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Nitrogen and Carbon Concentrations, Soluble Proteins and Free Amino Acids in Subterranean Turions of Hydrilla during Overwintering

FREDERICK J. RYAN[1]

ABSTRACT

The forms of nitrogen in subterranean turions of both biotypes of hydrilla found in the U. S. were quantified monthly from December 1989 to April 1990. Mean concentrations of free amino acids in subterranean turions ranged from 33.5 to 83.7 and 42.3 to 58.4 nmoles (mg dry weight)$^{-1}$ for the monoecious and dioecious biotypes, respectively. Concentrations of soluble proteins in subterranean turions ranged from 19.9 to 28.3 and 15.7 to 22.7 µg (mg dry weight)$^{-1}$ for turions of the monoecious and dioecious biotype, respectively. There were no significant linear time-dependent changes in the concentrations of free amino acids or soluble proteins during the overwintering period. Asparagine, alanine and arginine were major free amino acids in turions of both biotypes in October and April. Carbon concentrations decreased from 42 to 39% between December and April for turions of either biotype, while nitrogen concentrations fell from 1.41% to 1.21% for monoecious turions, with an approximately equivalent change in nitrogen composition for dioecious turions.

Key words: *Hydrilla verticillata*, nitrogen composition, storage organs.

INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) perennates by means of axillary and subterranean turions, the latter sometimes called tubers (Pieterse 1983). Two biotypes of hydrilla are presently known in the United States; dioecious female plants are in the southeast, Texas and California, while monoecious plants are in the Washington, D.C., area and in North Carolina. Other biotypes are known worldwide and can be distinguished by patterns of isoenzymes (Verkleij and Pieterse 1991). All strains produce large numbers of subterranean turions. For instance, Sutton et al. (1992) noted that monoecious plants grown from single turions produced a mean of 6046 and 4687 subterranean turions after 4 months, in experiments over two successive summers. Likewise, dioecious plants grown from single turions produced means of 3524 and 2126 subterranean turions during two successive winters. Turion production is promoted by decreasing photoperiod (Spencer and Anderson 1986), although in the case of the monoecious plant, evidence exists that plant size may also influence the timing of turion formation (Van 1989). In the experiment by Sutton et al. (1992) dioecious plants produced subterranean turions only during winter, while monoecious plants produced subterranean turions during winters and summers.

A number of studies have been concerned with aspects of turion formation, growth and nutrition (Steward 1984, Barko et al. 1988, Spencer et al. 1992); however, reports on the composition of subterranean turions are limited. Starch, sucrose, nitrogen and mineral content of subterranean turions of the dioecious plant have been determined from a single collection during a drawdown (Miller et al. 1976) and the carbon and nitrogen composition of axillary and subterranean turions of monoecious plants have been reported (Spencer and Ksander 1991). The major proteins of hydrilla turions of both biotypes have been characterized (Ryan 1988), although composition of all the nitrogenous components was not reported.

There is increasing recognition of the role of nitrogenous storage compounds in the overwintering process of many plants. Although these compounds comprise a relatively small proportion of plant tissue, the energetic requirements for nitrogen assimilation are considerable (Penning de Vries et al. 1974). Recent work has been concerned with the role of nitrogen compounds in the storage cycle of perennial and biennial plants (Coté, and Dawson 1986, Coté, et al. 1989, Cyr and Bewley 1989, 1990, Cyr et al. 1990, Granéli et al. 1992, Heilmeier et al. 1986, Hendershot and Volenec 1992, Suzuki and Kohno 1987, Wetzel et al. 1989).

The work reported here was undertaken to determine carbon and nitrogen composition of hydrilla turions during overwintering, to determine amounts of soluble proteins and free amino acids in turions, and to determine changes in these compounds during the overwintering period. This is part of a continuing study on the role of nitrogenous compounds in perennation and sprouting in propagules of aquatic plants.

MATERIALS AND METHODS

Plants of the monoecious and dioecious biotypes were maintained outdoors at the USDA ARS Aquatic Weed Facility, Davis, CA, USA, in 1000 L vaults on a substrate of 10% peat and 90% sand plus nutrients (Spencer and Anderson

[1] USDA ARS Aquatic Weed Research Laboratory, Robbins Hall, University of California, Davis, CA 95616. Received for publication: April 25, 1994 and in revised form: June 10, 1994.

1986) in well-water. Subterranean turions used in this experiment were produced during the previous growing season. Ten subterranean turions of each biotype were taken from the hydrosoil at monthly intervals from December 1989 through April 1990. At the April sampling time, some of the monoecious turions had begun to sprout; for these analyses, only non-sprouting tubers were used for this last determination. Turions were weighed, finely cut, frozen in liquid nitrogen, lyophilized and stored at -80 C until analysis.

For analysis of soluble protein, 10 mg of lyophilized plant material was ground in a mortar and pestle in 1.3 ml of 0.1 M Tris Cl, pH 7.8. Debris was removed by centrifugation for 1 min at approximately 5000 x g. For each sample, protein was determined as the mean of two analyses on this supernatant solution. Protein was precipitated with trichloroacetic acid in the presence of deoxycholate (Peterson 1977) and quantified by the method of Lowry et al. (1951). For quantitative analysis of free amino acids, 3 mg of lyophilized tissue was suspended in 1 ml of 95% ethanol at room temperature. After 18 h, insoluble material was removed by a 1 min centrifugation at approximately 5000 x g, then 0.8 ml of the supernatant solution was brought to dryness under N_2. Total free amino acids in the residue were quantified with ninhydrin (Moore and Stein 1948) using leucine as a standard. For identification and quantification of free amino acids, three turions of each biotype were harvested in late October and in early April. Each turion was ground at 4 C in 1 ml of 95% ethanol in a chilled mortar and pestle. The mixture was centrifuged at 5500 x g for 20 min and the supernatant solutions were dried under N_2. Analysis was conducted at the Protein Structure Laboratory (University of California, Davis, CA, USA) by ion-exchange chromatography in a citrate buffer system, with quantification by post-column reaction with ninhydrin.

Analyses for carbon and nitrogen were conducted on a Perkin-Elmer Model 2400 CHN Analyzer, calibrated with acetanilide according to the manufacturer's specifications. Mention of a manufacturer's name by the USDA does not constitute a warranty or endorsement and implies no approval of the product to the exclusion of others that may be suitable. Data were analyzed by simple linear regression.

RESULTS

The dry weights of monoecious and dioecious turions harvested during the overwintering experiment are shown in Figure 1. There were no time-dependent changes in dry weights, when a linear regression was used. The percentages of carbon or nitrogen associated with turions of both biotypes decreased during the overwintering period (Figure 2A and 2B). The rate of carbon loss, estimated by regression analysis of the data, was 0.70% per month for both monoecious (r^2 = 0.86, p=0.022) and dioecious (r^2= 0.79, p=0.044) turions. The rates of loss of nitrogen were 0.05% (r^2 = 0.82, p=0.0345) and 0.07% (r^2 = 0.24, p=0.359) per month for monoecious and dioecious turions, respectively. There were no significant linear trends with time for the concentrations of soluble protein or free amino acids during this period (Figure 2C and 2D).

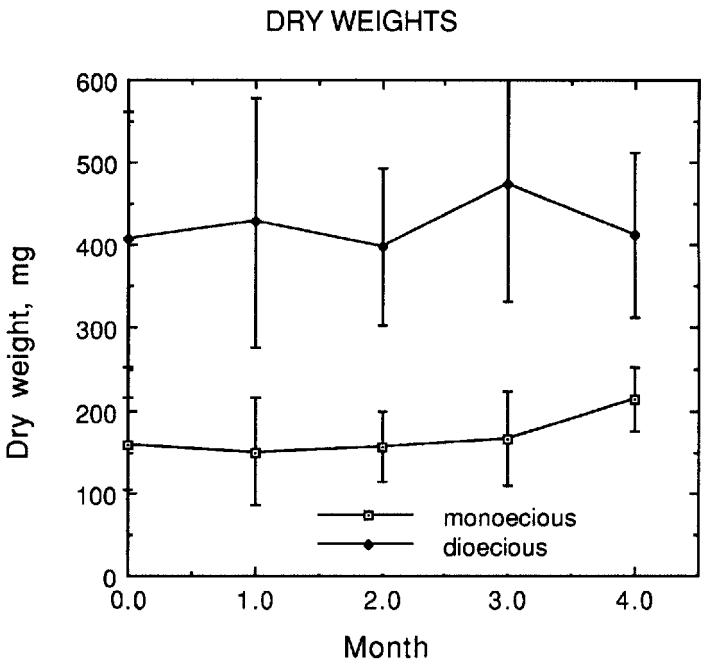

Figure 1. Dry weights of subterranean turions of monoecious and dioecious plants harvested during the course of the experiment. The means are indicated and the error bar is the standard deviation (N=10). Month 0 is December.

The principal free amino acids in ethanolic extracts of turions of both biotypes, prepared in either October or April, were asparagine, alanine and arginine (Table 1). In October, these three amino acids comprised 61.5 and 69.4 mole % of the total free amino acids of the monoecious and dioecious turions, respectively. In April, these amino acids comprised 41.5 and 60.1 mole % of the free amino acids of turions of the two respective biotypes. There were changes in relative amounts of amino acids; for instance, alanine decreased between October and April, while aspartate increased. Amino acids constituting less than 5 molar percentage of the sample are not shown in the Table.

DISCUSSION

There were no significant linear trends with time in the concentrations of free amino acids or soluble proteins in the turions of either biotype during this period. While it is possible that other models may fit the data, it is not possible to interpret the models biologically at this time. The present results contrast with those of Sagisaka (1987) and Suzuki and Ohno (1987), where substantial changes were noted in free amino acid concentrations in buds, bulbs, and other storage tissues of various plants during overwintering, even though these organs were exposed to freezing temperatures. Standard deviations in the present study were large and these may mask small changes in composition of the turions. Since only non-sprouting turions were analyzed, the results suggest that changes in the pools of soluble proteins and free amino acids might be closely linked to morphological changes apparent at the beginning of sprouting. This further suggests that sprouting itself is controlled by the environment of the

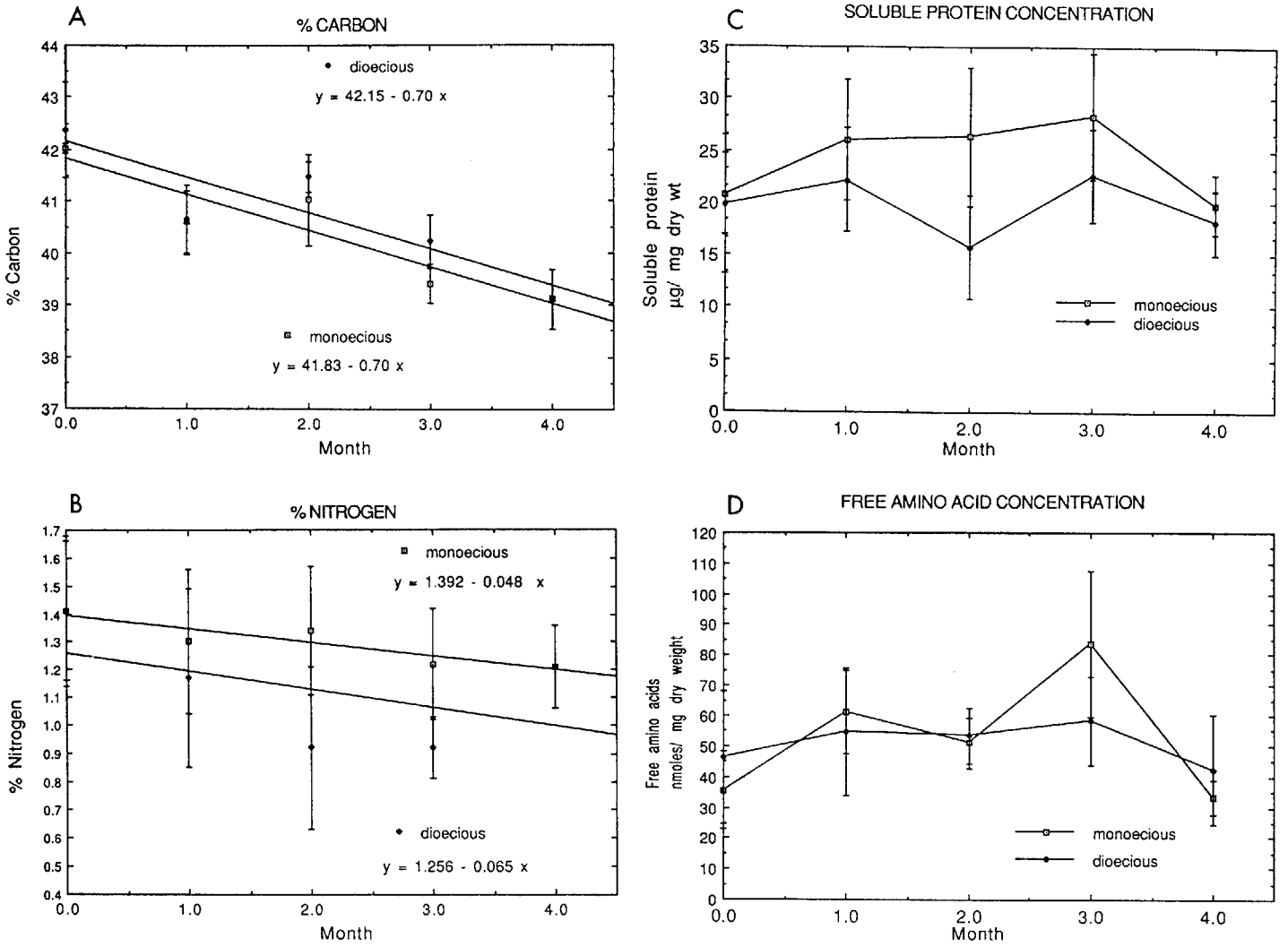

Figure 2. Percentage of carbon (A) and nitrogen (B), soluble proteins (C) and total free amino acids (D) for turions of monoecious and dioecious plants. The means are shown and standard deviations indicated by the error bars (N=10). Month 0 is December.

turion, with physiological changes occurring rapidly in response to favorable temperature or water status as has been shown for several species of pondweed (Madsen and Adams 1988, Spencer and Ksander 1992) and reviewed for other aquatic plants (Bartley and Spence 1987). Changes in free amino acid concentrations may occur subsequent to germination; this remains to be investigated.

The free amino acid pool comprises a substantial portion of the total nitrogen found in the turion. For instance, from the data in Table 1, it can be calculated that, for the monoecious turions in December, the free amino acid pool comprised 1.1 µg nitrogen (mg dry weight turion)$^{-1}$, a little more than 26% of the total nitrogen. The finding that asparagine and arginine are major components of the free amino acid

TABLE 1. MAJOR FREE AMINO ACIDS OF HYDRILLA TUBERS. VALUES ARE MOLE PERCENTAGES AND ARE THE MEANS ± STANDARD DEVIATIONS OF DETERMINATIONS ON THREE TURIONS OF EACH BIOTYPE. TOTAL AMINO ACIDS ARE CALCULATED FROM THE SUM OF ALL AMINO ACIDS DETECTED. UNITS FOR THE LATTER ARE µMOLES (MG DRY WEIGHT TUBER)$^{-1}$

Biotype	Month	Serine	Asparagine	Alanine	GABA[1]	Arginine	Aspartate
Monoecious							
	October	5.7 ± 3.2	34.1 ± 25.0	15.5 ± 8.7	5.8 ± 6.9	11.6 ± 2.1	2.0 ± 1.9
	April	6.2 ± 0.2	24.1 ± 4.5	8.1 ± 3.4	3.5 ± 0.4	9.3 ± 2.8	8.1 ± 1.8
Dioecious							
	October	2.32 ± 0.4	38.6 ± 7.3	19.3 ± 12.5	4.9 ± 6.0	11.5 ± 1.7	1.8 ± 0.4
	April	6.2 ± 1.7	30.3 ± 3.3	6.9 ± 0.7	1.5 ± 0.9	22.9 ± 0.2	10.0 ± 3.8

[1] GABA = gamma-aminobutyrate.

pool of hydrilla is in general agreement with studies on the amino acid composition of other plant storage organs. Sagisaka (1987) found that 20 of 40 plants studied accumulated arginine in storage organs, 23% accumulated arginine and proline, 8% glutamate and glutamine, 10% accumulated proline and 10% asparagine. Suzuki and Ohno (1987) found that, in mulberry (*Morus alba* L.), proline and arginine predominated in above ground storage tissue but asparagine and arginine were found in roots. Cyr et al. (1990) reported that aspartate, asparagine, glutmatate, glutamine, proline and arginine were the principal free amino acids in the roots of chicory (*Cichorum intybus* L.) and dandelion (*Taraxacum officinale* Weber) during the winter, and that asparagine underwent the largest change in concentration between summer and winter. Free amino acids, such as asparagine and arginine, may serve as nitrogen currency, and those that can exist in the amide form may detoxify ammonium ion produced during protein degradation (Cyr et al. 1990). Another role for them may be protection of cell membranes and proteins from environmental effects. Many of the plants examined by Sagisaka (1987) and the above ground storage tissue of mulberry (Suzuki and Ohno 1987) are tissues exposed to freezing and desiccation during winter. The free amino acids in hydrilla turions may protect these tissue from desiccation damage during the drawdown conditions they may sometimes experience (Spencer and Ksander 1992).

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