5. The buffer consisted of 118 m*M* NaCl, 6 m*M* KCl, 2 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 1.3 m*M* NaH_2PO_4, 25 m*M* $NaHCO_3$, and 5.6 m*M* glucose. When exposed to 95 percent O_2–5 percent CO_2 at 34°C, the *p*H was 7.4.
6. L. M. Beidler, *J. Neurophysiol.* **16**, 595 (1953).
7. Contact between the tongue and the amiloride solution was maintained by blocking the drain tube of the plexiglass flow chamber. The effect of amiloride was assessed by testing the three highest concentrations of NaCl and KCl. Recovery was monitored by sampling the response to 1*M* NaCl at various intervals. The phasic component recovered 72 percent and the steady state 50 percent in 15 minutes.
8. D. J. Benos, *Am. J. Physiol.* **242**, C131 (1982).
9. S. S. Schiffman, E. Lockhead, F. W. Maes, *Proc. Natl. Acad Sci. U.S.A.* **80**, 6136 (1983).
10. J. H. Teeter, W. L. Silver, J. G. Brand, *Soc. Neurosci. Abstr.* **9**, 1022 (1983).
11. We thank B. K. Coplon and S. K. DeSimone for technical assistance. Supported by NIH research grant NS 13767 and by the Brown and Williamson Tobacco Corporation.

20 October 1983; accepted 25 October 1983

Algal Chemical Defense Against Herbivores: Allocation of Phenolic Compounds in the Kelp *Alaria marginata*

Abstract. *Higher concentrations of phenolic compounds were found in the reproductive fronds (sporophylls) of the intertidal kelp* Alaria marginata *than were found in the vegetative blades. The sporophylls were consumed by herbivorous snails at a lower rate than were the vegetative blades, in both field and laboratory studies. These results indicate that differential internal production of defensive compounds in a marine alga can significantly affect the pattern of herbivory on the plant.*

Ecological and evolutionary theories often predict that the internal resources of an organism (such as energy, nutrients, and metabolites) should be allocated to different structures or functions in ways that maximize the organism's fitness (*1, 2*). Since fitness depends ultimately on the survival and growth of reproductive products, organisms should be under strong selection to allocate resources for protection of these reproductive parts against natural enemies.

Terrestrial plants have a wide variety of chemical and mechanical defenses in their fruits, seeds, or spores, that deter their natural enemies (*3*). The chemical defenses in the reproductive parts of terrestrial plants often differ quantitatively or qualitatively from those produced in the leaves or other vegetative parts of the same plant (*4*). Although many secondary compounds are common in marine plants (*5*), the interactions between these potentially defensive compounds and the herbivores that feed on them are just beginning to be investigated (*6–8*). Phenolic compounds, especially polyphenolics or tannins, are present in most or all species of brown algae (*9*) and deter feeding by invertebrate herbivores (*6, 7*). Differential feeding by invertebrate herbivores on vegetative and reproductive parts of marine algae has also been shown (*10*). I now report that the marine brown alga *Alaria marginata* allocates more phenolic compounds to reproductive parts than to vegetative blades, giving increased protection to its reproductive fronds against invertebrate herbivores.

Alaria marginata is a large (up to 3 m long), intertidal kelp (brown algae in the order Laminariales) from the Northeast Pacific Ocean (*11, 12*). Near the holdfast in the thallus of mature *A. marginata* are several to many pairs of reproductive fronds (sporophylls), which are morphologically distinct from the alga's single, large, vegetative blade (*11*).

In laboratory feeding experiments conducted over 2 years, sporophylls and vegetative fronds of *A. marginata* were offered to the intertidal herbivorous gastropod *Tegula funebralis* (*13*). The snails ate significantly more of the vegetative blade than of the sporophylls [*t*-test (*14*), $P < 0.001$ for both years] (Fig. 1A). The vegetative and reproductive tissues also differed chemically and physically. The sporophylls were significantly higher in total phenolics and tanning ability (*t*-test, $P < 0.001$ for both measures in both years) (Fig. 1B) (*15, 16*), not significantly different in nitrogen content (Fig. 1C), but somewhat tougher (*t*-test, $P < 0.05$) (Fig. 1D) than the vegetative blades.

I also assessed experimentally the amounts of herbivore damage on sporophylls and vegetative blades in the field by transplanting mature *A. marginata* plants into the intertidal in areas of "high" and "low" densities of *T. funebralis* (high, 35 to 105 snails per square meter; low, 8 to 15 snails per square meter) (Tables 1 and 2) (*17*). Algal tissue losses were analyzed by a two-way analysis of variance (herbivore density by tissue type; unequal but proportional cell sizes).

The results for measures of absolute (in square centimeters) and relative (as percentage of area) tissue loss are similar. The effect of tissue type was highly significant. More vegetative than sporophyllic tissue was lost in both high- and low-density herbivore treatments. The density of *T. funebralis* present was not a significant effect. The interaction between herbivore density and tissue type was significant. Loss of sporophyllic tissue was similar in the two levels of herbivores, but much more vegetative tissue was lost when herbivores were present in the higher density. These results show that when only a few herbivores or none are present, there is little tissue loss from both sporophylls and vegetative blades. Much of this is likely

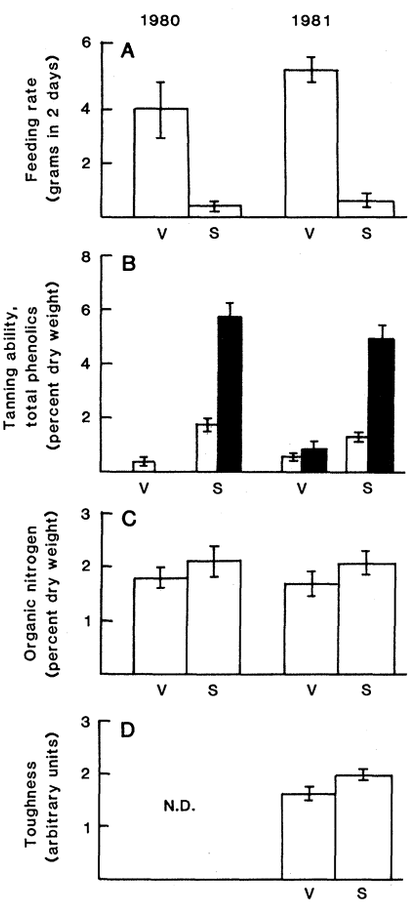

Fig. 1. Laboratory feeding experiments (A) and chemical (B and C) and physical (D) analyses of *A. marginata*; *V*, vegetative blades; *S*, sporophylls. Data are means ± standard errors (S.E.) for six plants. (A) Ten large (wet weight, about 10 g) *T. funebralis* were offered equal weights (about 10 g each) of *A. marginata* sporophylls and vegetative blades in 36-liter aquaria. After 48 hours, the algae were weighed again. Control aquaria contained algae but no snails. (B) For total phenolic content and tanning ability measurements, fresh algal tissue was ground in 50 percent methanol in a tissue macerator and extracted in the dark for 24 hours. Total phenolics were assayed by the Folin-Denis technique with phloroglucinol as the reference compound (*23, 24*). Tanning ability was measured by hemanalysis, with high molecular weight tannins from *Fucus vesiculosus* as reference compounds (*24–26*). (C) Organic nitrogen content was measured by the Kjehdahl technique (*27*). (D) Thallus toughness was measured with a "penetrometer" (*28*).

Table 1. Amounts, expressed as means ± 1 S.E., of sporophyllic and vegetative tissue of *A. marginata* lost in the field. Eight mature (sporophyllic) individuals of *A. marginata* were transplanted from Davenport Landing, Santa Cruz County to Pigeon Point, San Mateo, California, where *T. funebralis* are common. The plants were bolted to intertidal rocks (tidal height between +0.2 and −0.2 m). Four plants each were assigned to sites with high or low densities of *T. funebralis* (see text) (*17*). Any *Tegula* found within a 1-m^2 quadrat centered on the attachment bolt of the plants at the low-density sites were removed at each visit. All plants were within 40 m of one another. The initial area of the plants was measured by tracing the sporophylls and vegetative blades on sheets of transparent acetate with grease pencils. The tissue area lost was recorded every second day by further tracings, and the area lost was measured from cutouts of these tracings with a Li-Cor portable area meter (Li-Cor, Model Li-3000) (*29*). The experiment lasted 13 days (13 to 26 September 1981). One plant disappeared within the first 24 hours and was excluded from the analysis.

Herbivore density	Plants (No.)	Tissue area lost (cm^2)		Percentage of total area lost	
		Vegetative	Sporophyllic	Vegetative	Sporophyllic
High	4	437.0 ± 163.4	34.4 ± 7.2	22.5 ± 3.6	7.0 ± 1.8
Low	3	123.7 ± 36.1	64.1 ± 19.5	9.5 ± 1.5	8.0 ± 1.6

Table 2. Two-way analysis of variance. Areas were transformed logarithmically; percentages were transformed by arcsin $\sqrt{p}$. N.S., not significant.

Treatment	*F*	
	For area lost	For percentage of total area lost
Tissue type	22.61*	11.55†
Herbivore density	0.50 N.S.	4.19 N.S.
Tissue type by herbivore density	6.08‡	6.25‡

*$P < 0.001$. †$P < 0.01$. ‡$P < 0.05$.

due to physical abrasion. When herbivore density increases, however, damage increases only on the vegetative blades. These results parallel those from the laboratory experiments. The sporophylls of *A. marginata* are better defended against *T. funebralis* than are vegetative blades. This correlates with the higher levels of phenolic compounds present in the sporophylls.

Allocation of phenolics in the marine alga *A. marginata* is consistent with predictions from terrestrial plant–herbivore theory, according to which defensive compounds in different parts of terrestrial plants should be allocated to those parts in proportion to their risks of being eaten and their potential contribution to the fitness of the plant (*1*). Testing predictions such as these for terrestrial plants is complicated by the wide variety of compounds produced, their differing physiological costs, and their differing efficacies against herbivores (*18*), which in turn show a vast array of feeding preferences and patterns (*19*). However, in marine systems, tannins and other phenolics are apparently present in all brown algae (*9*), and in at least two ecologically dominant orders, the Laminariales and Fucales, they may be the primary chemical defense against herbivores (*20*). Unlike the terrestrial insect herbivores, which exhibit wide variations in their feeding preferences and patterns (*19*), many of the common invertebrate herbivores from the Northeast Pacific Ocean (at least) are generalist feeders (*21*) and exhibit similar feeding preferences (*22*), tending to eat the same species of brown algae. The relative simplicity of these marine systems (compared to terrestrial ones) may make them useful for testing some of the recent predictions of plant-herbivore theory.

PETER D. STEINBERG
Center for Coastal Marine Studies and Biology Board, Division of Natural Sciences, University of California, Santa Cruz 95064

References and Notes

1. D. McKey, in *Herbivores: Their Interaction with Secondary Plant Metabolites*, G. A. Rosenthal and D. H. Janzen, Eds. (Academic Press, New York, 1979), pp. 55–133.
2. C. R. Townsend and P. Calow, Eds., *Physiological Ecology: An Evolutionary Approach to Resource Use* (Sinauer, Sunderland, Mass., 1981).
3. D. H. Janzen, in *Biochemical Aspects of Plant and Animal Coevolution*, J. B. Harborne, Ed. (Academic Press, London, 1978), pp. 163–206; S. S. Rehr, E. A. Bell, D. H. Janzen, P. P. Feeny, *Biochem. Syst. Ecol.* **1**, 63 (1973).
4. W. A. Dement and H. A. Mooney, *Oecologia (Berlin)* **13**, 62 (1965).
5. P. J. Scheuer, *Marine Natural Products: Chemical and Biological Perspectives* (Academic Press, London, 1977, 1978), vols. 1 and 2; J. T. Baker and V. Murphy, *Handbook of Marine Science. Compounds from Marine Organisms* (CRC Press, Boca Raton, Fla., 1976), vol. 1; D. J. Faulkner and W. Fenical, Eds., *Marine Natural Products Chemistry* (Plenum, New York, 1977); M. A. Ragan, in *The Biology of Seaweeds*, C. S. Lobban and M. J. Wynne, Eds. (Univ. of California Press, Berkeley, 1981), chap. 17.
6. J. A. Geiselman, thesis, Massachussetts Institute of Technology and Woods Hole Marine Biology Laboratories (1980).
7. _____ and O. J. McConnell, *J. Chem. Ecol.* **7**, 1113 (1981); P. D. Steinberg, in preparation.
8. N. M. Targett and O. J. McConnell, *J. Chem. Ecol.* **8**, 115 (1982); J. N. Norris and W. Fenical, *Smithson. Contrib. Mar. Sci.* **12**, 417 (1982).
9. M. A. Ragan, *Bot. Mar.* **19**, 145 (1976); _____ and J. S. Craigie, in *Handbook of Phycological Methods*, J. Helleburst and J. S. Craigie, Eds. (Cambridge Univ. Press, Cambridge, 1978), vol. 2, pp. 157–179.
10. G. Sharp, in T. Carefoot, *Pacific Seashores* (Univ. of Washington Press, Seattle, 1977), p. 127; S. D. Gaines, *Am. Zool.* **20**, 808 (1980).
11. I. A. Abbott and G. J. Hollenburg, *Marine Algae of California* (Stanford Univ. Press, Stanford, Calif., 1976).
12. L. Druehl, *Phycologia* **9**, 237 (1970).
13. *Tegula funebralis* was used because (i) it is a large, common gastropod known to be a generalized herbivore [B. Best, *Veliger* **6** (Suppl.), 42 (1964)]; (ii) it overlaps the geographic and intertidal range of *A. marginata* [R. H. Morris, D. P. Abbott, E. C. Haderlie, *Intertidal Invertebrates of California* (Stanford Univ. Press, Stanford, Calif., 1980)].
14. Logarithmic transformations ensured homogeneity of variances [R. R. Sokal and J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), p. 382.
15. Percentage data (total phenolic content, tanning ability, and total organic nitrogen content) were transformed by arcsin $\sqrt{p}$ for *t*-tests.
16. These data (Fig. 1B) are a minimum estimate of the difference in total phenolic content between sporophylls and vegetative blades. Only mature sporophylls were used in these analyses, and I have found that the phenolic content (as percent dry weight) of *Alaria* sporophylls decrease as they mature. Linear regression: $y = 2.889 - 0.246x$, $P < 0.01$, for 15 sporophylls from four plants, where y is the total phenolic content (Folin-Denis technique), as percent dry weight of the sporophyll, and x is the ranked age of the sporophyll, as indicated by the position along the stipe of the plant [T. B. Widdowson, *Syesis* **4**, 11 (1971)]. This is not a simple dilution effect, since the proportionate decline in phenolic content of the sporophylls is less than the proportion increase in weight or area. Tanning ability of the sporophylls does not change with age. Linear regression: $y = 3.925 + 0.784x$, $P > 0.4$, where y is *Fucus vesiculosus* tannin equivalents (hemanalysis technique), as percent dry weight, and x is as above.
17. Grazing in the low-density sites was minimal, and these plants acted as "no grazing" controls for assessing tissue loss due to abrasion and other physical effects. At the low-density sites, snails were rarely observed on the thallus of *A. marginata*. At the high-density sites, the snails were commonly observed actively crawling on and feeding on the *A. marginata* plants. *Tegula funebralis* was the only abundant large herbivore at any of the sites.
18. G. A. Rosenthal and D. H. Janzen, Eds., *Herbivores: Their Interaction with Secondary Plant Metabolites* (Academic Press, New York, 1979); J. B. Harborne, *Biochemical Aspects of Plant and Animal Coevolution* (Academic Press, New York, 1978); T. Swain, *Annu. Rev. Plant Physiol.* **28**, 479 (1977)
19. L. R. Fox and P. A. Morrow, *Science* **211**, 887 (1981); D. J. Futuyma and F. Gould, *Ecol. Monogr.* **49**, 33 (1979); J. Lawton and D. R. Strong *Am. Nat.* **118**, 1 (1981).
20. Except for halogenated methanes in some Fucales [J. A. Geiselman (*6*)], no other chemical defenses against herbivores have been identified from these two orders.
21. J. Lubchenco and S. D. Gaines, *Annu. Rev. Ecol. Syst.* **12**, 405 (1981).
22. B. Best, in (*13*); D. L. Leighton, *Pac. Sci.* **20**, 104 (1966); R. L. Vadas, *Ecol. Monogr.* **47**, 337 (1977); D. W. Birch, *Crustaceana (Leiden)* **36**, 186 (1979); A. J. Paul, J. M. Paul, D. W. Hood, P. A. Neve, *Veliger* **19**, 303 (1979); P. D. Steinberg, in preparation.
23. T. Swain and W. E. Hillis, *J. Sci. Food Agric.* **10**, 63 (1959).
24. M. A. Ragan and A. Jensen, *J. Exp. Mar. Biol. Ecol.* **30**, 209 (1977).
25. E. C. Bate-Smith, *Phytochemistry* **12**, 907 (1973); R. Belserene, thesis, University of California, Santa Cruz (1980).
26. The Folin-Denis technique is used to assay for aromatic hydroxyl groups, which are characteristic of all phenolic molecules. The hemanalysis technique measures the ability of polyphenolics (tannins) to bind to proteins. The two procedures should agree qualitatively, but they measure different properties of phenolic molecules.

The results from *A. marginata* suggest that most of the phenolics are polymerized with high tanning ability. M. A. Ragan and A. Jensen (*16*) and A. Temple [*J. Chem. Ecol.* **8**, 1289 (1982)] critique different methods of assaying for phenolic compounds in brown algae and terrestrial plants, respectively.
27. Industrial method 334-74 A/A for the AutoAnalyzer II (Technicon Industrial Systems, Tarrytown, N.Y.).
28. P. P. Feeny, *Ecology* **51**, 656 (1970); M. M. Littler and D. S. Littler, *Am. Nat.* **116**, 25 (1980).
29. Growth of the plants did occur, but was minimal.
30. I thank D. C. Potts, L. R. Fox, and J. A. Estes for comments on the manuscript, and M. A. Ragan for useful general comments and for kindly providing the *Fucus vesiculosus* tannins used as standards in the hemanalysis assay.

21 March 1983; accepted 17 November 1983

Expression of Glial Fibrillary Acidic Protein in Immature Oligodendroglia

Abstract. *In the human fetal spinal cord at 15 to 16 weeks, glial fibrillary acidic protein (GFAP) was demonstrated within the cytoplasm and processes of cells having the cytological, ultrastructural, and immunocytochemical features of oligodendrocytes—including processes that extend into and contribute to the formation of myelin sheaths. By 17 to 18 weeks, however, GFAP immunoreactivity was no longer evident within such cells. Thus GFAP is expressed by myelin-forming oligodendroglia early in their development.*

Although oligodendrocytes were first discovered and described by del Rio Hortega more than 60 years ago (*1*), unequivocal demonstration of their role in myelin sheath formation was possible only with the advent of electron microscopy and immunocytochemistry (*2–4*). The cell of origin and mode of differentiation of oligodendrocytes in the developing central nervous system, however, are still uncertain. The prevailing view seems to be that oligodendrocytes are derived from "glioblasts," the nature of which has never been clearly defined (*5, 6*).

We showed earlier (*7*) that astrocyte-specific glial fibrillary acidic protein (GFAP) is present within radial glial cells in the developing human fetal spinal cord (HFSC) by 8 to 10 weeks. These cells are the first distinguishable neuroglial element among the cells within the ventricular zone. Correlative electron microscopic, Golgi, and immunocytochemical studies have suggested that radial glia are transformed into astroglial cells in the HFSC, human fetal cerebrum and cerebellum, and fetal monkey telencephalon (*8*). More recently, we showed, by electron microscopy and by immunocytochemical analysis under light microscopy, that "transitional forms" between astroglia and oligodendroglia may exist, and we suggested that radial glia may give rise to both astroglial and oligodendroglial cells (*9, 10*).

We tested this hypothesis further by light and electron microscopic immunocytochemical studies on serial sections (1 μm) of the subpial and marginal zones of the ventral columns of the HFSC obtained from 17 aborted fetuses at an ovulation age of 8 to 20 weeks. Alternating deeponized sections were processed for immunocytochemical determination of GFAP and myelin basic protein (MBP), respectively (*11*). At age 6 to 8 weeks the subpial region of the HFSC is relatively cell free. By 9 to 10 weeks, however, there is a significant increase in the population of cells within this region. Most of these cells show the cytological, ultrastructural, and immunocytochemical features of astroglial cells (*4*). At 11 to 12 weeks, when myelin formation begins, most of the cells still have the characteristics of astroglia, although a few show the features of oligodendrocytes (*6, 12*). By 16 weeks, however, almost all of the cells within this region are oligodendrocytes, as indicated by their morphology and by their intimate association with well-developed myelin sheaths.

Figure 1 shows representative immunocytochemical preparations of adjacent 1-μm sections of the HFSC at 16 weeks. At this stage, immunoreactivity to MBP is localized primarily within myelin sheaths and within the processes of cells with which they appear to be closely associated (Fig. 1A). Nearly all of these cells contain immunoreactive GFAP within their cytoplasmic processes, some of which appear to encircle thinly myelinated axons (Fig. 1B).

To investigate more fully the relation between GFAP-positive cells and myelin sheaths, we processed large numbers of vibratome sections for immunocytochemistry and examined under electron microscopy. At 13 to 15 weeks, many cells exhibited the features of oligodendroglia, the cytoplasm and processes of which, however, were strongly immunoreactive for GFAP (Fig. 2A). These processes often extended into myelin sheaths which, as judged from their thinness and the small number of lamellae, were relatively immature (Fig. 2B). By 17 to 18 weeks most of the axons within this region were surrounded by multilay-

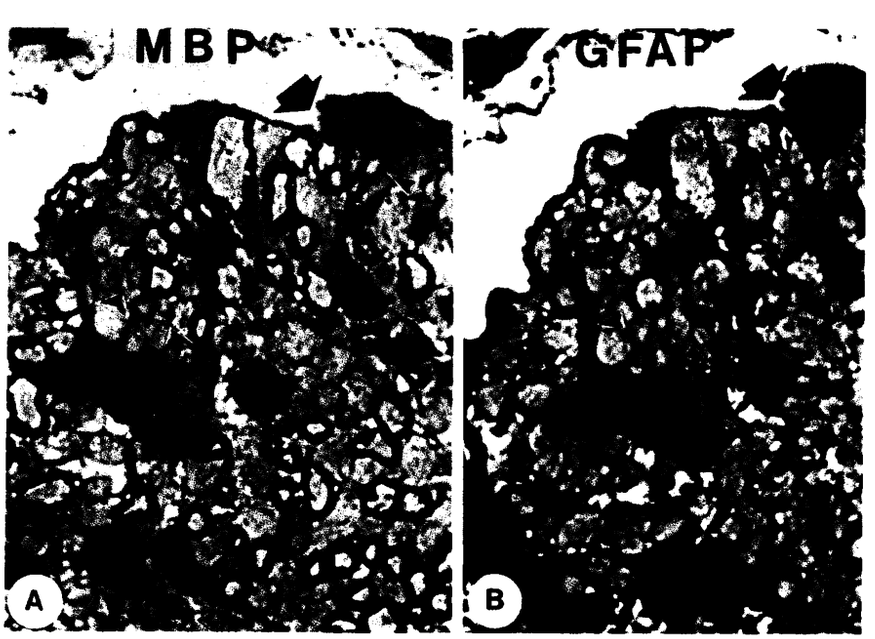

Fig. 1. Photomicrographs showing adjacent 1-μm sections of the ventral column of the HFSC at 16 weeks of age. The sections were prepared for (A) MBP and (B) GFAP immunocytochemistry with the unlabeled antibody enzyme technique. Dark staining represents immune precipitate. Immunoreactivity to MBP is localized primarily within myelin sheaths and within the processes of immature oligodendrocytes, with which they are closely associated. These cells show dense nuclear (*N*) and cytoplasmic staining and contain prominent nucleoli and marginated chromatin. Nearly all of these cells show strong immunoreactivity for GFAP within their cytoplasm and processes (thick arrow and arrowheads). Magnification, ×1350.