FEEDING PREFERENCES OF THE ENDEMIC GASTROPOD *Astraea latispina* IN RELATION TO CHEMICAL DEFENSES OF BRAZILIAN TROPICAL SEAWEEDS

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ABSTRACT

Seaweed preference by the Brazilian endemic gastropod *Astraea latispina* was examined in the laboratory to evaluate the role of secondary metabolites in determining food choice. Of three species of seaweeds examined, *Plocamium brasiliense* was highly preferred; less so were *Sargassum furcatum* and *Dictyota cervicornis* were preferred less. Extracts and/or pure major metabolites of the two potentially chemically-defended seaweeds (*P. brasiliense* and *D. cervicornis*) were tested as feeding deterrents against *A. latispina*. Algal extract assays demonstrated that three concentrations of crude organic extract of the red alga *P. brasiliense* (50%, 100%: natural concentration, and 200% of dry weight: dw) did not affect feeding of this gastropod. In contrast, the three concentrations of crude organic extract of the brown alga *D. cervicornis* (50%, 100% and 200% dw) inhibited feeding by *A. latispina*. The chemical deterrent property of *D. cervicornis* extract against the gastropod *A. latispina* occurred due to a mixture of the secodolastane diterpenes isolinearol/linearol (4:1 – 0.08% dry weight). This is the first report showing that *Dictyota cervicornis* produces a chemical defense against herbivores using secodolastane diterpenoid. In addition, these results widen the action spectrum of secondary metabolites found in seaweed belonging to this brown algal genus.

Key words: seaweed, *Dictyota cervicornis*, chemical defenses, herbivory.

RESUMO

Preferência alimentar do gastrópodo endêmico *Astraea latispina* relacionada a defesas químicas de algas bentônicas tropicais brasileiras

A preferência alimentar do gastrópodo endêmico *Astraea latispina* por macroalgas bentônicas foi avaliada em laboratório com o intuito de evidenciar o papel de metabólitos secundários em sua escolha alimentar. Dentre as macroalgas examinadas, *Plocamium brasiliense* foi preferencialmente consumida, enquanto *Sargassum furcatum* e *Dictyota cervicornis* foram menos consumidas. Os extratos brutos e/ou metabólitos majoritários puros das duas espécies de macroalgas potencialmente produtoras de defesa química (*P. brasiliense* e *D. cervicornis*) foram testados em relação à *A. latispina*. As três concentrações de extrato bruto (50%, 100%: concentração natural, e 200%: peso seco – ps) de *P. brasiliense* não inibiram a herbivoria dessa espécie de gastrópodo. Por outro lado, as três concentrações de extrato bruto de *D. cervicornis* (50%, 100% e 200% ps) inibiram a herbivoria de *A. latispina*. Constatou-se que a propriedade deterrente do extrato de *D. cervicornis* em relação ao gastrópodo *A. latispina* deve-se à mistura dos diterpenos isolinearol/linearol (4:1 – 0,08% ps). Este é o primeiro relato de que *D. cervicornis* produz defesa química contra herbívoros, particularmente diterpenos com esqueleto carbônico do tipo secodolastano. Além disso, esses resultados ampliam o espectro de ação dos metabólitos secundários encontrados em espécies desse gênero de alga parda.

Palavras-chave: alga bentônica, *Dictyota cervicornis*, defesa química, herbivoria.

INTRODUCTION

Today, and probably for most of evolutionary time, seaweed secondary metabolites are the most important chemical defense strategy against herbivory by fishes, urchins, and gastropods. Several of these herbivores strongly affect seaweed community structure and dynamics (John *et al*., 1992). To avoid, minimize, or tolerate the damages due to herbivory, seaweeds exhibit several adaptations such as decreased attractiveness or tolerance to herbivores (Duffy & Hay, 1990). Among a diverse array of adaptations to reduce attractiveness, chemical defenses are one of the most conspicuous strategies manifested by seaweed species living in tropical reef environments or regions under high herbivore pressure (Hay & Steinberg, 1992; Paul, 1992).

Recent research in marine chemical ecology has revealed high structural diversity of different secondary metabolites in seaweeds that are effective as chemical defenses against herbivores (Hay, 1991; Hay & Steinberg, 1992; Paul, 1992). Although several species of Chlorophyceae, Phaeophyceae, and Rhodophyceae exhibit secondary metabolites with broad ecological significance (Paul, 1992), brown algae belonging to the order Dictyotales are well known for producing similar secondary metabolites extensively tested as defenses against herbivores. The broad-spectrum of defensive activity of *Dictyota* metabolites against several types of herbivores is related to well-studied and common examples of chemical defenses belonging to the family of structurally similar diterpenes called the dictyols, including pachydictyol A, dictyol E, dictyol B, dictyol B acetate, and dictyol H (see Pereira *et al*., 1994, for amphipod *Parhyale hawaiensis*, and Hay & Steinberg, 1992, for a review about remaining herbivore species). Natural concentrations of pachydictyol A found in some species of *Dictyota* inhibit herbivory by tropical parrotfish species, by temperate fishes *Lagodon rhomboides* and *Diplodus holbrooki*, and by the sea urchin *Diadema antillarum*. In contrast, this same metabolite does not inhibit feeding by the sea urchin *Arbacia punctulata*, the cosmopolitan polychaete *Platynereis dumerilii*, the amphipod *Parhyale hawaiensis* and even increases feeding by the amphipod *Ampithoe longimana*. Dictyol E reduces herbivory by *L. rhomboides*, *D. holbrooki*, and *A. punctulata*, but is not an effective defense against *A. longimana* and *P. dumerilii*. Finally, three of these dictyol metabolite pachydictyol A, dictyol B, and dictyol H significantly inhibit herbivory by the rabbitfish *Siganus doliatus*. All these dictyol metabolites only represent a structural class of the prenylated guaiane diterpenoid metabolites.

However, two other structural classes of diterpenes distinct from dictyols, but also found in *Dictyota* species, can inhibit herbivory. An uncommon diterpene (dichotomane diterpene class) found in *D. menstrualis* from Brazil inhibits herbivory by the amphipod *Parhyale hawaiensis* (Pereira *et al*., 2000b). The feeding deterrent property of dictyodial (xeniane diterpene class) found in *D. menstrualis* from North Carolina is poorly studied due to its chemical instability (Cronin *et al*., 1995), but probably may deter feeding by the sea urchin *A. punctulata* (Cronin & Hay, 1996b).

Despite abundant literature about seaweed chemical defenses in the Caribbean sea (Bolser & Hay, 1996), in Guam – tropical Pacific (Pittik & Paul, 1997), and North Carolina, USA (Cronin & Hay, 1996a), little is known about the abundance and ecological significance of seaweed secondary metabolites in the South Atlantic (Fleury *et al*., 1994; Pereira *et al*., 1990, 1994, 2000a, b; Pereira & Yoneshigue-Valentin, 1999). Studies in the South Atlantic region may help in understanding both biogeographic and evolutionary aspects related to distribution and action of seaweed chemical defenses.

In this study, the endemic gastropod *Astraea latispina* was selected to examine the relationship between its feeding preferences and the presence of secondary metabolites found in some tropical seaweeds. Specifically we asked the following questions: 1. What species of seaweed are preferred by *A. latispina*? 2. Are *A. latispina* feeding preferences related to the presence of secondary metabolites found in these seaweeds? 3. Do the less preferred seaweeds contain deterrent compounds? and 4. What compound is responsible for the deterrent properties?

MATERIAL AND METHODS

Study site and organism

All organisms used in this investigation were collected at Enseada do Forno (Búzios, Rio de

Janeiro), a tropical region on the Brazilian Southeastern coast (22°45'S and 41°52'W). On its rocky shore, the crustose coralline *Amphiroa* spp. and *Jania adhaerens* are the most common seaweeds. The seaweeds *Dictyota cervicornis*, *Sargassum furcatum*, and *Plocamium brasiliense*, and the endemic gastropod *Astraea latispina* co-occur subtidally (0-2 m) in this region. However, *P. brasiliense* and *A. latispina* are more closely related spatially.

Feeding experiments

The gastropod *A. latispina* was selected as the test herbivore since it was the most abundant one found closely associated to red alga *P. brasiliense*. Prior to the experiments, *A. latispina* was maintained on a diversified diet (various algal species), because starvation may alter the exact feeding preference of some herbivores (Cronin & Hay, 1996b).

Seaweed susceptibility was evaluated by using a multiple-choice experiment in 20 small aquaria (replicates). In each, a previously weighed specimen of each species, *D. cervicornis*, *P. brasiliense*, and *S. furcatum* were placed together with 1 individual of *A. latispina*. All gastropod individuals used in the assays were previously weighed and measured to ensure specimens of the same size and probably similar age. After 24 h, seaweeds were reweighed to determine biomass loss due to herbivory. All weights were obtained from wet material from which excess water had been removed using a manual salad centrifuge. Control aquaria (20 replicates) without *A. latispina* contained one weighed specimen of each species. They were maintained under the same experimental conditions, allowing the measurement of biomass changes due to autogenic factors and not by herbivory (Peterson & Renaud, 1989). The consumed biomass was calculated using the equation $[(Ho \times Cf/Co) - Hf]$ suggested by Cronin & Hay (1996b) for this type of feeding experiment. Ho and Hf correspond to the initial and final wet masses of seaweeds exposed to herbivory, respectively, and Co and Cf the initial and final masses of the control.

Chemical procedures

The fresh specimens of *D. cervicornis* (54.5 g) were extracted using a mixture of dichloromethane/methanol solvents (2:1) at room temperature. Evaporation of the solvent under reduced pressure yielded a brownish residue (2.19 g), which was subjected to vacuum column chromatography on silica gel (60GF Merck) eluted with hexane/chloroform, chloroform, chloroform/ethyl acetate, ethyl acetate, ethyl acetate/methanol, and methanol. The fractions eluted with chloroform gave the mixture of the diterpenes isolinearol/linearol. The identities of these secodolastane diterpenes were confirmed by ^{1}H NMR spectral data (300 MHz, $CDCl_3$) with those reported in the literature (Teixeira *et al.*, 1986a). Extract of *P. brasiliense* was prepared using dichoromethane:methanol (2:3).

Effect of seaweed extracts and pure compounds

The effects of the *D. cervicornis* and *P. brasiliense* extracts were investigated by adding known amounts of extracts to a surface of *Ulva* disks. Disks of *Ulva* with three different concentrations (50%, 100% – natural concentration, 200%) of each extract and one control disk (n = 20 for each algal extract) were placed simultaneously in each 350 ml plastic chamber with one individual of *A. latispina*. Control disks were *Ulva* without extract added. The consumption percent was recorded after about 24 h.

Chemical deterrence of the pure major compounds found in *D. cervicornis* was verified by including natural concentrations of isolinearol/linearol (4:1) in an artificial food prepared according to Hay *et al.* (1994). The artificial food (control) was prepared by adding 0.72 g of agar in 20.0 ml of distilled water and heating in a microwave oven until boiling. This mixture was added to 16.0 ml of cold water containing 2.0 g freeze-dried *Ulva*. Experimental food (treatment) was similarly prepared but the pure compounds were first dissolved in diethyl ether, added to the 2.0 g of freeze-dried *Ulva*. Solvents were then removed by rotatory evaporation. This procedure is necessary to obtain a uniform coating of metabolite on the algal particles prior to being adding to the agar (Hay *et al.*, 1994). Treatments and controls were hardened onto a screen and cut to obtain small pieces (7 × 10 squares of about 1.2 × 1.4 mm) which were then simultaneously offered to *A. latispina*. Assays were carried out in small plastic chambers, each containing 400.0 ml of seawater (n = 9-15 replicates), each of which contained 1 individual of *A. latispina*. The deterrent activity of secondary metabolites was estimated by comparing the numbers of squares consumed in both experimental and control foods. New gastropod specimens were used in each assay (with extract and pure compounds).

Statistical analysis

The Friedman test suggested by Rhoa (1992) was used to analyze the existence of food preference between the species because this test allows independence between treatments. In addition, paired significant differences were assessed by the Student-Newman-Keuls test. Remaining results of the experiments were analyzed by single paired-sample *t* tests.

RESULTS

Seaweed susceptibility to herbivory

The assay revealed that *P. brasiliense* was strongly preferred or consumed by *A. latispina* (Fig. 1). On the other hand, *A. latispina* fed significantly less ($p < 0.001$; *t* test) on *S. furcatum* (9.98 mg ± 9.52 – ~10% consumed) than on either *D. cervicornis* (36.77 ± 20.69 ~37% consumed) or *Plocamium brasiliense* (66.99 ± 35.38 ~68% consumed). The Friedman test demonstrated that the three species assayed were differently consumed by *A. latispina* ($p < 0.001$). In fact, the pairwise comparison also revealed that the three species were differently consumed ($p < 0.05$, Student-Newman-Keuls).

Herbivory responses to extracts and pure compounds

The *P. brasiliense* extract was ineffective ($p > 0.05$, *t* test) as a chemical defense against herbivory by *A. latispina* (Fig. 2) in whatever of the three concentrations were tested. Thus, below: 50% ($p > 0.272$), natural: 100% ($p > 0.064$), and double natural: 200% ($p > 0.055$) concentrations of *P. brasiliense* extract do not inhibit feeding by this gastropod. In fact, *P. brasiliense* highly susceptible to herbivory by this gastropod species, as observed in the previous feeding assay (Fig. 1).

On the other hand, all three extract concentrations of *D. cervicornis* strongly deterred feeding by *A. latispina* ($p < 0.001$, *t* test), at below, natural, and double the natural concentrations (Fig. 3).

Finally, it was verified that the natural concentration (0.08%, dry weight) of the mixture containing the secodolastane diterpenes isolinearol (1)/linearol (2) (4:1) found in *D. cervicornis* (Fig. 4) significantly reduced feeding by *A. latispina* (Fig. 5, $p < 0.05$, *t* test).

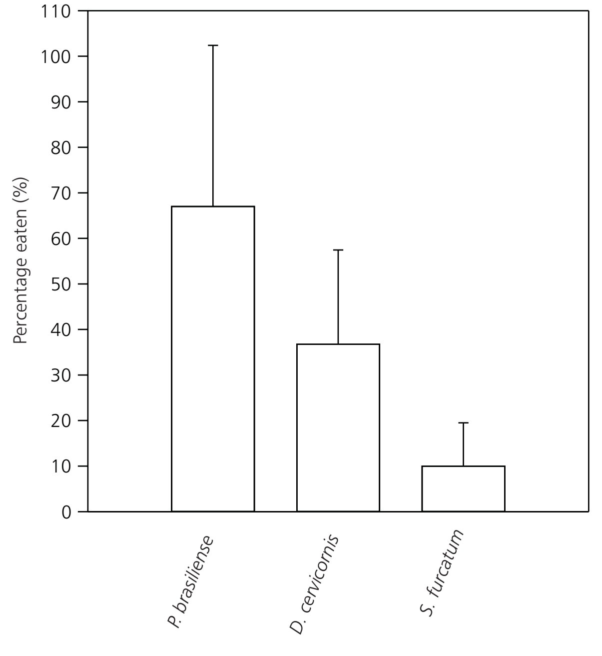

Fig. 1 – Algal biomass consumption percentage by the gastropod *A. latispina* of three seaweeds were offered simultaneously: *P. brasiliense*, *D. cervicornis*, and *S. furcatum*.

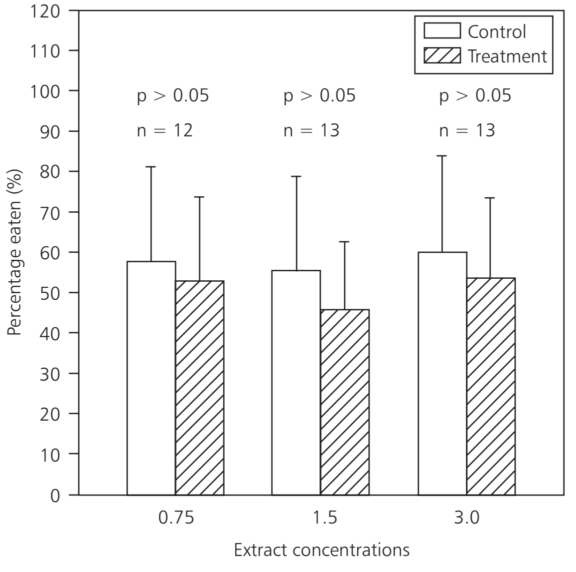

Fig. 2 — The effects of *P. brasiliense* crude extract on herbivory by the gastropod *A. latispina*. The p-values were generated by paired *t* test.

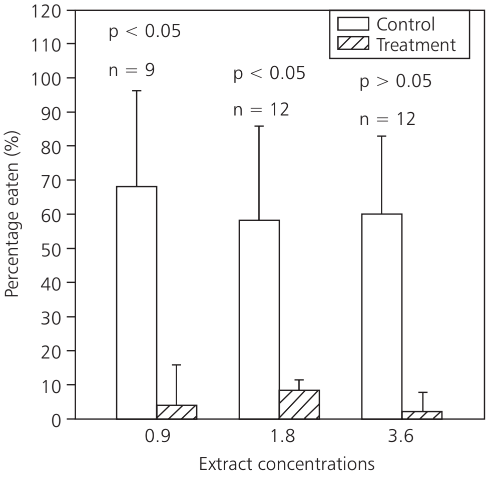

Fig. 3 — The effects of *D. cervicornis* crude extract on herbivory by the gastropod *A. latispina*. The p-values were generated by paired *t* test.

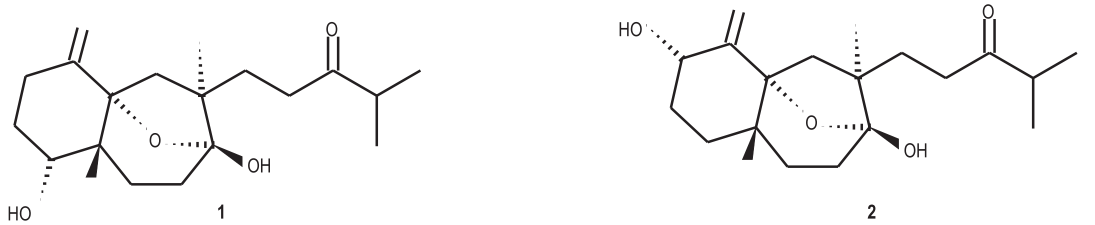

Fig. 4 — Major diterpenoid metabolites isolinearol (**1**) and linearol (**2**) found in *D. cervicornis*.

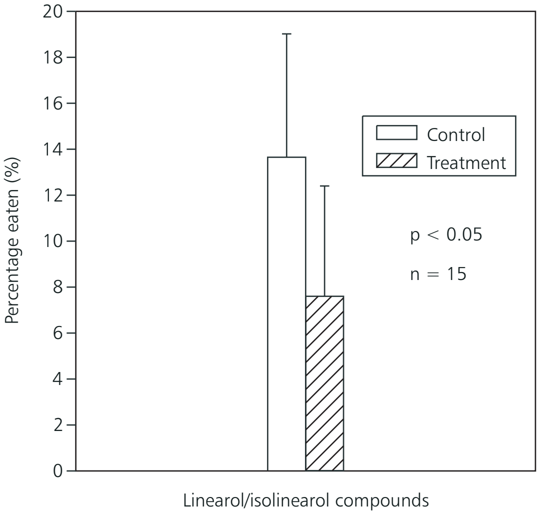

Fig. 5 — The effect of the mixture of isolinearol and linearol on herbivory by *P. hawaiensis*. The p-value was generated by paired *t* test.

DISCUSSION

For seaweed susceptibility to herbivory investigated here by a multiple-choice feeding experiment, it is clear that *S. furcatum* is less preferred by the gastropod *A. latispina* as compared to other remaining species (*P. brasiliense* and *D. cervicornis*). The following decreasing sequence was observed among species: *P. brasiliense* > *D. cervicornis* > *S. furcatum*.

Plocamium species have been extensively studied by chemists (Faulkner, 2000 and previous reviews cited therein) and are known to produce a diverse array of secondary metabolites including halogenated monoterpenes with potential ecological properties (Konig *et al*., 1999).

Specimens of Brazilian *P. brasiliense* also produce different secondary metabolites (the authors, not published), but these were not effective as chemical defenses against herbivory by *A. latispina*. These results demonstrate that *A. latispina* living closely associated with *P. brasiliense* in the natural environment may consume large amounts of this preferred alga.

In general, defensive compounds against fishes and urchins do not affect small and relatively sedentary herbivores such as amphipods, polychaetes, and crabs. In fact, mesograzer herbivores subjected to high predation rates may preferentially feed on these types of plants in order to minimize their susceptibility to natural enemies (Duffy & Hay, 1994). It is not surprising that the gastropod *A. latispina* by living associated to *P. brasiliense*, may minimize its susceptibility to predation. However, this hypothesis should be further investigated.

In general, *Sargassum* species may be either more or less preferred by herbivores (Steinberg & Paul, 1990; Steinberg *et al*., 1991). For example, contrary to the results obtained here, *S. furcatum*, also collected on the Brazilian coast, is highly preferred by the amphipod *P. hawaiensis* (Pereira & Yoneshigue-Valentin, 1999) and by the crab *Pachygrapsus transversus* (Pereira *et al*., 2000a).

Also, *Sargassum* species can produce high (Targett *et al*., 1992) and low quantities (Van Alstyne *et al*., 1999) of polyphenols, but these compounds have been shown to deter feeding by herbivores only at concentrations ranging from 2% to 6% (dry mass) or higher (Geiselman & McConnell, 1981; Pereira & Yoneshigue-Valentin, 1999; Steinberg, 1988; Winter & Estes, 1992). Specimens of Brazilian *S. furcatum* produce low amounts of polyphenols and these quantities are incapable of inhibiting herbivory by *P. hawaiensis* (Pereira & Yoneshigue-Valentin, 1999). In addition, potentially defensive terpenoid compounds were not detected and the sterol fraction (mainly fucosterol) of Brazilian *S. furcatum* did not inhibit herbivory by *P. hawaiensis* (Fleury *et al*., 1994). Of course, if polyphenols are the main defense against grazers on *Sargassum* species, Brazilian *S. furcatum* is not defended against herbivory, and the lower consumption by *A. latispina* may be due to characteristics other than chemical makeup.

The species *D. cervicornis* was less preferred by *A. latispina* and the observed lowest consumption was due to secondary metabolites found in this species. Previous studies of *D. cervicornis* from the Brazilian coast showed the occurrence of 17 different diterpenes belonging to secodolastane and dolastane skeletal classes (Fleury *et al.*, 1994; Teixeira *et al.*, 1986a, b; Kelecom & Teixeira, 1988). However, our study reports that *D. cervicornis* exhibits a natural mixture of two diterpenes with a secodolastane carbon skeleton as a chemical defense against the gastropod *A. latispina*. In addition, this is the first report showing that these compounds are a structural class of defensive diterpenes found in *Dictyota* species. In general, it is more usual that several defensive secondary metabolites produced by *Dictyota* species consist primarily of dictyols, a class of diterpenes with guaiane carbon skeleton (for reviews, see Hay, 1991; Hay & Steinberg, 1992; Paul, 1992).

There are several different results on susceptibility to and action of chemical defenses produced by *D. cervicornis* against herbivores. For example, some studies show that extract from this species can inhibit herbivory by the fish *Zebrasoma flavescens* (Wylie & Paul, 1988), yet in areas under high herbivory pressure, this alga is susceptible to many herbivorous fishes (Littler *et al.*, 1983; Lewis, 1986; Paul, 1987). On the other hand, this species was almost never used for domicile construction by the amphipod *Pseudoamphithoides incurvaria*, probably because this species does not produce chemicals which stimulate this amphipod to build domiciles (Hay *et al.*, 1990).

These conflicting or contrary results demonstrate that chemical variation, both qualitative and quantitative, may occur within a habitat or at differing time scales. Then, besides widing the action spectrum of secondary metabolites found in *Dictyota* species, the evidence of chemical defense in *D. cervicornis* is also important in understanding biogeographic and evolutionary aspects related to the distribution and functioning of seaweed chemical defenses.

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