Extrinsic factors influencing phlorotannin production in the brown alga *Ascophyllum nodosum*

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ABSTRACT: Many brown seaweeds contain high concentrations of phlorotannins (polyphenolics) that show extensive phenotypic variation, which can be generated by variation in a number of biotic and abiotic extrinsic factors. Extrinsic induced changes in the phlorotannin content of brown macroalgae can have significant consequences for secondary production in nearshore marine communities since they will affect the palatability and degradability of a dominating type of macroalgal tissue. In the present study the separate and interactive effects of natural herbivory and 3 abiotic factors on phlorotannin production in the brown alga *Ascophyllum nodosum* were tested in 2 different induction experiments. In the first experiment the hypothesis that grazing by the gastropod *Littorina obtusata*, nitrogen enrichment, and enhanced or ambient UV-B radiation (UVBR) can affect the phlorotannin content of *A. nodosum* was tested, and in the second experiment the effects of ambient UVBR and a variable water level (desiccation) were examined. In the first experiment it was found that 3 wk of grazing by *L. obtusata* caused a significant increase in the phlorotannin content of the algae and this effect was independent of the nitrogen and UVBR treatments which had no effects on phlorotannin levels. This is one of few demonstrated examples of induced chemical responses in seaweeds caused by natural herbivory. Measurements of biomass changes and tissue nitrogen content of the experimental algae showed that the nitrogen uptake of the algae was unaffected by herbivory and UVBR, and that the growth of *A. nodosum* plants was nitrogen-limited but not affected by UVBR. In the second experiment there was a significantly higher phlorotannin content in algae exposed to natural UVBR, compared to no UVBR, after 7 wk, but not after 2 or 4 wk. Furthermore, there was a significant interaction effect between the UVBR and the water level treatment. The phlorotannin content was higher in algae exposed to a variable water regime compared to continuously submerged algae when UVBR was filtered out, but not under ambient UVBR. In summary, the results of this study support the induced defence model (IDM), but not the carbon-nutrient balance model (CNBM), in explaining intraspecific variation in the phlorotannin content of *A. nodosum*. The results also support the previously proposed hypothesis that phlorotannins play a role as inducible screens against harmful UV radiation.

KEY WORDS: Brown algae · *Ascophyllum* · Herbivory · Inducible chemical defence · *Littorina obtusata* · Nitrogen · Phlorotannins · Polyphenolics · UV-B radiation

INTRODUCTION

Production of secondary metabolites in plants is generally a plastic trait that can be influenced by a number of biotic and abiotic extrinsic factors (Waterman & Mole 1989). Spatial and temporal variation in the

intensity of these extrinsic factors most likely causes much of the observed intraspecific phenotypic variation in secondary metabolite content in natural populations of both terrestrial plants (Bazzaz et al. 1987, Herms & Mattson 1992, Waterman & Mole 1994, Karban & Baldwin 1997) and marine macroalgae (Hay & Fenical 1992, Targett et al. 1992). Many brown macroalgae contain substantial concentrations of polyphenolic secondary metabolites known as phlorotannins

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(polyphloroglucinol phenolics). The phlorotannin content of brown seaweeds can show extensive intraspecific variation (see reviews by Ragan & Glombitza 1986, Steinberg 1992, Targett & Arnold 1998) which may be generated by variation in extrinsic factors such as nitrogen availability (Yates & Peckol 1993, Arnold et al. 1995, Peckol et al. 1996), solar radiation (H. Pavia unpubl.), ultraviolet-B radiation (UVBR; 290 to 320 nm) (Pavia et al. 1997), and physical damage (Van Alstyne 1988, Yates & Peckol 1993, Hammerstrom et al. 1998). Phlorotannins have been shown to deter herbivory by fishes, urchins and mesoherbivores, although their effectiveness as herbivore deterrents is highly variable (see Hay & Steinberg 1992, Targett & Arnold 1998 for reviews). Changes in phlorotannin production caused by extrinsic factors may consequently affect marine plant-animal interactions by changing the susceptibility of brown seaweeds to herbivory (e.g. Cronin & Hay 1996). Extrinsically induced alterations of phlorotannin levels may also have significant consequences for marine communities in coastal waters, since brown macroalgae are important sources of detritus in these habitats and changes in the phlorotannin content can strongly affect the quality of the algal detritus as a food source for detritus feeders (Duggins & Eckman 1997).

In natural populations of brown algae, phlorotannin production is likely to be affected by complex interactions between several biotic and abiotic factors. Experimental tests of the occurrence and magnitude of such interactions require that treatments be applied simultaneously in factorial combinations, something which has only rarely been done in studies on phlorotannin production (see Cronin & Hay 1996, Peckol et al. 1996, Pavia et al. 1997). In the only example, as far as we know, demonstrating interactive effects of extrinsic factors on phlorotannin production, *Fucus vesiculosus* plants were exposed simultaneously to simulated herbivory (clipping) and manipulations of nitrogen availability (Peckol et al. 1996). The results showed that both clipping and nitrogen availability affected phlorotannin production and that the effect of clipping was, at times, dependent on nitrogen availability, in support of both the induced defence model (IDM) (e.g. Karban & Myers 1989, Harvell 1990) and the carbon-nutrient balance model (CNBM) (Bryant et al. 1983, 1989), 2 of the dominant models for explaining intraspecific phenotypic variation in secondary metabolites of plants. The IDM is based on the assumption that defences are costly, and predicts that plants should allocate more resources to the production of costly defences when herbivores are present and active. The CNBM focuses on resource availability rather than herbivore activity, and predicts that the production of carbon-based secondary metabolites such as tannins will be governed by the relative supply of carbon (through photosynthe-

sis) and essential nutrients, e.g. nitrogen. Although the IDM and the CNBM differ in some of their basic assumptions and give alternative explanations for phenotypic variation in secondary metabolites of plants, they are not mutually exclusive models (Berenbaum 1995). Evaluations of their relative importance require factorial experiments where both herbivory intensity and resource availability are manipulated simultaneously (see Peckol et al. 1996).

In the present study we tested for the separate and interactive effects of extrinsic factors on phlorotannin production in the brown alga *Ascophyllum nodosum* (L.) Le Jol. on the Swedish west coast. Previous studies in the study area have shown that *A. nodosum* contains high concentrations (4 to 15% of dry weight [DW]) of phlorotannins with extensive variation among individual plants at different spatial scales and among years (Pavia & Åberg 1996, Pavia et al. 1999b). Two factorial experiments were done to examine the effects of 4 different extrinsic factors on the phlorotannin content of *A. nodosum*. In the first experiment we tested the hypothesis that natural grazing by the gastropod *Littorina obtusata* (L.), nitrogen availability and UVBR can, separately or interactively, induce changes in the phlorotannin content of *A. nodosum*. We used natural grazers rather than herbivory simulations, since clipping damages have previously failed to induce phlorotannin responses in *A. nodosum* (Lowell et al. 1991, Pavia et al. 1997). Moreover, studies on vascular plants have sometimes shown that mechanical simulations of herbivory through clipping do not adequately mimic natural herbivory, with regard to induced changes in secondary metabolism (see reviews by Baldwin 1990, Karban & Baldwin 1997). Nitrogen availability was chosen as a potentially interesting extrinsic factor, since nitrogen is commonly the most limiting nutrient for the growth of marine macroalgae (Chapman & Craigie 1977, Hanisak 1983, Lobban & Harrison 1994), and the CNBM would thus predict that the concentration of phlorotannins would decrease with increasing nitrogen availability. Furthermore, nitrogen enrichment experiments with other brown algae have revealed that nitrogen availability can affect phlorotannin production (Yates & Peckol 1993, Arnold et al. 1995, Peckol et al. 1996, but see Cronin & Hay 1996). The UVBR treatment was included because addition of UVBR has previously been shown to induce increased phlorotannin levels in *A. nodosum* (Pavia et al. 1997). In the second experiment we tested the hypothesis that the 2 factors, UVBR and water level (desiccation), can, separately or interactively, induce changes in the phlorotannin content of *A. nodosum*. Desiccation stress has been shown to influence production of other defence chemicals in brown seaweeds (Renaud et al. 1990, Cronin & Hay 1996), but the effect of desiccation,

or its interactive effects with ambient UV light, on the production of phlorotannins has not been previously explicitly tested. We hypothesized that UV light and desiccation would be likely to affect phlorotannin production of the algae interactively because algae that are periodically air-exposed, and not continuously submerged in UV-absorbing seawater, are exposed to higher levels of UV radiation.

MATERIAL AND METHODS

Study site and organisms. The experiments were carried out in 1996 and 1997 at Tjärnö Marine Biological Laboratory on the Swedish west coast. Field collections of algae and herbivores were done in *Ascophyllum nodosum* populations on several small islands in the archipelago around the field station. *A. nodosum* is a long-lived seaweed that grows on sheltered rocky shores in the intertidal zone on the coasts of the Northern Atlantic. At shores with large tidal amplitudes, *A. nodosum* plants can form large, almost monospecific stands. Because of the small tidal range (≤ 0.3 m) at the Swedish west coast, the vertical distribution of *A. nodosum* is limited and the width of the *A. nodosum* zone is 3 to 4 m max. and usually < 1 m. However, the abundance and population structure of *A. nodosum* within this narrow zone is similar to what has been observed on coasts with larger tidal amplitudes (Åberg & Pavia 1997). *A. nodosum* plants in the study area are inhabited by several small grazers, so-called meso-herbivores, mainly gastropods, isopods and amphipods (Pavia et al. 1999a). The most abundant gastropod on *A. nodosum* is the littorinid *Littorina obtusata* (Pavia et al. 1999a). Together with its sibling species *L. fabalis*, which feed on microepiphytes on the macroalgal thalli, *L. obtusata* is the only littorinid species that is specialized to live on fucoids (Vermeij 1992, McQuaid 1996), and it prefers to feed on fucoids (Watson & Norton 1987), despite their high phlorotannin content. Unlike rock-dwelling littorinid species, the radula teeth of *L. obtusata* have shovel-like cusps (Steneck & Watling 1982, Watson & Norton 1987), well adapted for excavating into the relatively tough thallus of *A. nodosum* and other fucoids, resulting in round holes through the algal fronds (Viejo & Arrontes 1992, H. Pavia pers. obs.).

Expt 1. For the first induction experiment, 48 similar-sized (≈ 50 g wet weight [WW]) *Ascophyllum nodosum* plants without visible grazing or other tissue damages were collected in July 1996 and brought to the laboratory at the starting day of the experiment. The experiment was run in an outdoor aquarium system consisting of 48 aquaria ($0.3 \times 0.3 \times 0.5$ m) made out of UVBR transparent Plexiglas (Esså Plast AB, Göteborg, Plexi GS OF 2458 UV, 5 mm). The aquaria were placed in a

cooling basin ($6 \times 2.5 \times 0.5$ m) and filled with filtered (cotton cartridge filters, 100 and 5 μ m) surface seawater which was exchanged every morning. After weighing, the *A. nodosum* plants were randomly and separately placed in the aquaria and anchored at the bottom to wooden pegs fastened in cement weights. Each alga was exposed to one of 12 factorial combinations of treatments (including controls), i.e. there were 4 replicate aquaria for each treatment combination. For the herbivory treatment 20 *Littorina obtusata* snails were placed in each of 24 aquaria. The UVBR treatment included both addition and filtering of UVBR, as well as ambient UVBR from solar radiation. To screen out UVBR, 16 of the aquaria were covered with Mylar film (Dupont, Mylar D). The Mylar film has a sharp wavelength cut-off with 50% transmittance at 320 nm and $\approx 90\%$ transmittance in the photosynthetically active radiation (PAR) range (Wängberg et al. 1996). Each of the 16 aquaria with enhanced UVBR were equipped with a battery-powered UVBR fluorescent tube (Philips TL 4W/12). To filter out wavelengths < 295 nm, the fluorescent tubes were covered with cellulose diacetate films (Erik S. Ekman AB, Stockholm) which were exchanged every 5 d. The tubes were mounted at a distance of 10 cm from the water surface and turned on between 11:00 and 13:00 h each day. The addition of UVBR was about 0.2 W m^{-2} at the water surface, while the addition of PAR and UV-A radiation from the fluorescent tubes was negligible. Light measurements were made with an International Light 1400A photometer equipped with cosine corrected sensors (detector types SUL 033 and SUL 240). The readouts were corrected for the spectral sensitivity of the sensors and the solar and UVBR fluorescent tube spectra. Correction factors were empirically determined from simultaneous measurements with the IL 1400A and an Optitronics OL 752 radiometer. The weather was relatively stable and sunny (except for 2 d) during the 3 wk experiment and average ambient UVBR between 11:00 and 13:00 h was $\sim 0.8 \text{ W m}^{-2}$ and PAR $\sim 1200 \mu\text{E m}^{-2} \text{ s}^{-1}$. For the nitrogen availability treatment, a spike of nitrogen, in the form of ammoniumnitrate, was given to the algae in 24 of the aquaria after the exchange of water each morning. The daily dose of ammoniumnitrate was chosen to increase the nitrogen concentration by 20 μM in the newly filled aquaria, which is about equal to the maximum levels of nitrogen found in the seawater around Tjärnö (B. Rex unpubl.). Nitrogen concentrations in the seawater of the aquaria were measured on 3 random days during the experiment. Water samples were taken at 3 different times: before nitrogen addition (natural background levels), and 2 h and 6 h after the nitrogen addition from 3 of the 12 treatment combinations: control aquaria (without herbivores or manipulations of nitro-

gen or UVBR), aquaria with enhanced nitrogen (without herbivores or UVBR manipulations) and aquaria with herbivores (without manipulations of nitrogen or UVBR). Samples were taken from the aquaria with *L. obtusata* to test for possible effects on nitrogen levels caused by the newly produced faeces of the gastropods (faeces were rinsed out every morning when water was exchanged). Ammonium and nitrate concentrations were analyzed on the day of sampling according to the method described in Carlberg (1972).

The experiment was run for 21 d, after which the algae were collected and their WW changes were recorded. Tissue samples (apical shoots) for phlorotannin analyses were immediately frozen at -80°C , while samples for nitrogen analyses were weighed, dried for 48 h at 60°C , and weighed again to determine individual DW to WW ratios. For phlorotannin analyses the frozen samples were ground to a fine powder with liquid nitrogen in a mortar, placed in aqueous acetone (60%) and shaken under nitrogen in the dark at 4°C for 24 h. After extraction the samples were centrifuged, evaporated in vacuo at less than 40°C to a small aqueous volume, filtered to remove precipitated lipophilic material, and diluted to a known volume. Phlorotannins were quantified colourimetrically using the Folin-Ciocalteus method (Waterman & Mole 1994, Van Alstyne 1995). Phloroglucinol (1,3,5-trihydroxybenzene, Merck art. 7069) was used as a standard. Samples for tissue nitrogen content were ground with a ball mill and analyzed in an elemental analyzer (FISONS Instruments NA 1500 NC).

Expt 2. For the second induction experiment 36 healthy *Ascophyllum nodosum* plants of similar size (~ 50 g WW) were collected in May 1997 and handled the same as the algae in the first induction experiment. However, instead of daily exchange of seawater, the 36 Plexiglas aquaria were continuously supplied with the filtered surface seawater through a tube system. The algae were continuously submerged in 18 of the aquaria, while the water level was adjusted 3 times d^{-1} in the other 18 aquaria, in order to correspond to the water level in the surrounding sea. The small tidal range (≤ 0.3 m) on the Swedish west coast results in stochastic, weather-induced changes in the water level, which affect all organisms in the upper littoral zone (Johannesson 1989). *A. nodosum* and other fucoids can be in or out of the water for several consecutive days, depending on the prevailing barometric conditions. During high pressure in the summer months the fucoids could thus experience exposure to solar radiation and extreme desiccation during periods of several days, which can lead to severe tissue damages on the algal thalli (H. Pavia pers. obs.). Algae exposed to a naturally varying water level during the 7 wk experiment were submerged 31 d and in the air for a total of

18 d, with no single period of desiccation lasting more than 48 h. The weather was clear and sunny on 26 out of 49 d. To screen out ambient UVBR, 18 of the aquaria were covered with Mylar films which were exchanged every 5 d. Samples for phlorotannin analyses were collected after 2, 4 and 7 wk, with one-third of the aquaria being sampled each time to obtain independent data for each sampling time. The tissue samples (apical shoots) were freeze-dried, ground to a fine powder in a mortar, extracted and analyzed using the Folin-Ciocalteus method as described above. WW changes were determined for the 12 algae remaining on the last sampling date.

Statistical analyses. Data were analyzed using different models of analysis of variance (ANOVA). Prior to the statistical analyses, data were tested for homogeneity of variances with Cochran's *C*-test (Winer et al. 1991, Underwood 1997). Multiple mean comparisons were made with the Student-Newman-Keuls (SNK) procedure (Winer et al. 1991, Underwood 1997). In the first experiment, data on algal WW changes and phlorotannin and tissue nitrogen content were analyzed using 3-way ANOVAs with herbivory, UVBR and nitrogen as fixed, orthogonal factors. In the second experiment, data on phlorotannin content were analyzed using a 3-way ANOVA with UVBR, water level and time as fixed orthogonal factors. Data on WW changes at the last sampling time of the second experiment were analyzed by a 2-way ANOVA with UVBR and water level as fixed, orthogonal factors.

RESULTS

Expt 1

Nitrogen analyses of seawater in the aquaria showed low ambient nitrogen concentrations, with concentrations of nitrate $< 0.4\text{ }\mu\text{M}$ and of ammonium $< 1.6\text{ }\mu\text{M}$. There was no effect of the presence of *Littorina obtusata* on nitrogen levels in the aquaria (Fig. 1). The nitrate and ammonium concentrations in the aquaria where nitrogen had been added were still high ($\geq 10\text{ }\mu\text{M}$) 6 h after nitrogen addition, with a somewhat steeper reduction in ammonium concentrations over time (Fig. 1).

At the end of the experiment, there was a distinct difference in colour between plants with and without nitrogen addition. Algae from nitrogen-enriched aquaria had a more brownish colour compared to the brighter untreated algae. Tissue nitrogen analyses revealed that the tissue nitrogen concentration was significantly ($F_{1,36} = 228.9$, $p < 0.0001$) higher in nitrogen-enriched *Ascophyllum nodosum* plants, with levels about twice as high in plants given extra nitrogen

Fig. 1. Concentrations of (a) nitrate and (b) ammonium in the filtered seawater of experimental aquaria from 3 different treatment combinations: control aquaria (without herbivores or manipulations of nitrogen or UVBR), aquaria with enhanced nitrogen levels (without herbivores or UVBR manipulations) and aquaria with herbivores (without manipulations of nitrogen or UVBR). Samples from $t = 0$ were taken before the addition of ammoniumnitrate at a concentration of 20 μ M. Error bars are $\pm$ SE (n = 3)

(Fig. 2). There were no significant separate or interactive effects of herbivory or UVBR treatments on tissue nitrogen content, indicating that the nitrogen uptake of *A. nodosum* was not affected by these treatments.

Ascophyllum nodosum plants from aquaria with *Littorina obtusata* snails showed extensive damage, and on some plants the snails had eaten through the basal parts of the primary shoots, resulting in detachment of algal pieces of various sizes. The detached pieces were included in the WW determination of the algae at the end of the experiment in order to analyze WW changes. The ANOVA showed that there was a significant interactive effect between the nitrogen and herbivory treatment on algal WW changes ($F_{1,36} = 4.84$, $p = 0.034$), while the UVBR treatment caused no significant separate or interactive effects. The SNK test revealed that nitrogen addition had a significantly ($p < 0.05$) positive effect on algal growth in the absence of grazers. Furthermore, *A. nodosum* plants exposed to grazing had a significantly smaller increase in WW than algae without grazers, and this difference was significantly larger for nitrogen-enriched algae (Fig. 3).

Fig. 2. Tissue nitrogen content of *Ascophyllum nodosum* plants exposed to enriched and ambient nitrogen conditions. Error bars are $\pm$ SE (n = 24)

The ANOVA for data on phlorotannin content showed that *Ascophyllum nodosum* plants which had been grazed by *Littorina obtusata* had significantly ($F_{1,36} = 29.0$, $p < 0.001$) higher phlorotannin concentrations (Fig. 4). This effect was independent of the nitrogen and UVBR treatments, which had no significant separate or interactive effects on phlorotannin concentrations.

Expt 2

At the end of the 7 wk experiment the *Ascophyllum nodosum* plants had increased their WW by an average of 39% of the initial WW, with no significant separate or interactive effects of the UVBR or water level treatments on WW changes of the algae.

The 3-way ANOVA for phlorotannin concentration showed that there were significant interactive effects between the UVBR treatment and the factor time, and between the UVBR and the water level treatment (Table 1). SNK tests revealed that there were significantly ($p < 0.05$) higher phlorotannin levels in the

Fig. 3. Relative change in the wet weight of *Ascophyllum nodosum* plants exposed to enriched and ambient nitrogen conditions and to grazing by the gastropod *Littorina obtusata*. Error bars are $\pm$ SE (n = 12)

Fig. 4. Phlorotannin content of *Ascophyllum nodosum* plants following grazing by the gastropod *Littorina obtusata*. Error bars are +SE (n = 24)

Fig. 5. Phlorotannin content of *Ascophyllum nodosum* plants following 2, 4 and 7 wk of exposure to ambient and no (Mylar-filter) UV-B radiation (UVBR). Error bars are $\pm$ SE (n = 6)

Ascophyllum nodosum plants exposed to ambient UVBR after 7 wk, but not after 2 or 4 wk (Fig. 5). Furthermore, there was significantly lower phlorotannin concentration in algae exposed to variable water level when UVBR was screened out, but not under ambient UVBR (Fig. 6).

Table 1. ANOVA results for the phlorotannin concentration in *Ascophyllum nodosum* plants following 2, 4 and 7 wk of treatments with UV-B radiation (UVBR) filters/ambient UVBR and variable water level/continuously high water level

Factor	df	MS	F	p
UVBR	1	7.60	45.24	<0.01
Water level	1	0.89	5.31	<0.05
Time	2	1.63	9.70	<0.01
UVBR $\times$ Water level	1	0.94	5.60	<0.05
UVBR $\times$ Time	2	1.11	6.59	<0.01
Water level $\times$ Time	2	0.20	1.17	>0.25
UVBR $\times$ Water level $\times$ Time	2	0.02	0.12	>0.75
Residual	26	0.17

Fig. 6. Phlorotannin content of *Ascophyllum nodosum* plants exposed to ambient and no (Mylar-filter) UV-B radiation (UVBR) and to a variable and a continuously high water level. Error bars are +SE (n = 9)

DISCUSSION

The results of this study show that both biotic (natural herbivory) and abiotic (UVBR and variable water level) extrinsic factors can affect the phlorotannin content of *Ascophyllum nodosum*. Three wk of grazing by *Littorina obtusata* resulted in a mean phlorotannin concentration of 9.1% DW in grazed *A. nodosum* plants, compared to 7.0% DW for ungrazed algae, indicating that the IDM can be relevant for explaining intraspecific variation in the phlorotannin content of *A. nodosum*. This is 1 of few demonstrated examples of herbivory-induced production of secondary metabolites in marine algae (see also Van Alstyne 1988, Cronin & Hay 1996). The shortage of evidence for inducible chemical responses in marine algae compared to vascular plants (see Karban & Baldwin 1997) has been attributed to differences in herbivory, e.g. in the relative frequency of specialist and generalist herbivores, between marine and terrestrial habitats, and to structural and functional differences between seaweeds and vascular plants (see Hay & Steinberg 1992, Steinberg 1994, Cronin & Hay 1996 for further discussion). However, there are only a few studies that have evaluated inducible production of defence chemicals in marine macroalgae for a limited selection of taxa, and most of these studies have looked for effects of artificial clipping or grazing by large and mobile herbivores, i.e. fishes and large urchins. Hay (1996) suggested that it is most likely to be grazing by smaller, less mobile mesoherbivores (sensu Brawley & Fei 1987) that cue for chemical induction in marine algae. These herbivores are active over spatial and temporal scales that would allow changes in defence chemicals to become effective in reducing grazing damages, even if the induction would take days or weeks to be

manifested. The hypothesis of Hay (1996) is supported by results from experiments on the interactions between *Fucus distichus* and the gastropod *Littorina sitkana* (Van Alstyne 1988), between *Dictyota mensturalis* and the amphipod *Amphitoe longimana* (Cronin & Hay 1996, and between *A. nodosum* and *L. obtusata* (present study, Pavia & Toth in press)

In the first induction experiment we found no significant separate or interactive effects of enhanced or ambient UVBR on phlorotannin concentrations in *Ascophyllum nodosum*. In contrast with these results, Pavia et al. (1997) found a significant increase in the phlorotannin content of *A. nodosum* after 2 wk of exposure to increased UVBR. There are several factors that could possibly explain the difference between these 2 experiments in the chemical response of *A. nodosum* to UVBR treatments. The addition of UVBR irradiance in Pavia et al. (1997) was more than twice as intensive, and the daily exposure time about 3 times as long (6 vs 2 h), than in the present study. Furthermore, the experimental algae in Pavia et al. (1997) were placed in the sea with natural variation in water level and the experiment was conducted during another season (early spring instead of summer), with generally lower phlorotannin concentrations in the experimental algae (4.8 vs 7.0% DW). The first induction experiment of the present study was preceded by a period of several weeks with clear weather and many hours of sunlight during most of the days, and any light-induced production of phlorotannins in the algae could have been already activated before the start of the experiment. The second experiment of the present study started in early May and the algae had relatively low initial phlorotannin levels (~4.5% DW). After 2 and 4 wk the phlorotannin levels in *A. nodosum* plants exposed to ambient UVBR were only slightly and not significantly higher than in algae from aquaria with UVBR filters, but after 7 wk the difference was substantial (5.9 vs 4.5% DW) and statistically significant. This result gives further support to the hypothesis of Pavia et al. (1997) (see also Berthold 1882) that phlorotannins may function as inducible screens against UV radiation, even though their absorption maxima are at wavelengths (260 to 280 nm) somewhat shorter than incident UV radiation. Unfortunately, changes in phlorotannin levels in natural populations of *A. nodosum* were not monitored during the course of the second experiment, but data from other years in the study area imply that phlorotannin concentrations in *A. nodosum* are generally low (~4 to 5% DW) in April-May, and increase up to levels >8% in July-September, although with substantial small-scale spatial variation and moderate among-year variation (H. Pavia unpubl., see also Ragan & Jensen 1978 for data on seasonal variation in an adjacent area). This implies that the increased phlorotannin

content with time in algae exposed to ambient UVBR was more similar to natural changes than the lack of significant change found in algae from aquaria with UVBR filters. It is notable that the time required (4 to 7 wk) for a detectable difference in phlorotannin levels to be manifested when screening out 100% of UVBR was much longer than the time required (≤ 2 wk) to induce increased phlorotannin levels under enhanced (~50%) UVBR (Pavia et al. 1997). This relatively slow response may also explain the lack of effect on phlorotannin production in the UVBR-screening treatment of the first induction experiment, which only lasted for 3 wk.

In the second induction experiment there was a significant interactive effect between UVBR and water level treatments on the phlorotannin content of *Ascophyllum nodosum*, but the effect was different from what we had expected. We hypothesized that the effect of ambient UVBR on phlorotannin production should be more pronounced when the water level was variable and the algae were periodically out of UV-absorbing seawater. The results, however, showed that under ambient UVBR there was no difference in the phlorotannin content between algae exposed to a variable water level and algae which were continuously submerged in seawater. In contrast, there was a statistically significant, although relatively modest (4.6 vs 3.9% DW) difference when UVBR was filtered out, with higher phlorotannin content in *A. nodosum* plants exposed to a variable water level. We can only speculate about the causes of this difference, e.g. that algae under a variable water level regime had a slightly higher carbon:nutrient balance, due to reduced nutrient uptake, which could have resulted in a somewhat higher production of phlorotannins, in accordance with the CNBM. In algae exposed to UVBR, any such relatively subtle effect may have been masked by the need to produce UV-absorbing metabolites.

The herbivory-induced increase of phlorotannin levels in *Ascophyllum nodosum* was independent of nitrogen availability. In the absence of grazers there was also a lack of effect of nitrogen enrichment on phlorotannin production in *A. nodosum*, in contrast with the predictions of the CNBM and with the results of previous studies on other brown algal species showing that nitrogen availability can affect phlorotannin levels (Ilvessalo & Tuomi 1989, Yates & Peckol 1993, Arnold et al. 1995, Peckol et al. 1996, but see Cronin & Hay 1996). The results of the present study alone cannot justify the conclusion that nitrogen availability or the CNBM are irrelevant for explaining intraspecific variation in the phlorotannin content of *A. nodosum*, since the effects of nutrient enrichment on secondary metabolism could be dependent on other environmental factors not tested in the present study, and also on the

developmental stage of the plant (Yates & Peckol 1993, Peckol et al. 1996). Given the pronounced seasonality of environmental conditions in the study area, the result might have been different if the induction experiment would have been conducted in another season with different light, temperature and ambient nutrient conditions. The lack of any effect of nitrogen availability on phlorotannin production in *A. nodosum* is, however, also supported by the absence of a relationship between the concentrations of tissue nitrogen and phlorotannins in *A. nodosum* plants from the Tjörnö area in spring (Pavia et al. 1997). Furthermore, in a 2 yr correlative field study comprising chemical analyses of 300 *A. nodosum* plants from the Tjörnö area and from the Irish Sea, it was found that the relationship between tissue nitrogen and phlorotannins was weak and variable (Pavia et al. 1999b). Together, these results suggest that nitrogen availability is not a good predictor of the phlorotannin content of *A. nodosum*.

The measurements of algal WW changes during the experiments clearly showed that the growth of the *Ascophyllum nodosum* plants in the aquaria was nitrogen-limited, while there was no apparent effect of the UVBR or water level treatments on algal growth. The statistical analysis of data on WW changes in the first experiment also revealed a significant interaction effect between the herbivory and nitrogen treatments. The post hoc comparisons of mean values showed that in the absence of grazers, nitrogen-enriched *A. nodosum* plants grow substantially more than algae under ambient nitrogen levels, but in the presence of *Littorina obtusata* snails there was no difference in WW changes between different nitrogen treatments. This effect could reflect the fact that the feeding rate of *L. obtusata* snails were positively affected by increased tissue nitrogen content of *A. nodosum*. If so, it would imply that when herbivores are abundant, the effect of increased nutrient availability may not have a positive net effect for the growth of *A. nodosum* plants. There is, however, an alternative model to explain the observed interaction effect between the nitrogen and herbivory treatments on the WW changes of the *A. nodosum* plants. The IDM and other optimality models for the evolution of defensive characters are based on the assumption that these characters are costly to produce (e.g. Rhoades 1979, Fagerström et al. 1987, Simms & Rausher 1987). A few correlative studies provide support for the assumption that phlorotannin production is costly, in terms of individual growth, for brown algae (Yates & Peckol 1993, Steinberg 1995), including *A. nodosum* (Pavia et al. 1999b). Theory predicts that the cost of production of carbon-based defence chemicals, such as phlorotannins, will be relatively higher in nutrient-rich environments, since the excess of carbon that can be used for production of

defence chemicals without affecting growth processes is smaller or lacking (Herms & Mattson 1992). The results of the first induction experiment fit these theoretical predictions. Assuming that the amount of algal tissue consumed by *L. obtusata* was relatively small, the interactive effect between the herbivory and nitrogen treatments could reflect a higher cost, in terms of growth, for the herbivory-induced phlorotannin production in nitrogen-enriched algae, compared to algae under low nitrogen levels. Unfortunately, there is no rigorous way, *a posteriori*, of separating the relative importance of actual algal tissue consumption by the herbivores, and any trade-off between phlorotannin production and growth, for the observed pattern of algal biomass changes. This would require a measure of herbivore consumption that is independent of algal biomass changes in the experiment, e.g. the quantity of faeces produced or the amount of tissue damages on the algae. We made no rigorous estimations of the amount of grazing damages on the algae in the present experiment, but there was no apparent difference between the 2 nitrogen treatments. This visual observation is supported by the almost equal mean amounts of detached algal pieces from the grazed *A. nodosum* plants in aquaria with and without added nitrogen (8.8 vs 8.7 g WW per aquaria). A significantly larger proportion of detached algal parts would have been expected in the aquaria with enhanced nitrogen levels, if these algae were exposed to more intense grazing than algae which were not given additional nitrogen. These findings could thus imply that the interactive effect of herbivory and nitrogen treatments on algal WW changes is, at least partly, an indirect effect of the induced increase in phlorotannin production.

In summary, the results of the present study show that both biotic and abiotic factors can affect phlorotannin production in *Ascophyllum nodosum*, and they provide support to the IDM, but not to the CNBM, as a relevant model for explaining intraspecific variation in phlorotannin content in natural populations of *A. nodosum*. The results also give further support to the hypothesis of Pavia et al. (1997), that UV radiation can be an important factor in explaining phenotypic variation in phlorotannin content of natural populations of intertidal brown seaweeds. The observed differences in phlorotannin levels caused by gastropod grazing as well as UVBR are probably large enough to affect the feeding behavior of *Littorina* snails. Van Alstyne (1988) found that a ~20% increase in the phlorotannin content of *Fucus distichus* reduced grazing by *L. sitkana* by ~50%, and experiments with *A. nodosum* and *L. obtusata* showed that a 20 to 40% difference in phlorotannin concentrations of algal shoots significantly affected the feeding preferences of the gastropods (Pavia & Toth in press). From studies in terrestrial

communities, there is substantial evidence that extrinsic factors can influence production of secondary metabolites in vascular plants and that these changes can have important secondary consequences by affecting the palatability and degradability of plant tissue (see reviews by Waterman & Mole 1989, Rozema et al. 1997). In marine communities much less is known about the effect of extrinsic factors on secondary metabolism of seaweeds and its importance for the nearshore foodweb (Hay 1996). Brown algae, especially furoids, are good candidates for more studies on these issues since they are important components of coastal marine communities and they contain substantial and highly variable concentrations of phlorotannins.

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