

Seaweed secondary metabolites as antifoulants: effects of *Dictyota* spp. diterpenes on survivorship, settlement, and development of marine invertebrate larvae

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Summary. A recent investigation showed that the brown seaweed *Dictyota menstrualis* was unfouled relative to co-occurring seaweeds, and that larvae of fouling invertebrates avoided settling on *D. menstrualis* due to chemicals on its surface. The secondary metabolites dictyol E and pachydictyol A are among the compounds found on this alga's surface. In the present study, we tested the effects of specific diterpenes from *Dictyota* on the survivorship, growth, and development of invertebrate larvae and developing juveniles that could foul seaweeds. Exposure to dictyol E, dictyol B acetate, pachydictyol A, and dictyodial from *Dictyota menstrualis* and *D. ciliolata* caused significant larval mortality, abnormal development, and reduce growth rates for three species of co-occurring invertebrates when their larvae were forced into contact with these metabolites. Larvae were damaged at metabolite concentrations as low as 5% of maximum possible surface concentrations of these compounds for the populations of *Dictyota* we studied. The negative effects of these secondary metabolites on potential foulers, in conjunction with data demonstrating larval avoidance of dictyol-covered surfaces, suggest that these compounds could function as chemical defenses against fouling, and could select for larvae that avoid hosts producing these metabolites.

Key words. antifouling – dictyol diterpenes – *Dictyota* – invertebrate larvae – physiological effects – secondary metabolites

Introduction

Seaweeds fouled by either plants or animals can experience reduced rates of photosynthesis, growth, and reproduction due to increased shading, competition for nutrients, and interference with reproductive processes (Orth & van Montfrans 1984; Brawley 1992; Williams & Seed 1992). Additionally, they may lose biomass if consumers feeding on fouling organisms take portions

of the host seaweed in the process (Bernstein & Jung 1979; Wahl & Hay 1995). Mortality also may increase because fouling organisms increase drag, causing plants to be dislodged by strong waves and currents (see Brawley 1992; Williams & Seed 1992). These processes could select for seaweed characteristics that minimize colonization by fouling organisms, including sloughing of surface cell layers (Moss 1982; Masaki *et al.* 1984; Johnson & Mann 1986), associations with consumers that feed on fouling organisms but cause minimal damage to the host (Brawley & Adey 1981; Steneck 1982; Duffy 1990; Brawley 1992), and the production of bioactive secondary metabolites that can inhibit the settlement or development of fouling organisms (Sieburth & Conover 1965; de Nys *et al.* 1995; Henrickson & Pawlik 1995; Schmitt *et al.* 1995).

Schmitt *et al.* (1995) showed that secondary metabolites produced by the brown seaweed *Dictyota menstrualis* were present on the alga's surface and appeared to deter invertebrate larvae from settling on this alga. They demonstrated: (1) In the field, *D. menstrualis* plants were less frequently and less heavily fouled than any of the other five co-occurring seaweed species they studied; (2) In lab assays, larvae of the common fouling bryozoan *Bugula neritina* failed to settle on *D. menstrualis* even though they contacted the surface of *D. menstrualis* as often as they contacted the surface of a preferred host alga; (3) Rejection of *D. menstrualis* occurred only after direct larval contact with the alga's surface and was not mediated by water-borne chemical cues or by surface wettability (a physical property of an organism's surface that can affect fouling); and (4) The lipid-soluble extract from surface rubbings of *D. menstrualis* inhibited larval settlement when placed on other surfaces and contained the diterpene alcohols pachydictyol A and dictyol E. These data suggest that dictyol diterpenes produced by *D. menstrualis* occur on the alga's surface and may function to reduce fouling by causing invertebrate larvae to reject this alga as a host.

Why larvae avoid *Dictyota menstrualis* surface chemistry is unclear. However, if survival, growth, or development is significantly diminished after larvae settle on *D. menstrualis* because larvae and juveniles contact fitness-reducing dictyols on the alga's surface, this could select for larvae, such as those of the bryozoan

Bugula neritina (Schmitt *et al.* 1995), that behaviorally avoid settling on *D. menstrualis* and other *Dictyota* species. To test the hypothesis that secondary metabolites produced by algae in the genus *Dictyota* negatively affect the fitness of invertebrate larvae and developing juveniles, we forced larvae from three invertebrates species that commonly co-occur with *Dictyota* spp. in North Carolina into contact with dictyols and monitored their survival and development.

Materials and methods

We used larvae of the bryozoans *Bugula neritina* and *Amathia convoluta* and the hydroid *Eudendrium carneum* in assays testing for potential negative effects of *Dictyota* secondary metabolites on invertebrate larvae. In North Carolina, *B. neritina* and *E. carneum* commonly occur on hard substrates, other sessile invertebrates, and macroalgae, but rarely on *Dictyota* spp. (Schmitt *et al.* 1995; N. Lindquist, pers. obs.). *A. convoluta* occurs mainly on hard substrates, and often co-occurs with *Dictyota* spp.

These invertebrates were collected as needed from Radio Island Jetty near Beaufort, North Carolina during the summer and fall of 1990 and maintained in flow-through seawater tanks at the University of North Carolina's Institute of Marine Sciences in Morehead City, North Carolina. We obtained *Bugula neritina* and *Amathia convoluta* larvae by keeping adult colonies in the dark for 1–2 days then exposing them to strong light. This exposure induced adults to liberate larvae that we collected and dispensed into assay dishes. Reproductive colonies of *Eudendrium carneum* exposed to natural light/dark cycles liberated large numbers of larvae between 0900 and 1100 h each morning.

The four *Dictyota* secondary metabolites used in our assays (dictyol E, dictyol B acetate, pachydictyol A, and dictyodial; see Fig. 1 for the structures of dictyol E and dictyol B acetate, Fig. 2 for pachydictyol A, and Fig. 3 for dictyodial) were extracted from *Dictyota menstrualis* and *Dictyota ciliolata* collected from Radio Island Jetty. Cronin and co-workers (1995) showed that, among North Carolina *Dictyota* spp., pachydictyol A and dictyodial occur in both *D. menstrualis* and *D. ciliolata* while dictyol E occurs exclusively in *D. menstrualis* and dictyol B acetate occurs exclusively in *D. ciliolata*. Initial fractionation of algal crude extracts was accomplished by vacuum-flash chromatography over silica gel, with final purification by silica-gel HPLC (a detailed description of these methods is given in Cronin *et al.* 1995). Compounds were identified by nuclear magnetic resonance (NMR) spectroscopy and other spectral techniques by W. Fenical and coworkers at Scripps Institution of Oceanography.

Schmitt *et al.* (1995) showed that larvae of *Bugula neritina* significantly avoided settling on polystyrene [a highly attractive surface for *B. neritina* settlement, (Maki *et al.* 1989)] coated with a surface extract of *Dictyota menstrualis*, which contained dictyol E and pachydictyol A. Because of this, in order to determine how *Dictyota* metabolites affect larval survivorship, settlement, and development, we needed to force continued exposure to the compounds and not allow larvae to avoid exposure simply by delaying settlement. To force larval-compound contact, we placed larvae in polystyrene petri dishes filled with seawater in which we had dispersed various concentrations of purified *Dictyota* secondary metabolites.

Because we presently are unable to rigorously determine the concentration of secondary metabolites a larva would experience when settling on *Dictyota menstrualis* or *Dictyota ciliolata*, we chose to test a broad range of concentrations to determine how adverse effects varied as a function of concentration. Seven concentrations of each compound were tested: 10, 5, 1, 0.5, 0.1, 0.01, and 0 (solvent control) $\mu\text{g}/\text{ml}$. For assays with *Bugula neritina* and *Eudendrium carneum*, compounds were dissolved in ethanol so that 100 μl of the ethanolic solutions added to 10 ml of unfiltered seawater would yield the desired test concentration. Because this amount of ethanol killed *Amathia convoluta* larvae, dimethyl sulfoxide, which had no apparent effect on the survival or development of *A. convoluta* larvae, was used

as the solvent in all assays with these larvae. One hundred microlitres of ethanol or dimethyl sulfoxide, respectively, also were added to the control dishes in these experiments.

By dispersing the compounds in the seawater, we knew that the larvae would likely contact the compounds, at least during the initial phase of each assay. When Schmitt (1991) dispersed pachydictyol A into seawater, only $47 \pm 5\%$ of this compounds could be recovered by extracting the seawater with methylene chloride after 1 h. Assuming that over the 48–75 h of the assays the dictyols absorbed onto the surface of the petri dishes, we calculated that our highest concentration treatment (10 $\mu\text{g}/\text{ml}$) could have had a surface concentration of $3.0 \mu\text{g} \cdot \text{cm}^{-2}$ based on the mass of compound added and the surface area of the dish covered by the seawater. This value exceeded the hypothetical mean maximum surface concentration (HMMSC) of pachydictyol A ($1.1 \mu\text{g} \cdot \text{cm}^{-2}$) and dictyol E ($2.3 \mu\text{g} \cdot \text{cm}^{-2}$) for *Dictyota menstrualis* but was less than that of dictyol B acetate ($5.6 \mu\text{g} \cdot \text{cm}^{-2}$) for *Dictyota ciliolata*. The HMMSC value is equivalent to taking all the compound contained within, and on, an organism and distributing it evenly across its surface. Because species in the genus *Dictyota* have a 1-cell thick surface layer overlying a 1-cell thick cortex composed of non-pigmented medullary cells, and because these medullary cells are devoid of certain metabolically active organelles, such as chloroplasts, the dictyols in these seaweeds probably lie within the surface cells, with some proportion of these secondary metabolites residing on their exposed surfaces (Schmitt *et al.* 1995). The HMMSC was calculated for each of these compounds using its mean percent of *Dictyota* dry mass [0.1, 0.2, and 0.5% for pachydictyol A, dictyol E, and dictyol B acetate, respectively, (Cronin *et al.* 1995)], the wet mass to dry mass ratio (0.15) for *D. menstrualis* (G. Cronin, pers. comm.), and the wet mass to surface area value [$7.5 \text{ mg} \cdot \text{cm}^{-2}$, (G. Cronin, pers. comm.)] for *D. menstrualis*. Because *D. ciliolata* is virtually identical to *D. menstrualis* morphologically, we used the wet mass to dry mass ratio and wet mass to surface area values for *D. menstrualis* to calculate the HMMSC for dictyol B acetate produced by *D. ciliolata*. Dictyodial's instability (Cronin *et al.* 1995), prevented us from reliably determining its mean concentration in *D. menstrualis* and *D. ciliolata*, and thus its HMMSC. In contrast to North Carolina *Dictyota* spp., these metabolites can occur at much higher concentrations in tropical species of *Dictyota* (see Appendix in Hay *et al.* 1987b); thus, even our highest assay concentration may be relevant, or even low, for some situations.

Because of the small size, rapid movement, and rapid settlement of *Bugula neritina* and *Amathia convoluta* larvae, we were unable to collect and count precise numbers of larvae added to each replicate container. For each of these species, we added larvae to each of our replicate containers as equal-sized aliquots from a stirred suspension of concentrated larvae. This procedure resulted in a variable number of larvae in each replicate. For assays with the slower moving and slower settling larvae of *Eudendrium carneum*, 9–12 larvae were used in every replicate and there were 8–13 independent samples for each concentration of each compound. For assays with *A. convoluta* and *B. neritina*, there were 5–25 larvae in each replicate and 7–15 independent samples for each concentration of each compound.

After being placed in our assay dishes, larvae were allowed to settle and develop in the dark. Settlement and development were checked 1 h after larvae were added, and then at 18–24 h intervals up to 75 h. Larval settlement and juvenile development for each species were easily identified to specific developmental stages using a dissecting scope and were recorded as follows. For *Bugula neritina*, individuals were scored as dead (before settling), swimming, on the bottom but not settled, settled, settled and metamorphosed, metamorphosed with 1 zooid, metamorphosed with 2 zooids, or metamorphosed and dead. In the case of pachydictyol A, many *B. neritina* individuals metamorphosed but developed abnormally; this was noted as well. No *B. neritina* developed beyond the 2 zooid stage during the course of these experiments. *Amathia convoluta* behavior and development differed from that of *B. neritina*; thus, we scored *A. convoluta* individuals as swimming, settled, settled but dead, settled and metamorphosed, metamorphosed with 1 zooid, or metamorphosed and dead. Because of the irregular growth form of newly-metamorphosed *A. convoluta*, deformation, if it occurred, could not be clearly determined. For *Eudendrium carneum*, we scored the settlement and development of larvae as not settled, settled, settled and metamorphosed, metamorphosed with 1 polyp, or metamorphosed and dead. We

tested all 4 metabolites for their effect on *B. neritina* larvae, but assayed only dictyol E, dictyol B acetate, and pachydictyol A against larvae of *A. convoluta* and *E. carneum*. If the assumptions of normality and homogeneity of variances among the data were satisfied, 1-way ANOVA with Tukey-Kramer (T-K) multiple comparisons tests were performed to analyze for significant differences in larval survival and juvenile development among treatments. Where the assumption of homogeneity of variances was violated, we used an arcsin or arcsin ($\sqrt{\quad}$) transformation prior to performing the ANOVA. Where this assumption was still seriously violated after data transformation, the nonparametric Kruskal-Wallis test with a Tukey type multiple comparisons (Zar 1984) were performed.

Results

Exposure to *Dictyota* secondary metabolites negatively affected *Bugula neritina* larvae and developing juveniles. When exposed to 10 $\mu\text{g/ml}$ of dictyol E, 96% of the larvae and settled individuals died within 24 hours (Fig. 1a), which differed significantly from mortality levels in

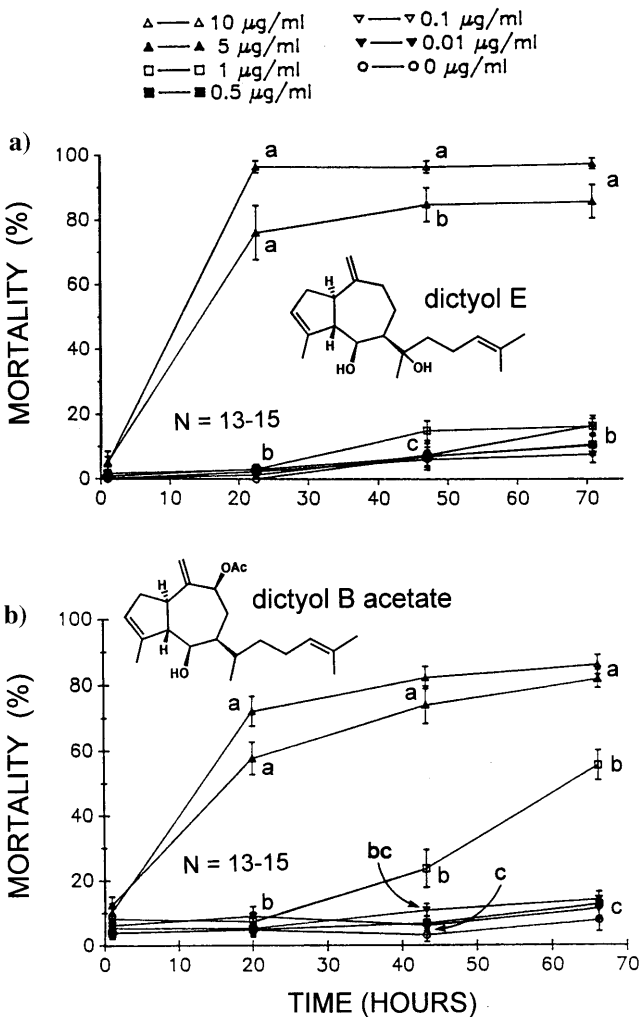


Fig. 1 Mean mortality (± 1 SE) of *Bugula neritina* larvae held in seawater containing various concentrations of dictyol E (a) and dictyol B acetate (b). Letters at each monitoring time indicate groups of treatments not significantly different from one another

the lower concentration treatments (Kruskal-Wallis/Tukey). At 5 $\mu\text{g/ml}$, 76% of the larvae died within 24 h and 86% of the larvae and settled individuals were dead by the end of the experiment (72 h). At metabolite concentrations ≤ 1 $\mu\text{g/ml}$, there were no significant effects on larval or juvenile mortality relative to controls (Fig. 1a, ANOVA/T-K with arcsin ($\sqrt{\quad}$) transformation was applied to the 43 and 67 h data sets); after 72 h, >93% of surviving juveniles in each of these treatments had developed to the 2 zooid stage. The effects of 10 and 5 $\mu\text{g/ml}$ of dictyol E on metamorphosis and juvenile development could not be reliably assessed because so few larvae survived to settle in these 2 treatments.

Bugula neritina larvae and juveniles exposed to dictyol B acetate also experienced significant mortality (Fig. 1b, ANOVA/T-K was applied to the 23, 47, and 71 h data sets). At 10 and 5 $\mu\text{g/ml}$, 72% and 58%, respectively, of larvae and newly settled individuals were dead within 20 h; mortality increased to 87% and 82%, respectively, by 66 h. At 1 $\mu\text{g/ml}$, larval mortality within 20 h was low and not significantly different from the lower concentration treatments; however, at 1 $\mu\text{g/ml}$, mortality increased to 56% within 66 h, which differed significantly from all other treatments. At dictyol B acetate concentration ≤ 0.5 $\mu\text{g/ml}$, we detected no negative effects on survival of *B. neritina* larvae and developing juveniles.

In contrast to the toxic effects of dictyol E and dictyol B acetate on *Bugula neritina* larvae, pachydictyol A did not affect larval survival or settlement ($P = 0.80$); however, it did affect juvenile survival and development. Within 19 hours, only 54–67% of individuals in the 10, 5, and 1.0 $\mu\text{g/ml}$ treatments had progressed to the 1-zooid stage which differed significantly ($P < 0.0001$, ANOVA/T-K with arcsin transformation) from the 86–92% of individuals at the 1-zooid stage in the lower concentration treatments (Fig. 2a). In the 10, 5 and 1 $\mu\text{g/ml}$ treatments, approximately one third of the individuals had metamorphosed but were deformed. The deformed individuals appeared bulbous and bent when compared to individuals in the lower concentration treatments and the control, which were stalk-like and straight. After 42 h of exposure to pachydictyol A, the percent of individuals at the 2-zooid stage in the 3 highest concentration treatments was significantly lower ($P < 0.0001$, ANOVA/T-K) than treatments having 0.5 $\mu\text{g/ml}$ or less of pachydictyol A (Fig. 2b). At 67 hours, the percent of 2-zooid individuals in the 10, 5, and 1 $\mu\text{g/ml}$ treatments (40, 42, and 62%, respectively) differed significantly ($P < 0.0001$, ANOVA/T-K with arcsin transformation) from the >84% of 2-zooid individuals in the control and 3 lower concentration treatments (Fig. 2b). Most individuals in the 10, 5, and 1 $\mu\text{g/ml}$ treatments that did not reach the 2-zooid stage had metamorphosed, developed abnormally, and then died.

As with pachydictyol A, exposure to dictyol did not effect *Bugula neritina* larvae prior to settlement for

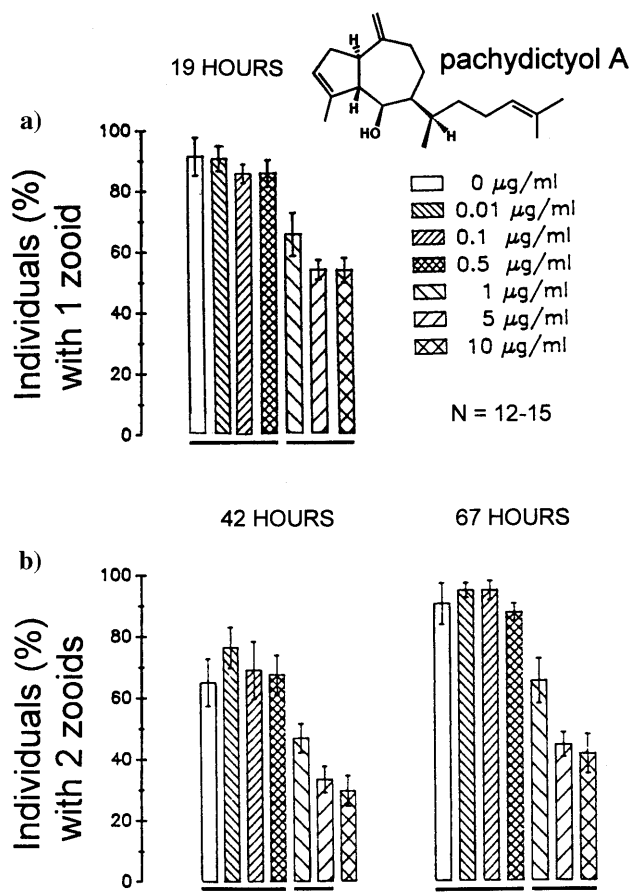


Fig. 2 Mean percent (± 1 SE) of newly settled and metamorphosed *Bugula neritina* with one zooid after exposure to various concentrations of pachydietyl A for 19 h (a) and with 2 zooids after exposure for 42 h and 67 h (b). Solid lines at the base of the bars connect treatment not significantly different from one another

any treatment; it, however, did increase post-settlement mortality and inhibit metamorphosis. After 70 h of exposure to dictyodial, no individuals in the 10 and 5 µg/ml treatments had developed to the 2-zooid stage. In contrast, the percentages of 2-zooid individuals in the lower concentration treatments were significantly higher ($>73\%$) and not significantly different ($P < 0.0001$, Kruskal-Wallis/Tukey) from the control (Fig. 3). Among larvae in the 10 and 5 µg/ml treatments, 66% and 52%, respectively, had settled (*i.e.*, adhered to the polystyrene) but died before metamorphosis, and 32% and 42%, respectively, of the larvae settled but neither died nor metamorphosed within 70 h.

Unlike larvae of *Bugula neritina*, larvae of *Amathia convoluta* had a relatively uniform response to the dictyols tested. The larval stage appeared unaffected, but after settlement, levels of metamorphosis were diminished at the higher metabolite concentrations (10 and 5 µg/ml) and unmetamorphosed settlers died. After exposure to 10 and 5 µg/ml of dictyol E, dictyol B acetate, or pachydietyl A for 68, 75, and 48 h, respectively, significantly fewer individuals had developed to

the 1-zooid stage than in the lower concentration treatments and the control (Fig. 4, $P < 0.0001$, Kruskal-Wallis/Tukey, ANOVA/T-K with arcsin transformation, and ANOVA/T-K, respectively). Across all treatments, virtually all those individuals not at the 1-zooid stage had settled but died before metamorphosis. At or below 1 µg/ml there was no significant compound effect on juvenile survivorship or development, except for pachydietyl A in which the percent of juveniles with 1 polyp at a concentration of 1 µg/ml was not significantly different from any of the other treatments (Fig. 4).

When larvae of the hydroid *Eudendrium carneum* were exposed to 10 µg/ml of dictyol E for 66 h, significantly fewer individuals (17%) settled and developed to the 1-polyp stage than in treatments with ≤ 5 µg/ml of this metabolite ($\geq 42\%$, $P < 0.0001$, ANOVA/T-K with arcsin ($\sqrt{\quad}$) transformation, Fig. 5). Exposure to 10 and 5 µg/ml of dictyol B acetate significantly suppressed juvenile development ($P < 0.0001$, ANOVA/T-K with arcsin ($\sqrt{\quad}$) transformation). After 73 h of exposure, $<10\%$ of individuals at 10 and 5 µg/ml of dictyol B acetate developed to the 1-polyp stage, while 38–60% of the individuals in the other treatments had developed 1-polyp. The majority of individuals ($>79\%$) in the 10 and 5 µg/ml treatments were alive but had not settled within 73 h. Unlike dictyol E and dictyol B acetate, pachydietyl A had no significant effect ($P = 0.10$, ANOVA) on the settlement, metamorphosis, or development of *E. carneum* larvae (Fig. 5).

Discussion

Proposed antifouling mechanisms of seaweeds include: (1) periodic sloughing of surficial cells (Moss 1982; Masaki *et al.* 1984; Johnson & Mann 1986), (2) associ-

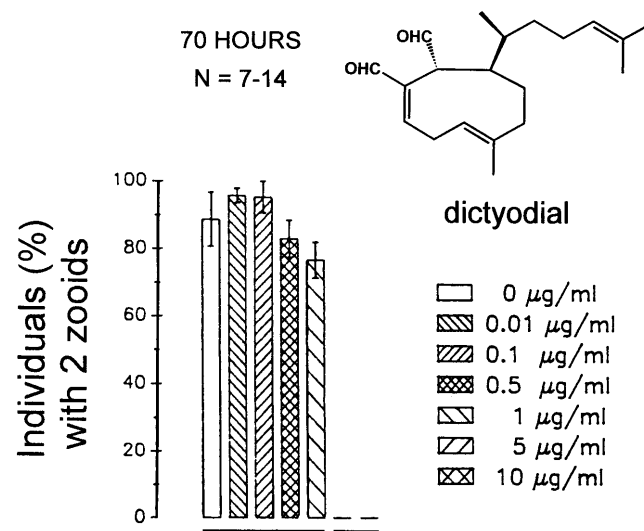


Fig. 3 Mean percent (± 1 SE) of newly settled and metamorphosed *Bugula neritina* with 2 zooids after exposure to various concentrations of dictyodial for 70 h. All symbols as in Fig. 2

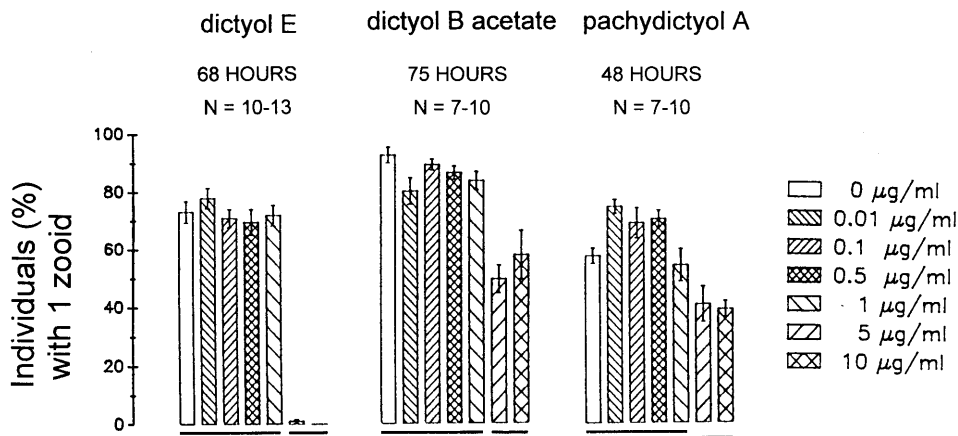


Fig. 4 Mean percent (± 1 SE) of newly settled and metamorphosed *Amathia convoluta* with 1 zooid after exposure to various concentrations of dictyol E, dictyol B acetate, or pachydictyol A for 68, 75, and 48 h, respectively. All symbols as in Fig. 2

ations with consumers that feed on fouling organisms but that cause minimal damage to the host alga (Brawley & Adey 1981; Steneck 1982; Duffy 1990; Brawley 1992; Stachowicz & Hay 1996), and (3) production of secondary metabolites that can inhibit the settlement or development of fouling organisms (de Nys *et al.* 1991, 1995; Henrickson & Pawlik 1995; Schmitt *et al.* 1995). Brown algae of the genus *Dictyota* are rarely fouled (Schmitt *et al.* 1995), but because these algae have only a 1-cell thick layer of surface cells overlying a cortex composed of a 1-cell thick layer of large, non-pigmented medullary cells, sloughing of surficial cells is not an option for this genus. Additionally, although some amphipods are known to selectively associate with *Dictyota*, these amphipods eat *Dictyota* as readily as some of its epiphytes, suggesting that they might impose a high cost for their cleaning services (Hay *et al.* 1987a,b; Hay *et al.* 1990; Duffy & Hay 1991, 1994). Surface wettability, which can influence larval settlement (Brewer 1984; Rittschof & Costlow, 1989a,b; Roberts *et al.* 1991; Pawlik 1992), also does not explain the low level of fouling on *Dictyota menstrualis* (Schmitt *et al.* 1995). Alternatively, *Dictyota* spp. are a rich source of bioactive secondary metabolites

(Faulkner 1996 and references cited therein) that previously have been shown to deter feeding by common marine herbivores, including fishes, urchins and amphipods (Hay & Fenical 1992). These compounds also may function to repel larvae of fouling invertebrates (Schmitt *et al.* 1995), kill larvae (Fig. 1), inhibit larval settlement, or harm newly settled foulers (Figs. 2–5).

In contrast to the rapid progress made in documenting the ecological advantages of chemically repelling consumers (see reviews by Paul 1992; Pawlik 1993; Hay 1996; Hay *et al.* 1998), ecologically realistic demonstrations of secondary metabolites as antifouling agents have been hampered, in general, by a lack of data on where compounds occur in hosts and how the compounds are deployed (but see Stoecker 1978; Coll *et al.* 1982; Thompson 1985; Lindquist *et al.* 1991a,b). However, results of this study, in conjunction with the demonstration by Schmitt *et al.* (1995) that pachydictyol A and dictyol E occur on the surface of *Dictyota menstrualis*, provide strong evidence that secondary metabolites from *Dictyota* spp. can function to prevent fouling. When we forced invertebrate larvae into contact with *Dictyota* secondary metabolites, which they behaviorally avoid by not settling on *Dictyota* spp.

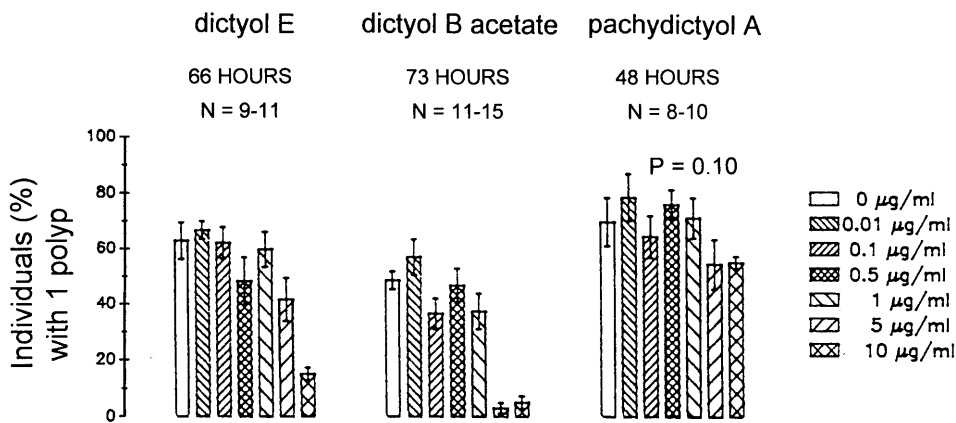


Fig. 5 Mean percent (± 1 SE) of newly settled and metamorphosed *Eudendrium carneum* with 1 polyp after exposure to various concentrations of dictyol E, dictyol B acetate, or pachydictyol A for 66, 73, and 48 h, respectively. All symbols as in Fig. 2

(Schmitt *et al.* 1995), these compounds caused larval mortality, reduced rates of settlement, abnormal development, and reduced rates of growth at concentrations down to 1 µg/ml (Figs. 1–5). The morphology of *Dictyota* spp. strongly suggests that their secondary metabolites occur exclusively in surface cells and are deployed to some extent on their exposed surfaces (Schmitt *et al.* 1995). In several assays we observed negative compound effects on larvae and juveniles at levels well below the maximum possible surface concentration. For example, 1 µg/ml of dictyol B acetate killed *Bugula neritina* larvae (Fig. 1b) – if all the compound added to the seawater in this assay had adhered to the container surface, this treatment would have represented only 5% of the maximum possible surface concentration for dictyol B acetate in *Dictyota ciliolata* from North Carolina where our plants were collected. Because the dictyols can occur at much higher concentrations in tropical than in temperate species of *Dictyota* (Hay *et al.* 1987b), even our highest concentration treatment may be relevant, or even low, for some tropical species.

It is clear that *Dictyota* secondary metabolites **could** harm settling larvae (Figs. 1–5), and thus, potentially select for larvae that behaviorally avoid settling on *Dictyota*, as Schmitt *et al.* (1995) reported for *Bugula neritina* larvae; the degree to which they **do** harm fouling larvae and juveniles settled on *Dictyota* spp. is unclear. Our results (Figs. 1–5) suggest that these algal metabolites may act as chemical defenses against fouling for *Dictyota menstrualis*, and perhaps other *Dictyota* spp. (e.g., *Dictyota ciliolata*). However, our confidence in this conclusion is tempered by our lack of knowledge concerning: (1) the actual surface concentration of these compounds on *D. menstrualis* and *D. ciliolata*, (2) the effects on larvae of secondary metabolites adsorbed onto surfaces vs. dispersed in seawater, and (3) additive or synergistic effects among *Dictyota* secondary metabolites. As discussed in greater detail by Hay (1996) and Hay *et al.* (1998), for substantial progress to occur in research on chemical antifouling mechanisms of marine organisms, ecological and chemical underpinnings need to be established that: (1) rigorously identify host preferences of common fouling species (e.g., Schmitt *et al.* 1995), (2) employ new technologies for measuring levels of secondary metabolites that settling larvae would experience on contact with a chemically rich, low-preference host (see de Nys *et al.* 1998 for a possible example), and (3) develop assay methodologies that deploy compounds in ecologically realistic ways.

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