Inducible resistance to herbivory in *Fucus vesiculosus*—duration, spreading and variation with nutrient availability

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ABSTRACT: Inducible resistance in plants is a defense strategy for avoiding the negative consequences of herbivory on plant fitness. Since resistance is costly, the induction of resistance may depend on resource availability. Resource-based plant–herbivore hypotheses predict that the cost of producing carbon-based defensive metabolites will be higher when an excess of nutrients is available for growth. We tested experimentally the effect of nutrient availability on the occurrence, duration and within-alga spread of inducible resistance in the brown alga *Fucus vesiculosus* L. Based on feeding preference bioassays using the herbivore *Idotea baltica* (Pallas), simulated grazing of *F. vesiculosus* caused a clear, rapid, induced resistance that disappeared after 10 to 38 d. Furthermore, the induction of resistance does not spread to neighboring fronds or to growing tips above the point of simulated grazing. Induced resistance against isopod grazing, however, was not explained by increased production of phlorotannins, despite their putative role in the defense against herbivory. Phlorotannin production responded most strongly to nutrient enhancement, which reduced the phlorotannin concentration of the alga. Nutrient enhancement, however, did not affect the induction of resistance. The occurrence of induced resistance together with the lack of correlation between the phlorotannin concentration and the food preferences of herbivores imply that *I. baltica* is not deterred by the total quantity of phlorotannins; there may, however, be other, as yet unknown, rapidly inducing substances in *F. vesiculosus* that are capable of functioning as feeding deterrents to isopod grazers. Resistance may also arise as a side-effect of substances with a functional role, for instance in wound-healing processes. The localized nature and short duration of such deterrence imply that it may be inefficient as a general defense against herbivory, but may benefit the alga by dispersing future damage within the individual and avoiding the breakage of whole fronds.

KEY WORDS: *Fucus vesiculosus* · Inducible resistance · Nutrients · Eutrophication · Herbivory · Chemical defense · Phlorotannins · Plant–herbivore interactions

INTRODUCTION

The production of secondary metabolites is often suggested to protect vascular plants and algae against herbivory. Chemical defenses occur on either a constitutive or an inducible basis (inducible defense hypothesis), their mutual profitability to the plant depending on a number of environmental, biochemical and life-history parameters (Karban & Myers 1989). Inducible defenses are considered more profitable than constitutive ones when a plant is relatively long-lived (Karban

& Baldwin 1997), the presence of herbivores is variable and unpredictable (Harvell 1990, Clark & Harvell 1992, Herms & Mattson 1992, Hay 1996), defensive secondary chemicals are costly to produce (Rhoades 1979), and defenses are quantitative (Karban et al. 1997). Attack by herbivores may induce resistance in the plant either quite rapidly (Carroll & Hoffman 1980) or after a delay, and the resistance may either fade quite rapidly or be sustained for a longer period of time (Haukioja 1982, Yates & Peckol 1993). Moreover, the costs of resistance for the plant may vary according to

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the availability of resources (Karban & Myers 1989, Herms & Mattson 1992).

The occurrence of chemical resistance induced by true or artificial herbivory has been widely studied and documented in a variety of terrestrial plants (reviewed in Karban & Baldwin 1997, Tollrian & Harvell 1999, Koricheva 2002). Despite its ecological relevance, however, only a few studies have explored the occurrence of induced chemical resistance in perennial seaweeds, and knowledge on the spread and duration of induced responses is almost lacking (but see Van Alstyne 1988, Peckol et al. 1996). The concentration of phlorotannins in brown seaweeds has been widely used to indicate the level of chemical resistance of a plant to herbivory. Phlorotannins are phenolic compounds specific to brown algae. In addition to their putative role in defenses against herbivory, other suggested ecological functions of phlorotannins include acting as a UV screen (Pavia et al. 1997) or as antimicrobial, antipathogenic or antifouling substances (Pawlik 1992, Schmitt et al. 1995, Targett & Arnold 1998). Van Alstyne (1988), for example, observed induction in phlorotannin production in *Fucus distichus* after simulated grazing. Yates & Peckol (1993) and Peckol et al. (1996) found some evidence of induced phlorotannin production in *F. vesiculosus* due to simulated grazing and Arnold et al. (2001) found evidence of a role of methyl jasmonate in inducing phlorotannin production in *F. vesiculosus*. Pavia & Toth (2000) showed that *Ascophyllum nodosum* responds to grazing by the gastropod *Littorina obtusata* by increasing its phlorotannin concentration and that increased phlorotannins provided resistance to further gastropod grazing. On the other hand, phlorotannins were not affected after grazing by the isopod *Idotea granulosa* or simulated herbivory, implying that the consequences of herbivory to induced responses are species-specific. Also, inducible responses to natural or simulated grazing have been found, e.g. in the non-fucacean brown algae *Dictyota menstrualis* (Cronin & Hay 1996a) and several kelp species (Hammerström et al. 1998). However, generalizations about the prevalence of induced responses, and especially about their defensive role, are difficult to make since inducible responses to natural or simulated grazing in brown algae have not always been found (see e.g. Lowell et al. 1991, Pfister 1992, Steinberg 1994, Pavia et al. 1997, Toth & Pavia 2000, 2002).

The variance in the occurrence of induced responses may be due to environmental variation. Resource-based theories on the allocation of resources in plants (the carbon-nutrient balance hypothesis and the growth-differentiation balance hypothesis; Bryant et al. 1983, Herms & Mattson 1992) predict that when external nutrient resources are abundant, primary allocation will be to growth at the expense of the production of carbon-based, quantitative chemical defenses. Conversely, when nutrient resources are scarce, growth will be suppressed and defense increased. The costs of allocating carbon to secondary metabolites are expected to increase with improving nutrient resources (Herms & Mattson 1992). The inducible allocation of resources to defensive substances after herbivore grazing may thus be reduced in conditions where ambient nutrient concentrations are elevated. Eutrophication in coastal waters, for instance, may alter resource allocation patterns in the algae, affecting plant–herbivore interactions. Although the covariation of phlorotannin production with resource availability has been shown in several studies (Ilvessalo & Tuomi 1989, Yates & Peckol 1993, Arnold et al. 1995, Steinberg 1995, Pavia et al. 1999, Pavia & Toth 2000), there is little information as to the effect of nutrient availability on the capability of algae to induce chemical responses to loss of tissue. Existing studies provide indirect evidence that resource availability may modify algal responses to herbivore grazing (Yates & Peckol 1993, Peckol et al. 1996).

When grazing of one frond increases the probability of the other fronds being grazed too, the ability to spread the induction of defenses should be beneficial and favored by selection. Given this, it would pay for the alga to spread the induction among adjacent fronds and among different tissue types of different fitness value, e.g. between non-growing older tissues and growing apical meristems. Although algae have a poorly developed vascular system, they may contain a series of tracheal cells forming a primitive transport system within the algal thallus (Moss 1983, Diouris & Floc'h 1984). To our knowledge, there are only 2 previous studies which have examined the spread of induction in brown algae. Van Alstyne (1988) concluded that *Fucus vesiculosus* is capable of increasing its phlorotannin concentration (i.e. resistance) after simulated grazing, but the evidence of the spread of induction remained uncertain. Toth & Pavia (2000) studied inducible responses in *Ascophyllum nodosum*, and suggested that water-borne cues due to herbivory may induce resistance even in ungrazed nearby individuals. Locally induced resistance may benefit the plant by dispersing future damage over a larger area within the plant individual. The total biomass loss due to herbivory would then be equal but the pattern of damage different (Marquis 1992), reducing the probability of losing whole fronds due to disintegration of the thallus.

To minimize the costs of maintaining resistance, the duration of induction will presumably be adjusted to the prevailing risk of herbivore attack. In general, rapidly occurring and fading resistance will be most effective against relatively mobile herbivores (e.g. fishes

or isopods), while long-lasting induction is likely to evolve in response to less mobile herbivores (e.g. gastropods) and when duration of the herbivory peak is prolonged (Karban & Baldwin 1997).

The present study is the first to observe experimentally the effect of resource availability on the occurrence, spread *and* duration of inducible resistance in *Fucus vesiculosus*. The following 5 questions are explored: (1) Are different parts of the alga equally able to induce resistance? (2) How rapidly does induction occur and how long does it last? (3) Does the induction spread within the algal individual? (4) Does the loss of biomass affect the subsequent growth of *F. vesiculosus*? (5) Does nutrient enhancement modify the occurrence, spread or duration of induced resistance?

MATERIALS AND METHODS

Study organisms. *Fucus vesiculosus* L. (Phaeophyceae: Fucales) is widely distributed along the western and eastern sides of the Atlantic Ocean (Lüning 1990). In our study area, the Archipelago Sea, SW Finland, it is the only perennial brown rockweed of the littoral community, forming dense vegetation belts in shallow, hard-bottom habitats. Specimens for the experiments were collected from a single growing site close to the Archipelago Research Institute of the University of Turku (60° 14′ N, 21° 40′ E) in June 1999.

Idotea baltica (Pallas; Isopoda; Crustacea) is a common generalist herbivore occurring in the littoral zone of the brackish Baltic Sea. After the juvenile stage its diet consists mainly of *Fucus vesiculosus* (Salemaa 1987, Schaffelke et al. 1995, Jormalainen et al. 2001a) and, to a lesser extent, of filamentous epiphytic algae. The presence of *I. baltica* in *F. vesiculosus* habitats is highly variable over the course of the year. Juveniles are released in July, and the highest herbivore density and grazing pressure is reached in August. The number of individuals declines gradually towards winter and spring, after which the over-wintered individuals start to reproduce.

Manipulation of *Fucus vesiculosus*. Partial removal of the algal thallus, mimicking loss of biomass due to herbivore grazing, was carried out by artificial clipping, after which the alga was reared in either control or nutrient-enhanced conditions. Each clipped algal part was left with a sufficient amount of leafy tissue around the midrib. From each algal individual, 24 vegetative tips were collected and randomly assigned to each treatment combination. The treatments included 2 kinds of algal parts—clipping and nutrient manipulation. Half (n = 12) of the tips were 'short tips' and half were 'long tips'. Half (n = 6) of both types of tips were clipped and the other half acted as a non-clipped con-

trol. The tips were then assigned to either ambient (n = 3) or enriched nutrient (n = 3) conditions. One of the 3 tips in each of these groups was used in herbivore preference tests 2, 10 and 38 d after clipping the parts used and the initial clipping patterns are shown in Fig. 1a. Part A was a small part with 2 unclipped apical fronds; Part B was similar to Part A, with 2 apical fronds but 1 of these was clipped from the sides of the thallus leaving meristematic tissue and midrib unclipped to allow further growth; Part C was a large part containing older unclipped thallus; and Part D was similar to Part C, but was clipped from the basal thallus around the midrib. Shortly after clipping, the algal parts were photographed, fastened to a clothes-pin (with all apical tips adjusted to the same depth of 15 cm to ensure equal light conditions for all the algae), and randomly assigned to either nutrient-enhanced or control pools. The 60 l flow-through pools (n = 16) were situated outdoors in natural light conditions. The water was changed continuously at a rate of 180 l d^{-1}.

Half the pools received 30 mg NPK fertilizer (Kemira Agro; N:P:K ratio 20:2:8) 3 times a day through automatic feeders to raise the ambient nutrient concentration. The ambient nutrient levels during the manipulation period were monitored with water samples taken randomly in relation to the timing of nutrient addition. Nutrients were analyzed by the standardized methods used by the Finnish National Board of Waters (Koroleff 1976). The nutrient concentrations (µg l^{-1}; mean ± SD, [n]) were as follows—total nitrogen: 225 ± 120 (2),
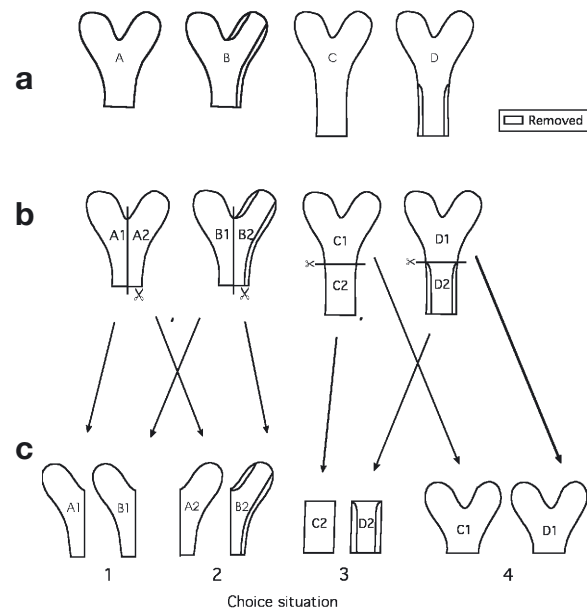

Fig. 1. *Fucus vesiculosus*. (a) Parts used in simulated grazing experiment; (b) division patterns of algal parts after manipulation periods of 2, 10 and 38 d, prior to feeding preference bioassays; (c) 4 different choice situations for feeding preference bioassays. Removed: parts clipped off fronds

404 ± 182 (8); ammonia: NH_4 1.0 ± 0 (2), 5.3 ± 3.4 (8); phosphate: PO_4 8.0 ± 2.8 (2), 21.1 ± 17.8 (8), in the control and nutrient-enhanced pools, respectively. The nutrient levels in the nutrient-addition treatment corresponded closely to the ambient levels observed along the eutrophicated littoral coasts of the study area.

Herbivore preference tests. After cultivation periods of 2, 10 and 38 d following simulated grazing, the algal parts were each cut in half (cutting patterns shown in Fig. 1b) for the feeding-preference bioassays. The bioassays were conducted under laboratory conditions to assess the preference of *Idotea baltica* for clipped or control algae reared in control and nutrient-enhanced environments. Because of sexual differences in the magnitude of feeding preferences (Jormalainen et al. 2001a,b), we used only male *I. baltica* in the feeding preference experiments. The isopods for the bioassays were collected from the same site as the alga.

The preference bioassays were conducted in 1 l glass containers, with one *Idotea baltica* individual able to choose between 2 algal pieces: 1 clipped or adjacent to a clipped specimen, the other unclipped as a control. Each trial lasted 5 d. We used 4 different pairs of algal pieces (hereafter 'choice situations') to investigate the occurrence and spreading of induced resistance within the alga. The different choice situations are shown in Fig. 1c. Thus inducible resistance was assumed to have occurred if Parts B2 and D2, in Choice Situations 2 and 3, respectively, were consumed less than Parts A2 and C2. Similarly, a spread of induction to the neighboring frond or from the basal thallus to the apices was assumed if Parts B1 and D1, in Choice Situations 1 and 4, respectively, were consumed less than Parts A1 and C1. In Choice Situations 1 and 4, in which the 2 algal pieces were otherwise indistinguishable, one randomly chosen piece was marked with a thin sewing thread. The algal pieces were digitally photographed at the beginning and end of the 5 d feeding trials, and the surface area consumed by the herbivore was calculated as the absolute area consumed (rather than for instance the final area of the pieces minus the initial area). An example of a pair of digital photographs of the algae at the beginning and end of the feeding preference bioassays is shown in Fig. 2.

In the feeding preference experiments, the algae reared in the nutrient-addition treatment received water slightly enriched with inorganic nutrients (20 µg l^{-1} nitrate NO_3-N, 60 µg l^{-1} ammonia NH_4-N and 15 µg l^{-1} phosphate PO_4-P) to prevent the algal quality from changing during the 5 d assay. Nutrient-enriched water does not directly affect *I. balthica*, as shown in Hemmi & Jormalainen (2002).

The test containers were kept at 12 to 13°C, under a 17:7 h light:dark regime and aeration.

Illumination was provided by four 400 W sodium vapor lamps (Sylvania) to enable algal photosynthesis during the feeding preference trials.

Chemical analyses and measurements of algal growth. The total content of phenolics (referred to in the following as phlorotannins [Ragan & Glombitza 1986]) in the algae was measured by a modification of the Folin-Ciocalteau technique (Nurmi et al. 1996). Phloroglucinol was used as a standard agent. For practical reasons, the phlorotannin determination was carried out on algal parts different than those used in the bioassays. The chemical analyses were run on freeze-dried and finely ground algal material stored at –20°C until determination.

To control the initial concentration of phlorotannins in all parts used in the bioassays, we measured the phlorotannin concentration immediately (within a few minutes) after simulated grazing by the algae, when no induction was assumed to have yet occurred. No initial differences were found between the 7 different parts of the algae (A1+A2 combined, B1, B2, C1, C2, D1, D2 in Fig. 1b; phlorotannin concentration: 9.38 ± 0.1% of dry matter, mean ± 1 SE, n = 43; $F_{6, 36}$ = 0.71, p = 0.65). Hence any subsequent differences between the parts must have been due to possible induction arising from simulated grazing and from the subsequent rearing conditions.

Algal growth was determined as the wet weight increment (final wet weight – initial wet weight) during the manipulation period of 38 d. Prior to weighing the algae, the epiphytes were removed and excess water dried off between paper tissues.

Statistical analyses. Induced resistance, in terms of feeding preferences after simulated grazing, was analyzed by repeated-measures ANOVA (unstructured covariance matrix based on Akaike's information criteria), with consumption of the clipped piece or its neighbor versus the control piece in the same container as the repeated measure. Since time from the simulated grazing affected the preferences, we ran separate analyses for the bioassays conducted 2, 10 and 38 d after simulated grazing. Also, we conducted
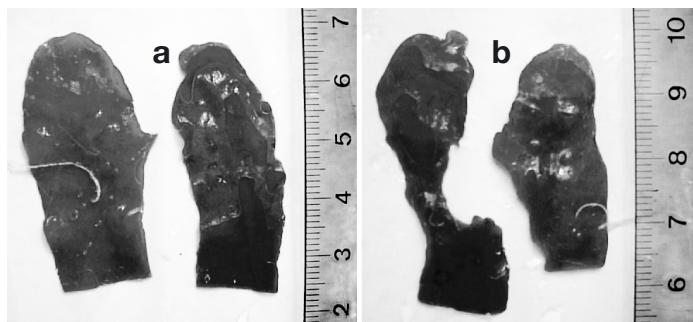

Fig. 2. *Fucus vesiculosus*. Digital photographs of the alga at (a) the beginning and (b) the end of the feeding preference bioassays

separate analyses to test the occurrence of induction in the clipped pieces (including Choice Situations 2 and 3 in Fig. 1c) and to test the spreading of induction within the alga (including Choice Situations 1 and 4 in Fig. 1c). Within each analysis, nutrient treatment and choice situation were used as grouping factors; thus their interaction with the repeated factor (preference) would indicate variation in induced resistance as a function of nutrient environment, or dissimilar occurrence in clipped and neighboring fronds. Interactions between nutrient treatment × choice situation × preference were not significant (p > 0.10) in any of the tests, nor did they influence the significance of the other factors; thus they could be excluded from the statistical models.

Differences in phlorotannin concentrations between parts of the alga and nutrient treatments were analyzed by 3-way ANOVA using simulated grazing, nutrient treatment and time as grouping factors. We conducted separate analyses for short and long tips. The factor 'time' and its interactions appeared to be significant in the analyses conducted with pooled data consisting of either the small or the large parts of the algae. We therefore ran separate 2-way ANOVAs again for the different time intervals.

Growth of *Fucus vesiculosus* was analyzed by 2-way ANCOVA, with data adjusted for the initial size of the algal part.

To ensure that the assumptions for ANOVA were fulfilled, Levene's test was used to test for homogeneity of variances and the Kolmogorov-Smirnov test to test for normality of the residual variation. All statistical analyses were performed using linear mixed models or general linear models (procedures MIXED and GLM in SAS 8.01 statistical software; SAS Institute 2000).

RESULTS

Herbivore preference

In the experiment conducted with the clipped parts (A2 vs B2, C2 vs D2 in Fig. 1c) 2 d after simulated grazing, the herbivores clearly preferred the parts without clipping: mean ± SE (n) consumption (cm^2) = 0.20 ± 0.04 (49) and 0.07 ± 0.02 (46) for control and clipped parts, respectively) (Table 1, Fig. 3a). Nutrient treatment did not affect the average consumption rate, but the apical parts in Choice Situation 2 were consumed significantly more than the basal parts in Choice Situation 3: 0.18 ± 0.03 (48) and 0.09 ± 0.03 (47), respectively (Table 1). There were no significant interactions between preference and either nutrient treatment or choice situation, indicating that induced resistance occurred in both nutrient environments and in both apical and basal parts (Table 1).

Induced resistance was still effective in the clipped parts 10 d after simulated grazing—0.35 ± 0.06 (53) and 0.18 ± 0.03 (55) for control and clipped parts, respectively—and apical parts were again consumed more than basal parts—0.37 ± 0.04 (54) and 0.16 ± 0.04 (54), respectively—(Table 1, Fig. 3a). In addition, there was a significant nutrient × choice situation interaction in the total consumption of the algal parts. In the control algae, apical (Choice Situation 2) and basal (Choice Situation 3) parts were consumed in equal amounts— cm^2, adjusted mean ± SE (n) = 0.30 ± 0.05 (28) and 0.21 ± 0.05 (27), respectively—while in the nutrient-treated algae the average consumption of apical parts was considerably higher than that of basal ones— 0.43 ± 0.05 (26), 0.12 ± 0.05 (27), respectively—(Table 1).
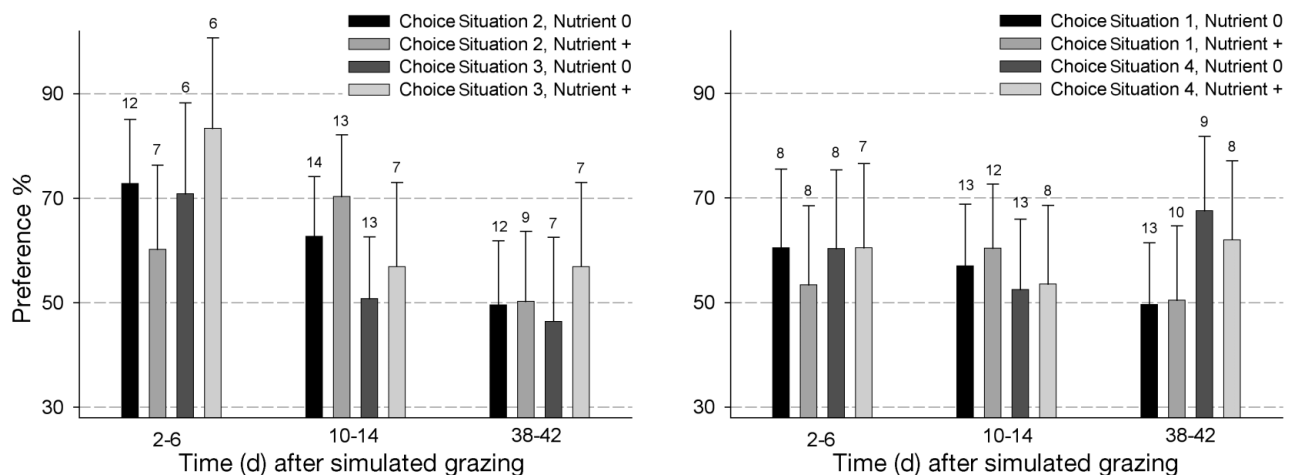

Fig. 3. *Idotea baltica* grazing on *Fucus vesiculosus*. Relative preference during 5 d bioassay (mean + SE; n above bars) showing control alga consumed as proportion of total consumption (consumption of control alga/[consumption of clipped + control algae] × 100) in each treatment combination. (a) Choice Situations 2 and 3 (control vs simulated grazing), (b) Choice Situations 1 and 4 (control vs fronds adjacent to parts subjected to simulated grazing). Value of 100% indicates complete preference for control pieces, i.e. highest possible inducible resistance, while value of 50% indicates complete absence of preference

Table 1. *Idotea baltica* grazing on *Fucus vesiculosus*. Statistical results of repeated-measures ANOVA testing herbivore food preferences between control and clipped alga and effects of nutrient enhancement and choice situation on average consumption and preferences. Choice situations were the choice between apical parts (Choice Situation 2) and basal parts (Choice Situation 3) (see Fig. 1c). Preference data are shown in Fig. 3a. Statistically significant results are in **bold**

Source	df	F	p
2 d			
Preference	1,49	7.62	**0.008**
Nutrient	1,49	2.35	0.132
Choice Situation	1,49	5.12	**0.028**
Preference × Nutrient	1,49	0.07	0.790
Preference × Choice Situation	1,49	0.35	0.559
Nutrient × Choice Situation	1,49	2.14	0.150
10 d			
Preference	1,52	5.38	**0.024**
Nutrient	1,52	0.23	0.634
Choice Situation	1,52	14.02	**<0.001**
Preference × Nutrient	1,52	0.86	0.359
Preference × Choice Situation	1,52	3.47	0.068
Nutrient × Choice Situation	1,52	5.64	**0.021**
38 d			
Preference	1,55	0.57	0.454
Nutrient	1,55	0.46	0.499
Choice Situation	1,55	0.80	0.376
Preference × Nutrient	1,55	0.25	0.618
Preference × Choice Situation	1,55	0.08	0.773
Nutrient × Choice Situation	1,55	0.44	0.511

Table 2. *Idotea baltica* grazing on *Fucus vesiculosus*. Statistical results of repeated-measures ANOVA testing herbivore food preferences between control algal parts and parts adjacent to clipped parts and effects of nutrient enhancement and choice situation on average consumption and preferences. Choice situations were choice between neighboring frond (Choice Situation 1) or between apical part above clipped part (Choice Situation 4) and control part (see Fig. 1c). Preference data are shown in Fig. 3b

Source	df	F	p
2 d			
Preference	1,48	3.53	0.066
Nutrient	1,48	0.02	0.881
Choice Situation	1,48	0.90	0.349
Preference × Nutrient	1,48	0.27	0.603
Preference × Choice Situation	1,48	0.08	0.779
Nutrient × Choice Situation	1,48	0.37	0.548
10 d			
Preference	1,52	2.18	0.146
Nutrient	1,52	1.39	0.244
Choice Situation	1,52	5.18	**0.027**
Preference × Nutrient	1,52	0.48	0.494
Preference × Choice Situation	1,52	0.01	0.932
Nutrient × Choice Situation	1,52	2.07	0.157
38 d			
Preference	1,54	1.06	0.309
Nutrient	1,54	0.42	0.520
Choice Situation	1,54	1.77	0.189
Preference × Nutrient	1,54	0.42	0.521
Preference × Choice Situation	1,54	0.51	0.479
Nutrient × Choice Situation	1,54	0.08	0.772

Later, 38 d after simulated grazing, there was no induced resistance or any other treatment effects or their interactions with preference (Table 1, Fig. 3a).

In the feeding preference experiment conducted between the control and parts neighboring those subjected to simulated grazing (A1 vs B1, C1 vs D1 in Fig. 1c), 2 d after simulated grazing *Idotea baltica* tended to prefer control pieces over parts neighboring clipped parts; the difference, however, was not significant—0.21 ± 0.04 (52) and 0.11 ± 0.03 (49), respectively—(Table 2, Fig. 3b), suggesting that induced resistance may not spread to neighboring fronds. The preference was similar in both choice situations, as revealed by non-significant preference × choice situation interaction; this suggests an absence of spread of induction to both neighboring fronds and to the apices above the simulated grazing.

After 10 d there was no indication of induced resistance. This implies that the weak spreading of induced resistance to nearby fronds possibly occurring 2 d after simulated grazing had faded away

between Days 3 and 10 (Table 2, Fig. 3b). The average food consumption of the herbivores differed between the 2 choice situations: the algal parts in Choice Situation 4 were consumed more than in Choice Situation 1—0.33 ± 0.03 (48) and 0.23 ± 0.03 (55), respectively—

Table 3. *Fucus vesiculosus*. Results of 3-way ANOVA testing effects of simulated grazing (clipping), nutrient enhancement and time on phlorotannin concentration. Clipping refers to differences between (a) small (A, B1 and B2) or (b) large (C1, C2, D1, D2) parts of alga. Data are shown in Figs. 4 & 5

Source	df	Type III SS	MS	F	p
(a) Small algal parts A, B1, B2					
Clipping	2,84	0.178	0.089	0.25	0.777
Nutrient	1,84	20.48	20.48	58.33	**<0.0001**
Time	2,84	33.45	16.73	47.65	**<0.0001**
Clipping × Nutrient	2,84	0.019	0.010	0.03	0.973
Clipping × Time	4,84	0.770	0.193	0.55	0.701
Nutrient × Time	2,84	22.75	11.38	32.41	**<0.0001**
Clipping × Nutrient × Time	4,84	1.817	0.454	1.29	0.279
(b) Large algal parts C1, C2, D1, D2					
Clipping	3,91	1.478	0.493	1.14	0.337
Nutrient	1,91	11.71	11.71	27.10	**<0.0001**
Time	2,91	16.43	8.216	19.02	**<0.0001**
Clipping × Nutrient	3,91	0.736	0.245	0.57	0.638
Clipping × Time	6,91	10.95	1.825	4.22	**<0.001**
Nutrient × Time	2,91	9.686	4.843	11.21	**<0.0001**
Clipping × Nutrient × Time	6,91	4.328	0.721	1.67	0.137

(Table 2). This may follow from the larger total number of apices consumed in Choice Situation 4.

In the test 38 d after simulated grazing, we found no significant preference, nutrient effect or difference in choice situations, or any interactions among them; nutrient enhancement did not affect induced resistance in any of the bioassays (Tables 1 & 2).

Chemical quality and growth of *Fucus vesiculosus*

Simulated grazing of small parts of the alga (Parts A, B1, B2) did not affect its phlorotannin concentrations (Table 3a, Fig. 4). Nutrient treatment had a major effect on phlorotannins, the size of the effect depended on time after simulated grazing (Table 3a, Fig. 4), leading us to analyze clipping and nutrient effects separately for each time interval. Nutrient treatment significantly decreased the phlorotannin concentrations of the alga, the magnitude of the effect increasing with increasing duration of nutrient treatment and being statistically significant after 38 d (Table 4a, Fig. 4).

Table 4. *Fucus vesiculosus*. Results of 2-way ANOVA testing effects of simulated grazing (clipping) and nutrient enhancement on phlorotannin concentration. Clipping refers to differences between (a) small (A, B1 and B2) or (b) large (C1, C2, D1 and D2) parts of alga. Data are shown in Figs. 4 & 5

Source	2 d			10 d			38 d		
	df	F	p	df	F	p	df	F	p
(a) Small algal parts A, B1, B2									
Clipping	2	0.19	0.829	2	0.49	0.619	2	0.57	0.572
Nutrient	1	0.13	0.721	1	2.24	0.146	1	205.7	**<0.0001**
Clipping × Nutrient	2	0.30	0.746	2	0.81	0.454	2	1.68	0.205
Error	30			27			27		
(b) Large algal parts (C1, C2, D1, D2)									
Clipping	3	3.83	**0.017**	3	1.11	0.362	3	4.19	**0.016**
Nutrient	1	0.05	0.821	1	8.02	**0.009**	1	39.29	**<0.0001**
Clipping × Nutrient	3	0.58	0.631	3	1.21	0.326	3	2.66	0.071
Error	40			27			24		

For the large algal parts, the effects of nutrient treatment and time after simulated grazing were interdependent, and the effect of clipping depended on time (Tables 3b & 4b). Since we had data for both the basal and the apical parts, with the basal part undergoing simulated grazing and the apical part reflecting the possible transfer of induction, we tested the differences between control and clipped algae, contrasting apical pieces with each other and basal pieces with each other: 2 d after simulated grazing, the phlorotannin level was significantly higher in the clipped parts (Table 4b, Fig. 5). The difference between clipped and control algae was marginally significant in the apical parts (*a priori* contrasts: $F_{1,40} = 3.97$, p = 0.053) (Fig. 5a), and especially clear in the basal parts (*a priori* contrasts: $F_{1,40} = 6.25$, p = 0.017) (Fig. 5b). The initial phlorotannin levels, however, were generally higher compared to the situation 2 d after beginning the manipulation (Fig. 5). Nutrient enhancement did not have an effect on phlorotannin concentration 2 d after simulated grazing (Table 4b, Fig. 5).

Phlorotannins did not differ between the parts 10 d after clipping, but nutrient enhancement had started to reduce the phlorotannin concentration (Table 4b, Fig. 5).

Long-term (38 d) nutrient enhancement resulted in an overall decrease in phlorotannins (Table 4, Fig. 5). Clipping significantly affected phlorotannin concentration, but in the opposite direction than 2 d after simulated grazing: the clipped parts contained fewer phlorotannins than the control parts. These differences were greatest among the nutrient-treated algae, as indicated by the trend in the clipping × nutrient enhancement interaction (Table 4b). *A priori* contrasts were therefore calculated separately for the control and nutrient treatments: in control water the differ-
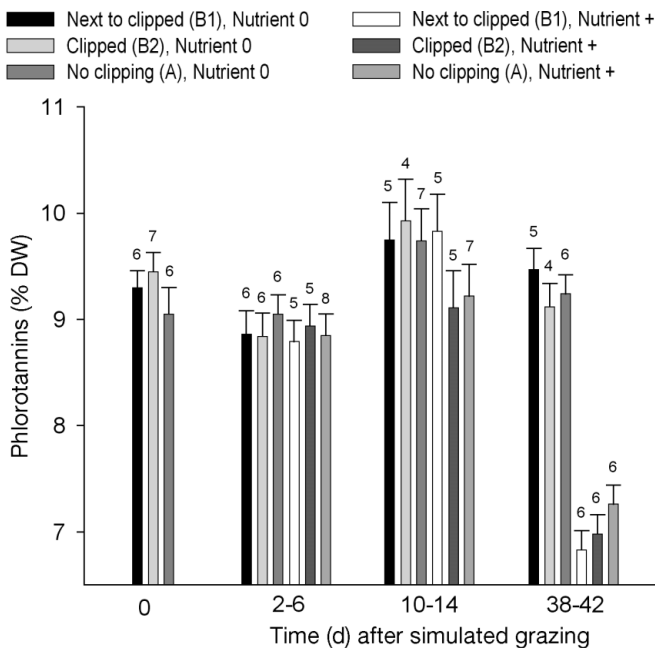

Fig. 4. *Fucus vesiculosus*. Phlorotannin concentration (adjusted mean + SE; n above bars) of clipped fronds, of fronds adjacent to clipped fronds and of control fronds of small parts A and B before (Time 0) and 2, 10 and 38 d after simulated grazing. DW: dry weight
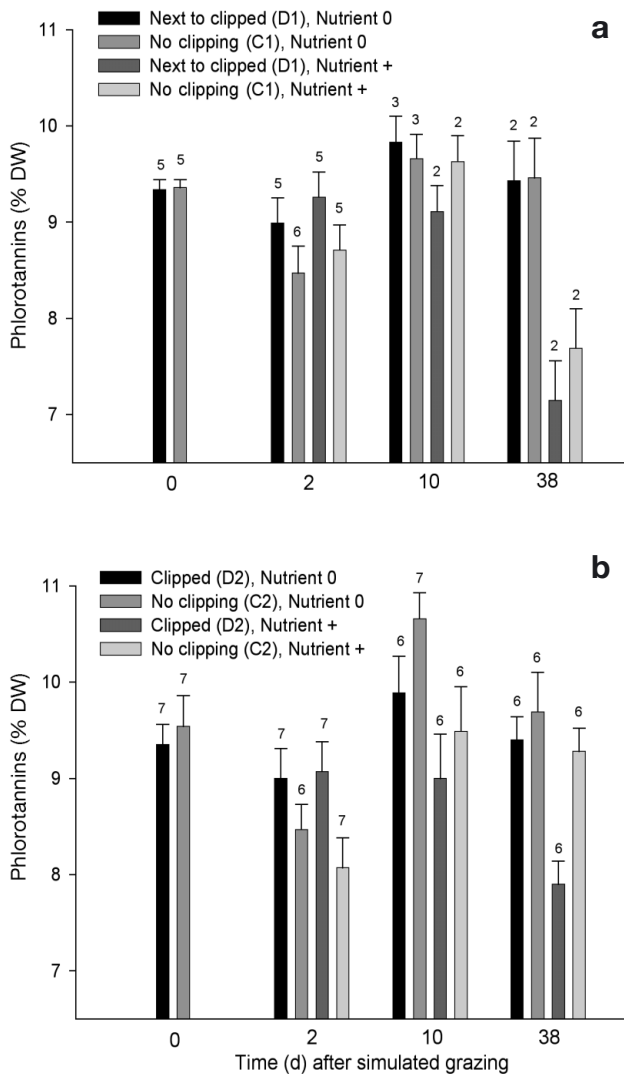
Fig. 5. *Fucus vesiculosus*. Phlorotannin concentration (adjusted mean + SE; n above bars) of (a) apical and (b) basal thallus of clipped and control large parts C and D before (Time 0) and 2, 10 and 38 d after simulated grazing

ences were non-significant for both basal and apical parts ($F_{1,12} = 0.30$, p = 0.595 and $F_{1,12} = 0.01$, p = 0.915, respectively), while in nutrient-enriched water they were marginally significant in basal parts ($F_{1,12} = 4.78$, p = 0.049) and non-significant in apical parts ($F_{1,12} = 2.19$, p = 0.164).

Simulated grazing had no effect on the subsequent growth of the algae; the increase in wet weight did not differ between clipped and control parts (Table 5). Algae grown in a nutrient-enhanced environment grew less than those grown in ambient nutrient conditions. This was the case for both small and large pieces of *Fucus vesiculosus* (Table 5).

In a comparison of the group means of different parts at 2 nutrient levels, algal growth tended to correlate positively with phlorotannin concentration (r = 0.657, p = 0.077, n = 8; Fig. 6).

The food consumption of the herbivores did not correlate significantly with phlorotannin concentration of the algal parts in any treatment or at any point of time: pooled data; values calculated as control part minus clipped part or part neighboring the clipped part (Fig. 1b), 2 nutrient treatments and 3 times: r = 0.089, p = 0.678, n = 24.

DISCUSSION

Simulated grazing by clipping of the thallus did induce resistance in clipped parts in *Fucus vesiculosus*, independent of nutrient environment. In the feeding preference bioassays shortly after simulated grazing, *Idotea baltica* preferred the unclipped control alga to the clipped alga. This implies that inducible resistance of *F. vesiculosus* occurs quite rapidly after wounding and starts to diminish after 10 d. The observed resistance, however, was not related to total phlorotannin concentrations; clipping did not induce changes in

Table 5. *Fucus vesiculosus*. Growth (g wet weight increments: mean ± SE) of small and large parts of alga 38 d after simulated grazing, and results of ANCOVA. Initial wet weight of alga was used as covariate

| Treatment	Nutrient 0	Nutrient +	Source	df	F	p
Small parts						
Control	0.42 ± 0.03 (20)	0.32 ± 0.03 (20)	Clipping	1,115	0.02	0.885
Clipped	0.43 ± 0.02 (40)	0.30 ± 0.02 (40)	Nutrient	1,115	21.14	**<0.0001**
			Clipping × Nutrient	1,115	0.27	0.602
			Initial weight	1,115	85.06	**<0.0001**
Small parts						
Control	0.60 ± 0.04 (20)	0.57 ± 0.04 (20)	Clipping	1,73	0.68	0.413
Clipped	0.69 ± 0.04 (20)	0.55 ± 0.04 (18)	Nutrient	1,73	4.54	**0.036**
			Clipping × Nutrient	1,73	1.74	0.192
			Initial weight	1,73	42.05	**<0.0001**
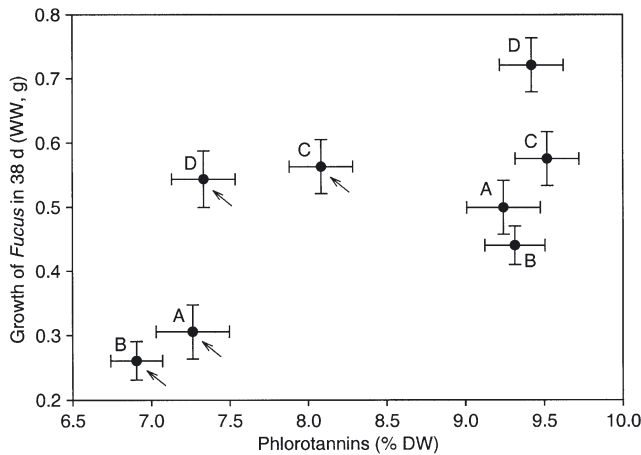
Fig. 6. *Fucus vesiculosus*. Correlation between growth and phlorotannin concentration of 4 different treatments (mean ± SE). Data points indicated by arrow represent alga grown in nutrient-enriched water. DW: dry wt; WW: wet wt. A–D: clipping treatments shown in Fig. 1a

these, and the phlorotannin concentration in *F. vesiculosus* did not predict the preference of *I. baltica*. Phlorotannin concentration was highly dependent on the nutrient environment: the concentration decreased with time in nutrient-enhanced conditions, and this decrease was independent of simulated grazing.

The isopod's preference for control alga over clipped alga together with no induction of changes in total phlorotannin content strongly suggests that there are substances other than phlorotannins involved in the induction of grazing deterrence in *Fucus vesiculosus*. Deal et al. (2003) quantified metabolites responsible for grazing deterrence of sea urchins in *F. vesiculosus*, and concluded that galactolipids and some non-phenolic water-soluble compounds rather than phlorotannins produced the strongest deterrence. Alternatively, qualitative changes in different fractions of phlorotannins may explain the herbivore preference and consumption rate of *F. vesiculosus*, since the deterrence capacity of phlorotannins may vary according to their molecular structure (Boettcher & Targett 1993, Arnold & Targett 1998, Targett & Arnold 1998). The chemical basis underlying the early induction of deterrence in *F. vesiculosus* against the isopod herbivore *Idotea baltica* remains unclear and requires further research.

The ecological and life-history traits of *Fucus vesiculosus* and *Idotea baltica* provide favorable preconditions for the evolution of inducible defenses. In our study area, *I. baltica* is the major herbivore of *F. vesiculosus* and, by feeding on the thallus, the only species capable of causing considerable biomass losses (Kangas et al. 1982 and pers. obs. of present authors). *F. vesiculosus* is a perennial, long-lived species, which

encounters several peaks of herbivory by isopods during its lifespan (Salemaa 1979, 1987). The peak period in herbivory occurs during the few months in fall when the generation of isopods born in the summer begin feeding on *Fucus*, and isopod density may reach dozens to hundreds of isopods per individual *F. vesiculosus*; the values are 10-fold those in late spring and summer (Jormalainen et al. 1995 and unpubl. data of present authors). Moreover, *I. baltica* is highly mobile within and between algal individuals, and its presence is thus variable among the algae.

Since resistance induction was local and did not spread to neighboring fronds, it may not be a very efficient defense against biomass loss to highly mobile herbivores. On the other hand, a rapid local deterrence may be capable of dispersing grazing away from already grazed spots of thallus, thereby reducing the size of single grazing marks and also reducing the risk of the thallus being completely removed, which would greatly increase the magnitude of biomass loss due to grazing. Furthermore, the relatively rapid relaxation of the local induction would hardly provide an efficient defense against herbivory during a herbivory peak lasting for months. We therefore suggest that although the observed resistance may clearly provide adaptive benefits in defense against herbivory, the possibility remains that resistance arises as a side-effect of metabolic processes which have evolved in another context, for instance wound-healing of the damaged tissue.

Contrary to our expectations, based on earlier studies on brown algae (Van Alstyne 1988, Pavia & Toth 2000), the total phlorotannin concentration clearly did not play any crucial role in the induced response. Simulated grazing increased the phlorotannin concentration only in the clipped basal parts after 2 d, regardless of nutrient addition. Since phlorotannins are involved in the hardening of cell walls (Faberberg & Dawes 1976, Schoenwaelder 2002), the rapid increase may be due to the possible wound-healing role of phlorotannins in the clipped, non-growing tissues. In addition, there was an overall decrease in phlorotannins after 2 d and an increase after 10 d in almost every treatment combination. We suspect this to be a consequence of natural changes in growing conditions (e.g. light and/or temperature) occurring in the outdoor pools at that time. Such responses can be expected if the levels of soluble phlorotannins vary with the growth rate of the alga (Arnold & Targett 2003).

Nutrient enhancement reduced the concentration of phlorotannins, consistent with the prediction of the carbon–nutrient balance (CNB) hypothesis. However, as nutrient enhancement did not affect production of phlorotannins after simulated grazing, the CNB hypothesis was unable to predict correctly the consequences of nutrient enhancement on the occurrence

of induced phlorotannin production by the alga. Nutrient enhancement likewise had no effect on feeding preferences, suggesting that the nutrient environment does not affect the induced response in any way. However, nutrient enhancement increased the consumption rate of apical pieces compared to that of basal pieces, suggesting possible changes in the primary metabolism of the growing tips that make them more susceptible to herbivory. This is consistent with the results by Hemmi & Jormalainen (2002), who showed that *Idotea baltica* consumes more food and achieves a higher growth rate when reared on nutrient-treated *Fucus vesiculosus*.

The decrease in phlorotannins in the nutrient-enhanced environment was not due to increased growth of the alga. Instead, we found a positive though weak correlation between growth rate and phlorotannin concentration, which contradicts the predictions of the CNB and GDB (growth-differentiation balance) hypotheses. Previous studies on brown algae have reported a variety of mixed results on the phlorotannin–growth relationship, suggesting a complex system with several factors influencing growth and secondary chemistry. The findings have been inconclusive: some authors have found a positive correlation between the growth and defense of *Fucus vesiculosus* (Cronin & Hay 1996b under laboratory conditions), some have observed a negative correlation (Yates & Peckol 1993 at high but not low N concentrations, Pavia et al. 1999), others have found a negative correlation in the brown alga *Ecklonia radiata* in spring but not in autumn (Steinberg 1995), and others have failed to track any resource-based trade-offs (Cronin & Hay 1996b under field conditions, Pavia & Åberg 1996, Targett 2001). Rather than a straightforward trade-off between growth and phlorotannin concentration, the non-existing or positive phenotypic correlation in our case may thus have been due to the nutrient-addition treatment, which often increases the amount of epiphytic algae and other microorganisms on the surface of the thallus (authors' pers. obs.). Shading by epiphytes reduces the availability of light, causing carbon limitation; this may explain the lower levels of phlorotannins as well as the growth of *F. vesiculosus* in the nutrient-addition treatment.

If losing biomass to herbivory generates costs for the alga, then the induction of resistance may be regarded as a defensive strategy against further herbivory. A negative relationship between growth and artificial grazing has previously been found in the kelp *Laminaria hyperborea* (Toth & Pavia 2002). However, clipping did not affect growth of *Fucus vesiculosus*. This indicates that the consequences of biomass loss may depend on growing conditions and do not necessarily affect the fitness of the alga. *F. vesiculosus* may be able to compensate for the loss of biomass by utilizing stored resources in the remaining thallus to maintain growth (Lehvo et al. 2001, Honkanen & Jormalainen 2002). Another possibility is that under good conditions with high nutrient and light availability, *F. vesiculosus* does not need to rely on internal storages, but may sustain growth by resorting to external resources available in excess. Transfer of resources within the alga is probably most important under conditions in which external resources are scarce; in such a situation, thallus loss should affect growth. The availability of resources may have been relatively high in this study, and prevented artificial grazing from negatively affecting growth. The absence of this trade-off may partly be explained by the location of the artificial grazing marks: we intentionally avoided clipping the apical parts of the thallus, containing meristematic tissue, to avoid exaggeration of the magnitude of simulated grazing. The loss of meristematic tissue to herbivores would necessarily terminate the growth of the focal frond.

We conclude that *Fucus vesiculosus* is capable of inducing rapid resistance against grazing by its major herbivore *Idotea baltica*. The induction of resistance is highly local, diminishes after 10 d, and does not spread within the algal individual. We thus suggest that it may be inefficient as a general defense against herbivory, but may benefit the alga by dispersing future damage within the individual and avoiding the breaking off of whole fronds. Whether such deterrence represents a defense adaptation against herbivory or arises as a side-effect of wound-healing metabolism cannot be concluded from the data and needs further research. In addition, the chemical substance behind the induction needs to be further clarified, as the deterrence is not derived from the alga's total phlorotannin concentration. Nutrient enhancement does not affect the inducible resistance of *F. vesiculosus* but reduces the long-term concentration of phlorotannins, which do not seem to play a role in induced defense.

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