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Phenolic Acid Content of Vegetative Propagules of *Potamogeton* spp. and *Hydrilla verticillata*

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ABSTRACT

Plant phenols serve as defenses against attack by microorganisms and herbivores. Phenolic acid content of propagules of submersed aquatic plants were measured for three *Potamogeton* species and two biotypes of *Hydrilla verticillata*. Mean phenolic acid content ranged from 6.9 $\pm$ 0.9 (mean $\pm$ standard error) to 53.1 $\pm$ 7.3 µM g dw^{-1}. Turions of monoecious *Hydrilla verticillata* had the lowest phenolic acid content while *Potamogeton gramineus* winter buds had the highest phenolic acid content. In general *Potamogeton* propagules had higher phenol contents than *Hydrilla* propagules. Within species there was no significant negative relationship between propagule nitrogen content (%) and phenolic acid content, but across species phenolic acid content increased with increasing nitrogen content. This information will be useful in assessing the susceptibility of aquatic weeds to biological control techniques.

Keywords: pondweeds, plant defense, tissue nitrogen, tissue carbon, turions, winter buds.

INTRODUCTION

Phenolic compounds are present in plant seeds (Hendry 1993) and are believed to enhance survival by functioning as deterrents to herbivore grazing and attack by fungi and soil microorganisms (Walker 1975, Levin 1976). In contrast to terrestrial plants, there is a strong tendency for many species of rooted submersed aquatic plants to rely on vegetative structures (tubers, turions, and winter buds) more than upon seeds for perennation (Sculthorpe 1967, Hutchinson 1983). These structures are often produced underground. Although levels and types of phenolic compounds present in above ground portions of aquatic plants have been reported for some marine (Harborne and Williams 1976, Zapata and McMillan 1979, McMillan *et al.* 1980, Harrison and Durance 1989, Buchsbaum *et al.* 1990) and freshwater species (Su *et al.* 1973, Woodward *et al.* 1974, Planas *et al.* 1981, Martyn *et al.* 1983, Martyn and Cody 1983, Pip 1992) those in underground reproductive structures of rooted aquatic plants have not. Populations of microorganisms in sediments are typically orders of magnitude greater than those present in the water column (Wetzel 1983) and plant propagules are important sources of food for waterfowl (Kantrud 1990) and invertebrates (Balciunas and Purcell 1991, O'Brien and Pajni 1989). In some habitats (i.e., those that experience seasonal desiccation; see Spencer and Ksander 1992) underground reproductive structures are a critical stage in the plant's life cycle. Understanding characteristics that influence propagule survival in the sediment may be important in developing novel management approaches such as enhancing attack by microorganisms (Kremer 1993). This paper presents data on the level of phenolic compounds present in vegetative propagules produced by three *Potamogeton* species and two biotypes of *Hydrilla*.

METHODS

Tubers were collected from *Potamogeton pectinatus* L. growing in the Byrnes Canal (Solano County, California: see Spencer and Ksander 1992) on November 9, 1993. On November 22, 1993 winter buds from *P. nodosus* Poir. and *P. gramineus* L. were collected from cultures maintained at the USDA Aquatic Weed Control Research Laboratory, Davis, California. Subterranean turions (tubers) and axillary turions (turions) were collected from cultures of monoecious and dioecious *Hydrilla verticillata* (L.f.) Royle also maintained at the USDA Aquatic Weed Control Research Laboratory on that date. Within 1 hour of collection, 10 propagules of each plant were placed in liquid N. Tubers of *P. pectinatus* were stored at -70 C for 14 days prior to lyophilization and phenol determination. The other propagules were lyophilized and analyzed for phenolic acid content within 24 h of collection. Individual lyophilized propagules were ground to a fine powder with a mortar and pestle and 25 mg used for phenol determination using the method described by Hendry (1993) which is based on procedures described by Forrest and Bendall (1969). Briefly, this method determines the concentration of dihydroxyphenols (simple to polymeric) by their reaction with potassium titanium oxalate which forms a red/orange color that can be measured spectrophotometrically at 445 nm. Pyrogallol was used as the standard. The method should be suitable since dihydroxyphenols have been reported for submersed aquatic plant tissues. For example, the following dihydroxyphenols have been reported for shoots of *P. gramineus*: gentisic acid, protocatechuic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid, and syringic acid; and *P. pectinatus*: ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, and syringic acid (Pip 1992). Woodward *et al.* (1974) reported that ferulic acid, p-

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coumaric acid, vanillic acid, protocatechuic acid, and caffeic acid were present in *Hydrilla* shoots. In *Hydrilla,* caffeic acid levels were 50 to 100 times those of the other phenolic compounds.

In addition, to the individual fresh weight and lyophilized dry weight, the nitrogen and carbon contents (%) were determined for each propagule using a Perkin Elmer 2400 CHN analyzer. Results of phenol determination were expressed as μM g dw^{-1} or μM g N^{-1} or C to facilitate comparison with published values for seeds.

To determine if there were differences in phenolic acid content among the seven propagule groups (i.e., a group was the combination of species and propagule type) we used the general linear model programs in SAS to calculate an analysis of variance and Tukey's HSD procedure for an *a posteriori* means comparison. For each group of propagules, we also performed linear regression of phenolic acid content on N content to test the hypothesis that phenolic acid content was related to nitrogen status of the propagule. We performed a similar analysis for all propagules, i.e. across groups.

RESULTS AND DISCUSSION

Mean phenolic acid content (μM g dw^{-1}) for the seven groups of propagules varied more than seven-fold (Table 1) with significant ($P < 0.01$) differences among the groups. On a dry weight basis there was a tendency for *Potamogeton* spp. to have higher levels than *Hydrilla*. The lower phenolic content of *Hydrilla* propagules may mean that they would be more susceptible to biological control techniques that use herbivores and/or pathogens. Mean phenolic acid content g N^{-1} or g C^{-1} also differed significantly ($P < 0.01$) among propagule groups (Table 1).

Mean phenolic acid content for vegetative aquatic plant propagules were similar to reported values for seeds of terrestrial plants (Hendry 1993). According to Hendry (1993), seeds with phenol contents around 25 μM g dw^{-1} were described as low and those with phenol contents of 32 or 45 μM g dw^{-1} were described as intermediate. Values of 93 to 96 μM g dw^{-1} characterized seeds with high phenolic acid content. On this basis, vegetative propagules of aquatic plants belong in the low or intermediate categories.

Phenolic content of vegetative propagules were similar to levels reported for aquatic plant shoots. Planas *et al.* (1981) reported that shoots of *Myriophyllum spicatum* contained 7% phenolic compounds on average. Expressed in a similar way, values in Table 1 range from 6.7 to 0.8% for *P. gramineus* winter buds and monoecious *H. verticillata* tubers, respectively. Harrison and Durance (1989) reported that phenolic content of *Zostera marina* L. shoots varied seasonally from 0.65 to 1.54% dry weight. Phenolic compounds constituted about 0.7% of dry weight for *H. verticillata* growing in an unnamed lake in south Florida (Woodward *et al.* 1974).

Buchsbaum *et al.* (1990) reported that leaf nitrogen content and phenolic content were inversely related for *Zostera marina* growing in running sea water mesocosms with either mud or sand substratum. Similar inverse relationships between tissue nitrogen and some nitrogen-free constituents (i.e., phenolics, carbohydrates) have been observed in several species of vascular plants (Mattson 1980, Buchsbaum *et al.* 1981, Bryant *et al.* 1987) and macroalgae (Neish *et a*l. 1977, Macler, 1986). When nitrogen limits growth and light does not, there may be more fixed carbon than can be metabolized into proteins, thus plants accumulate phenolics or other nitrogen-free constituents (Tuomi *et al.* 1984, Bryant *et al.* 1987). The results of linear regression of phenolic content (μM g dw^{-1}) against nitrogen content (% dry weight) for

TABLE 1. PHENOL, C AND N CONTENT FOR PROPAGULES OF ROOTED AQUATIC PLANTS. VALUES ARE MEAN ± STANDARD ERROR, N = 10. THE 'D' IN PARENTHESIS INDICATES DIOECIOUS PLANTS AND THE 'M' DENOTES MONOECIOUS PLANTS. WITHIN A COLUMN, THE UPPERCASE LETTERS BELOW THE MEAN AND STANDARD ERROR SHOW THE GROUPING OF MEANS THAT RESULTED FROM TUKEY'S HSD PROCEDURE FOR *A POSTERIORI* MEANS COMPARISON.

Species	Propagule Type	Phenolic Acid Content ($\mu M\ g\ dw^{-1}$)	Phenolic Acid Content ($\mu M\ g\ N^{-1}$)	Phenolic Acid Content ($\mu M\ g\ C^{-1}$)	C (%)	N (%)
Potamogeton gramineus	Winter Bud	53.1 ± 7.3 A	2175 ± 341 AB	126.7 ± 17.3 A	41.9 ± 0.3 B	2.5 ± 0.1 B
Potamogeton nodosus	Winter Bud	37.7 ± 3.1 AB	2563 ± 243 A	87.9 ± 7.3 B	43.0 ± 0.2 A	1.5 ± 0.1 CD
Potamogeton pectinatus	Tuber	32.3 ± 5.0 BC	1072 ± 165 CD	77.2 ± 11.9 BC	41.8 ± 0.3 B	3.1 ± 0.2 A
Hydrilla verticillata (d)	Tuber	20.4 ± 1.5 CD	1591 ± 146 BC	50.9 ± 3.9 BCD	40.1 ± 0.2 C	1.3 ± 0.1 D
Hydrilla verticillata (d)	Turion	20.2 ± 2.2 CD	1100 ± 132 CD	48.8 ± 5.5 CD	41.5 ± 0.4 B	1.9 ± 0.1 C
Hydrilla verticillata (m)	Tuber	6.9 ± 0.9 D	532 ± 67 D	16.8 ± 2.1 D	41.0 ± 0.2 BC	1.3 ± 0.0 D
Hydrilla verticillata (m)	Turion	7.6 ± 0.8 D	470 ± 60 D	18.3 ± 1.9 D	41.6 ± 0.2 B	1.7 ± 0.1 CD

Table 2. Linear regression equations relating phenolic acid content (μM g DW^{-1}) to nitrogen content (N, %) for rooted aquatic plant vegetative propagules. The 'd' in Parenthesis indicates dioecious plants and the 'm' denotes monoecious plants.

Species	Equation	Significance of Overall Equation	R^2
Potamogeton nodosus	Phenol = 69.3 - 20.9 (N)	0.18	0.21
Potamogeton gramineus	Phenol = 114.3 - 24.4 (N)	0.34	0.11
Potamogeton pectinatus	Phenol = 19.8 - 4.0 (N)	0.69	0.02
Hydrilla verticillata tuber (d)	Phenol = 32.6 - 9.3 (N)	0.36	0.11
Hydrilla verticillata turion (d)	Phenol = 12.5 + 4.1 (N)	0.53	0.05
Hydrilla verticillata tuber (m)	Phenol = 1.7 + 4.0 (N)	0.75	0.01
Hydrilla verticillata turions (m)	Phenol = 10 - 1.4 (N)	0.47	0.07
All Groups	Phenol = 4.5 + 11.1 (N)	0.001	0.16

propagules are listed in Table 2. Although the slope was negative for five of seven groups, in no case was there a significant negative relationship between phenolic acid content and nitrogen content. When all data were pooled and the linear regression recalculated, there was a significant positive relationship between phenolic acid content and propagule nitrogen content. This implies that the mechanisms regulating phenol and nitrogen content for perennating structures may operate differently than those for leaves. Because of the important role played by perennating structures plants may preferentially allocate nitrogen to them. Unpublished data from *P. gramineus* and *P. pectinatus* grown in sediments of differing nitrogen fertility showed that mean propagule nitrogen content was unaffected by sediment nitrogen fertility. The preferential allocation of nitrogen to propagules may then enhance nitrogen limitation for shoots, thus favoring the accumulation of phenolic compounds.

Kremer (1993) suggested that it may be possible to use microorganisms to deplete seed banks for terrestrial weeds. Phenolic acids are important defenses against invasion by bacteria and fungi (Harborne 1993, Ebukanson 1989) and information on phenolic content of aquatic plant propagules may identify species amenable to this management practice.

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