fucales) are also common, including *S. vestitum*.

Sites for the experiments at Cape Banks were chosen based on the rarity of herbivores, in order to minimize the possibility of previous induction of phlorotannins in the experimental plants. For *Ecklonia radiata*, the site chosen was at 4 m depth, and lacked both *Odax cyanomelas*, an herbivorous fish which feeds exclusively on *E. radiata* (Andrew & Jones 1990), and *Holopneustes purpurescens*, an echinoid which lives on and consumes *E. radiata* (Steinberg unpubl.). Less is known about herbivory on *Sargassum vestitum*, but the site for this species (depth = 5 m) was selected based on a subjective impression of the rarity of semicircular fish-bite marks on the vegetative blades of *S. vestitum* plants at the site, and likewise a rarity of surface grazing marks typically associated with amphipods, molluscs, or other small grazers.

Clipping experiments. Damage by herbivores was simulated by clipping plants with scissors. Experiments with *Sargassum vestitum* were done in autumn (April 25 to June 7, 1987) and spring (September 21 to November 16, 1987); experiments with *Ecklonia radiata* were done in autumn (April 3 to 19) only. Individual plants of *E. radiata* and *S. vestitum* were tagged with Dymo tape and cable ties and randomly assigned to 3 treatments consisting of different levels of clipping: 0 (control), 10, and 25 % of thallus tissue removed. These treatments were accomplished as follows:

For *Sargassum vestitum*, the number of vegetative blades (or secondary axes near the distal portion of the plant where the vegetative blades become small) was counted, and half the tissue clipped from 0, 20, or 50 % of the blades; this corresponded to 0, 10, and 25 % tissue removal, respectively. A similar procedure was followed for *Ecklonia radiata*. The number of secondary laminae was counted, after which plants were treated by one of the following: (a) no tissue was removed (0 % treatment); (b) the distal halves of 20 % of the laminae were clipped off (10 % tissue removed); or (c) the distal halves of 50 % of the laminae on a plant

were removed (25 % tissue removed). Note that these treatments for *E. radiata* refer to the amount of secondary laminae removed, which represent the bulk of the biomass of the plant. However, the total fraction of the alga's biomass removed was less than the nominal treatment values, because stipes and primary laminae were not damaged.
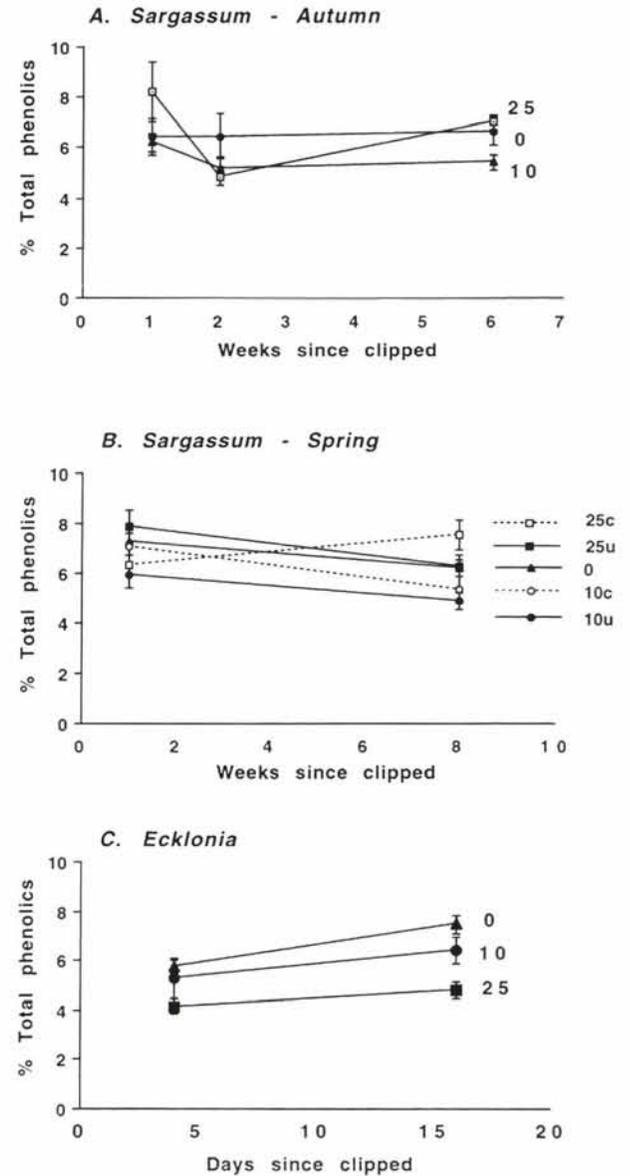

Fig. 1. *Sargassum vestitum, Ecklonia radiata.* Total phenolic (phlorotannin) levels following clipping in (A) *S. vestitum* in autumn, (B) *S. vestitum* in spring, and (C) *E. radiata.* 0, 10, and 25 indicate % tissue removed for plants in a given treatment. For clipped *S. vestitum* plants in spring, phenolic levels were measured for both clipped (10c, 25c) and unclipped (10u, 25u) blades from each clipped plant (see 'Methods'). N = 8 (4 plants × 2 duplicate samples per plant) for each combination of Clipping × Time

Algae were harvested at between 4 d and 8 wk after clipping, with the frequency and timing of sampling varying somewhat between experiments (Fig. 1), and their phlorotannin content measured by the Folin-Denis technique (Ragan & Jensen 1977, Steinberg 1985, 1989). At each harvest, 2 duplicate samples from each of 4 plants from each treatment were analyzed (i.e. N = 8 samples for each treatment at each time). For the autumn *Sargassum vestitum* experiment, and the *Ecklonia radiata* experiment, all tissue samples were from undamaged blades or laminae. For plants in the clipped treatments in the spring *S. vestitum* experiment, both clipped and unclipped blades (taken from separate clipped plants at each harvest) were analyzed. Thus in this experiment at each date there were 5 treatments; unclipped plants (0), and clipped (10c, 25c) and unclipped (10u, 25u) blades from clipped plants.

All experiments were analyzed by 3-factor ANOVA with Clipping, and Time since clipped, as fixed orthogonal factors, and replicate Plants nested within each combination of Clipping × Time. Phlorotannin levels (as % dry weight) were transformed by arcsin $\sqrt{p}$ to ensure normality and to homogenize variances.

RESULTS AND DISCUSSION

Changes in phlorotannin levels following clipping are shown in Fig. 1 and the results of analyses of variance for these data are shown in Table 1. The factor Clipping and its interaction with the factor Time were not significant in any of the 3 experiments, indicating that there was no significant induction of increased phlorotannin levels in response to clipping. The effect of clipping on *Ecklonia radiata* was marginal (p = 0.094), but in this instance there was a *decrease* in phlorotannins levels in clipped plants relative to unclipped ones.

The duration and timing of these experiments, the methodology, and the number of plants used (24 to 40 per experiment) are all similar to previous experiments demonstrating induction of phlorotannins in *Fucus* spp. (Van Alstyne 1988, Yates & Peckol 1993). Thus *Ecklonia radiata* and *Sargassum vestitum* appear to be less susceptible to the induction of phlorotannins than are species of *Fucus*.

The generality of inducible defenses in macroalgae is an important issue for our understanding of the ecology and evolution of algal/herbivore interactions. Most research to date has focused on inducible changes of phlorotannins in brown algae. Van Alstyne (1988) and Yates & Peckol (1993) demonstrated induction of increased levels of phlorotannins in *Fucus* spp. following simulated herbivory, although in both cases the effect only occurred at some times or places. Pfister (1992), while measuring the cost of reproduction in the kelp *Alaria nana*, also measured phlorotannin levels in plants which were undamaged, or had had 30 to 40 % of either reproductive (sporophylls) or vegetative tissue removed. She found no differences in phlorotannin levels in either sporophylls or vegetative tissue as a function of damage (Fig. 3 in Pfister 1992). Steinberg (unpubl.) caged different densities of the canopy-dwelling echinoid *Holopneustes purpurescens* with individual *Ecklonia radiata* plants and measured changes in phlorotannin levels after 1 mo. There was no correlation, in either autumn or spring, between damage suffered by individual plants and changes in phlorotannin levels. This last study was the only one of those described above in which the damage caused to the plants was due to a natural herbivore of the algae (simulated herbivory may not always mimic the effects of actual herbivores; e.g. Baldwin 1990, Renaud et al. 1990).

Even less is known about the induction of chemical defenses in other (non-brown) seaweeds, or about compounds other than phlorotannins. Paul (1992), in her review on chemical defenses in tropical seaweeds, mentions that she has found little evidence for induction (as opposed to 'activation') of defenses in the tropical green algal genera *Halimeda*, *Udotea*, and

Table 1. *Sargassum vestitum, Ecklonia radiata*. Analyses of variance for algal clipping experiments. All analyses done on % dry mass phlorotannin content, transformed by arcsin $\sqrt{p}$

			Sargassum vestitum								*Ecklonia radiata*		
		Autumn				Spring							
Factor	df	MS	F	p	df	MS	F	p		df	MS	F	p
Time	2	53.72	5.67	0.009	1	20.81	4.17	0.050		1	14.64	2.56	0.127
Clipping	2	10.63	2.24	0.125	4	8.95	1.79	0.156		2	14.58	2.71	0.094
Time × Clipping	4	8.95	1.89	0.141	4	6.94	1.39	0.261		2	0.23	0.07	0.932
Plant (Time × Clip.)	27	4.74	2.63	0.004	30	4.99	1.60	0.083		18	3.32	2.97	0.007
Error	36	1.80			40	3.12				24	1.12		
Total	71				79					47			

Caulerpa. Renaud et al. (1990) observed changes in the palatability of *Padina gymnospora* following damage by the echinoid *Arbacia punctulata*; however, any possible chemical basis of such changes was not identified.

The strength of selection on plants for constitutive vs inducible defenses is affected by many factors (Adler & Harvell 1990), but should strongly depend on (a) the timing, distribution and intensity of herbivory and (b) the sensitivity of herbivores to the induced compounds. Several authors (Hay 1984, 1985, Estes & Steinberg 1988, Hay & Steinberg 1992) have suggested that spatial variation — at a number of scales — in the intensity of herbivory in benthic algal communities is often highly predictable, and moreover that this variation is strongly correlated with variation in the frequency of algae which are rich in secondary metabolites. Such a pattern suggests that there has been long-term selection for constitutive defenses in macroalgae, and this may lessen the relative importance of inducible defenses in these systems. Indeed, in some tropical communities where the intensity of herbivory is very high (Hay 1992), unless the response time for induction is very short, by the time inducible defenses are mobilized the alga is likely to have been eaten. An alternative and perhaps more effective mechanism for deterring herbivores in such systems is the 'activated' defenses of *Halimeda* spp. (Paul & van Alstyne 1992), in which damage to the plant results in essentially instantaneous changes to secondary metabolites via enzymatic action.

Herbivores are notorious for adapting to or tolerating a wide variety of secondary metabolites, and presumably plants should not be selected to increase levels of compounds in response to damage caused by adapted herbivores. This is particularly relevant for temperate Australasia, because a number (though not all; Steinberg & van Altena 1992, Steinberg unpubl.) of Australasian herbivores are tolerant of high levels of phlorotannins in their diet, including those from *Ecklonia radiata* and *Sargassum vestitum*. Thus induction of phlorotannins in these algae may not be advantageous because increases in phlorotannins simply do not increase the seaweed's resistance to most herbivores. However, Australasian brown algae consistently maintain quite high levels of phlorotannins (Steinberg 1989), and such reasoning should also apply to the production of high levels of metabolites as constitutive defenses. Possible reasons for the production of high levels of phlorotannins by Australasian algae in systems where many herbivores are tolerant to these compounds are discussed by Steinberg (1992) and Steinberg & van Altena (1992).

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