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The *Azolla-Anabaena azollae* Relationship. XIV. Chemical Composition of the Association and Soluble Carbohydrates of the Association, Endophyte-free *Azolla*, and the Freshly Isolated Endophyte

DRORA KAPLAN[1] and GERALD A. PETERS[2*]
[1]*Laboratory of Environmental and Applied Microbiology, The Jacob Blaustein Desert Research Institute, Ben Gurion University, Sede Boqer Campus, Israel 84990; and* [2]*Department of Biology, Virginia Commonwealth University, Richmond, VA 23284-2012 USA, Tel. +804-828-1562, Fax. + 804-828-0503, E-mail. gpeters@saturn.vcu.edu*

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Abstract

Grown under controlled conditions with dinitrogen as the sole N source, the *Azolla caroliniana-Anabaena azollae* association exhibited a doubling time of 2.3 +/–0.2 days. Dry matter content was 5.2 +/–0.2% of the fr. wt. and contained 41.2 +/–0.3% C and 5.2 +/–0.1% N. Other dry wt. analyses yielded about 32% crude protein (N X 6.25), 17% Lowry protein, 23% total sugars, 14% neutral sugars, and 9% minerals. The water soluble carbohydrate content of the association was determined and compared with that of endophyte-free *Azolla*. The total sugars/g FW of the endophyte-free plants grown with nitrate or ammonium as the sole nitrogen source were consistently significantly higher than the total sugars/g FW of the association grown on dinitrogen alone or on dinitrogen with nitrate. The water soluble carbohydrates obtained from each of these sources and from the endophyte isolated from the dinitrogen grown association were fractionated into three size groups roughly corresponding to large polysaccharrides, smaller polysaccharides and oligosaccharides other than disaccharrides, and di-/ monosaccharrides. Gas-liquid chromatography analysis

*The author to whom correspondence should be sent.

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revealed that the sugars in the individual fractions were qualitatively similar in all cases. In the examples shown, the association grown on dinitrogen alone contained 1 mg total sugars/g FW, the association grown on dinitrogen and nitrate 1.2 mg/g FW, the endophyte-free *Azolla* grown on nitrate 1.5 mg/g FW and the endophyte-free grown on ammonium 1.9 mg/g FW. The increase in the soluble sugar content of the endophyte-free plants appears to be at least in part attributable to an increase in the mono- and dissacharrides, especially sucrose. Relative to the association, this fraction from the endophyte-free *Azolla* accounted for a significantly greater proportion of the total sugars. This finding is consistent with a prior study (Kaplan and Peters, 1988) which revealed that the fern provides the endophyte with sucrose in the symbiotic association.

Keywords: Soluble carbohydrates, sucrose, *Azolla-Anabaena* symbiosis, nitrogen fixation, combined nitrogen

1. Introduction

Azolla is widely distributed in relatively placid tropical and temperate freshwater environments. All species of this free-floating, heterosporous, aquatic fern normally harbor a nitrogen-fixing heterocystous cyanobacterium as a symbiont (Peters and Meeks, 1989; Plazinski, 1990). Although the cyanobacterium of all *Azolla* species is historically referred to as *Anabaena azollae* Stras., it may well be a *Nostoc* rather than an *Anabaena* (Meeks et al., 1988; Peters and Meeks, 1989; Plazinski et al., 1990). Undifferentiated filaments of the cyanobacterium associated with the fern's shoot apices are partitioned into leaf cavities which form in the dorsal, aerial lobe of every deeply bilobed leaf (Calvert and Peters, 1981; Peters and Calvert, 1983). During the ontogenetic sequence of leaf development, the cyanobacterium rapidly differentiates heterocysts and concomitantly exhibits nitrogenase activity (Hill, 1977; Kaplan et al., 1986). As the cyanobacterium is capable of providing these associations with their total N requirement by the fixation of dinitrogen (Peters and Mayne, 1974 a, b; Peters et al., 1980, 1982), the associations are capable of prolific vegetative propagation in environments deficient in combined nitrogen. Moreover, the cyanobacterium retains appreciable nitrogenase activity under situations where combined nitrogen sources are available to, and assimilated by, the fern (Peters et al., 1981a; 1982). These attributes have been exploited in the use of these associations as a biofertilizer for rice. Recent reviews of the basic biology of these associations and of their use as a biofertilizer for rice include: Peters and Meeks (1989); Braun-Howland and Nierzwicki-Bauer (1990); Nierzwicki-Bauer (1990); and, Plazinski (1990).

In contrast to the numerous studies pertaining to N content of *Azolla-Anabaena* associations, there are relatively few dealing with the general composition of these associations, especially under defined growth conditions, and virtually none that have addressed their total and soluble carbohydrate content. Several observations pertaining to the carbohydrate content of these associations merit comment. First, the C/N ratio of the dinitrogen grown association has been found to be lower than that of endophyte-free plants (Peters et al., 1981b) but generally quite comparable to those obtained for the association grown on dinitrogen and a combined N source (Peters et al., 1981a, b; 1982). Second, while the freshly isolated endophyte was found to fix CO_2 at rates approximating those exhibited by free-living heterocystous cyanobacteria, sucrose was not a short term fixation product (Ray et al., 1979). Third, it was subsequently shown that sucrose nevertheless was a major constituent of the freshly isolated endophyte's soluble sugars (Peters et al., 1985). Finally, evidence was presented indicating that, in contrast to the freshly isolated endophyte, the endophyte within the leaf cavities fixes very little CO_2 and showing that in the symbiotic state labeled sucrose is transported from the fern to the endophyte (Kaplan and Peters, 1988). These observations led to the question of whether the soluble carbohydrate content, and especially the mono- and disacharride fraction, of endophyte-free plants might differ from those of the association grown on dinitrogen alone or on nitrate and dinitrogen. Here we provide: 1) a proximate analysis of the *Azolla caroliniana-Anabaena azollae* symbiosis grown with dinitrogen as the sole N source under defined growth conditions; and, 2) a direct comparison of the soluble carbohydrates of the association grown on dinitrogen alone and dinitrogen along with combined nitrogen (nitrate) with those of the endophyte isolated from the dinitrogen grown association and the endophyte-free *Azolla* grown on two different combined N sources, specifically nitrate and ammonium.

2. Materials and Methods

Growth conditions

Azolla caroliniana Willd., population wt-v-cf in the collection of G.A. Peters and CA3001 in the germplasm collection at the International Rice Research Institute, was grown under conditions previously shown to optimize biomass increase and N content (Peters et al., 1980) for the proximate analysis. For the soluble carbohydrate studies a 16/8 h, 26/19° light/dark cycle, was employed. A combination of cool-white fluorescent and incandescent lights provided a photosynthetic photon flux density of 200 μmoles/m^2/sec as measured with a Lambda L1-1905 quantum sensor. *Azolla* with the endophyte

was grown on an N-free liquid (IRRI) medium (Watanabe et al., 1977; Peters et al., 1980) and on this medium supplemented with 5 mM KNO_3. The endophyte-free *Azolla* was grown on the N-free liquid medium supplement with either 5 mM KNO_3 or 2.5 mM NH_4Cl buffered at pH 6 using 10 mM MES.

Proximate analysis

The dinitrogen grown association was harvested, blotted, and oven dried for 24 h at 60°C. Dried plant material was pulverized prior to CHN analysis and values for C and N are expressed as a percentage of the dry wt. Approximately 2.5 mg samples of the dried material, weighed on a Cahn Model G electrobalance, were analyzed using a Perkin-Elmer Model 240 Elemental Analyzer equipped with a Control Equipment Corporation MC-341-HA microinjector. Dinitrodurene was used as a standard. Elemental analysis of the dried material was carried out at the Ohio Agriculture Development Center (OARDC). Neutral sugars of these samples were determined as alditol acetate derivatives (Mort and Bauer, 1980; 1982) and total sugars, after hydrogen fluoride degradation, as the trimethylsiyl (TMS) methyl glycoside derivatives following the general procedure in Pierce Catalogue, Method 21. Extractable protein was estimated by the method of Lowry et al. (1951).

Carbohydrate extraction

The association and endophyte-free *Azolla* were extracted with boiling water (approximately 1 ml/g FW of plant material) for 10 min, briefly homogenized, passed through 4 layers of cheesecloth, and centrifuged in a clinical centrifuge to remove cellular debris. The endophyte was isolated from the association using the "gentle" procedure (Peters and Mayne, 1974a) and subjected to the same protocol. In an initial series of studies these supernatants were applied directly to an ion retardation column (Dowex AG 11A8). The desalted fractions found to contain carbohydrate were pooled and then passed through a Bio-Gel P-2 column basically as described below. Recovery of total carbohydrates was less than desired due to the retention of some of the higher molecular weight components in the extract on the ion retardation column. Therefore, in subsequent studies we carried out an initial fractionation prior to desalting. Following centrifugation in the clinical centrifuge, the supernatant was centrifuged at 80,000g for 1 h. The pellet from this high speed centrifugation was designated fraction a. The supernatant was passed through a 0.45 μ Millipore filter and then through an Amnicon PM-10 Diaflo membrane. The material retained on the Millipore filter was designated fraction b. Fractions a and b were subsequently combined and termed fraction 1

(F1). The material retained on the PM-10 membrane was termed fraction 2 (F2). The PM-10 filtrate, designated fraction 3 (F3), was desalted using a 100 cm × 2.5 cm ion retardation column (Dowex AG 11A8). Fractions of 3 ml were collected using an ISCO Model 1850 fraction collector and conductivity was monitored using a Radiometer Copenhagen type CDM 2d. F1 and F2 were resuspended in warm deionized water and, as with aliquots of F3, analyzed for total sugars by the phenol sulfuric acid method (Ashwell, 1966). Desalted carbohydrate containing samples of F3 were pooled, lyophilized, and redissolved in a small volume of deionized water.

For further characterization, resuspended F1, F2 and redissolved F3 were applied individually to a 100 cm × 1.4 cm Bio-Gel P-2 column. Samples were eluted with deionized water and collected fractions analyzed as above for carbohydrate/total sugars to obtain elution profiles. The Bio-Gel P-2 column had been calibrated using a mixture of Dextran-10, sucrose and glucose at 1 mg/ml each. The elution profile of those carbohydrates corresponding to the elution profile of the Dextran-10 were considered to be polysaccharides while those eluting between the Dextran-10 and sucrose were arbitrarily considered to be comprised of those oligosaccharrides larger than disaccharrides and of higher polymers smaller than polysaccharrides.

Analysis of carbohydrates in individual fractions

As noted, the total sugar in each fraction was quantitated using the phenol-sulfuric acid assay (Ashwell, 1966). Individual neutral sugars of the fractions were identified directly as either their alditol acetate derivatives (Mort and Bauer, 1980; 1982) or as their TMS-oxime derivatives using gas-liquid chromatography (GLC). The TMS-oxime method used here was a microscale modification of that described in the Pierce catalogue, method 21. One-tenth ml of a solution containing 25 mg hydroxylamine hydrochloride in 1.0 ml pyridine was added to dried samples in 1.0 ml Pierce reacti-vials. Samples were heated for 30 min at 70–75°C and cooled to room temperature before adding 1.0 ml hexamethyl dizalazane (HMDS) and 0.01 ml triflouroacetic acid (TFA).

An SE-30, 2 m glass column was used in conjunction with a temperature program of 25 min at 180°C followed by a linear increase of 10°C/min to 250°C and holding for 20 min. All derivatized samples were analyzed using a Hewlett-Packard Model 5830A GLC equipped with a 1885 QA Hewlett-Packard terminal.

Statistical analysis

One factor analysis of variance (ANOVA) was used to compare the soluble carbohydrate content of the endophyte-free plants with the association. Significant differences among treatment means were identified with Tukey test. All statistical procedures follow Sokal and Rohlf (1981). Means are presented +/– the standard deviation.

3. Results

Using optimized growth conditions (Peters et al., 1980), the *Azolla caroliniana -Anabaena azollae* association grown with dinitrogen as the sole N source exhibited a doubling time of 2.3 +/–0.2 days. Nitrogenase activity, as determined with the acetylene reduction assay, was 43.0 +/–7.6 nmoles C_2H_4/min/g fr wt. The dry matter was 5.2 +/–0.2 % of the fr wt and contained 41.2 +/–0.3% C, 5.6 +/–0.0% H and 5.2 +/–0.1% N. Crude protein (N X 6.25) accounted for about 32% and Lowry protein for 17% of the dry wt. Neutral sugars accounted for 13.5% and total sugars, determined after hydrogen fluoride degradation, for 23.5% of the dry wt. The contribution of individual sugars to the neutral and total sugars is shown in Table 1. The hydrogen fluoride treatment leads to the degradation of cell walls as evidenced by the increase in the glucose, galacturonic acid, glucuronic acid and mannose residues and indicates that wall components account for about 10% of the dry weight. Minerals accounted for about 9% of the dry wt. with the following distribution: K, S, P, Mg, Ca, Na, and Fe at 63.7, 12.0, 8.8, 2.9, 2.6, 0.9 and 0.3 mg/g, respectively; and, Zn, Mn, B, Cu and Al at 92.1, 66.9, 53.0, 32.7 and 15.0 μg/g, respectively. The C/N ratio of 7.9 for the samples analyzed here compares well with those reported previously (ranging from 7.6 to 8.8) for the dinitrogen grown association (Peters et al., 1980; 1981a, b; 1982). In comparison, C/N ratios for the endophyte-free plants grown under the same light intensity and temperature regime with ammonium as the N source generally fall in the range of 9.1–10.2 (Peters et al., 1981b; Peters unpublished observation).

Soluble carbohydrates

Boiling water extracts were obtained from: the association grown with dinitrogen as the only N source; the association grown on nitrate as well as dinitrogen; and, the endophyte-free *Azolla* grown on medium containing either nitrate or ammonium nitrogen. The total sugars/gfw of plant material were consistently higher in the endophyte-free plants than in the association (Fig. 1) and the difference between the two was statistically significant

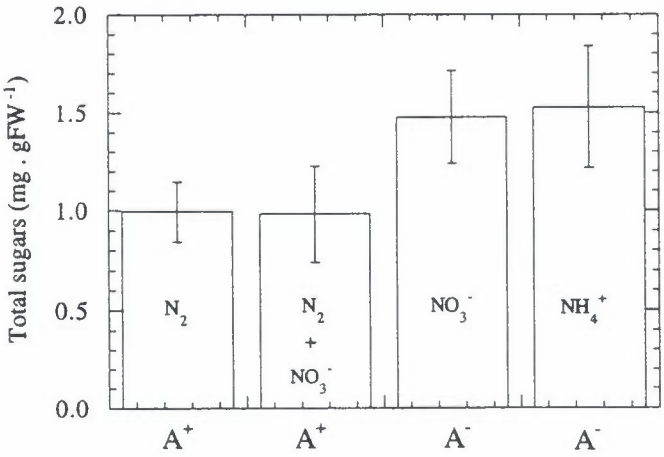

Figure 1. Comparison of the total soluble sugars obtained with boiling water extracts of the *Azolla-Anabaena* association (A^+) grown on dinitrogen alone and on dinitrogen with nitrate with those obtained for endophyte-free *Azolla* (A^-) grown on nitrate and on ammonium. Values are the mean +/− standard deviation. (n = 8 for each treatment of the association and n = 3 for each treatment of the endophyte-free *Azolla*).

Table 1. Neutral and total sugar content

	(% Dry weight)							
	A	B	C	D	E	F	G	H
Neutral sugars[1] (13.5%)	1.4	0.8	2.0	1.5	2.3	5.5	ND	ND
Total sugars[2] (23.6%)	0.9	1.2	1.8	2.1	2.1	10.6	0.9	4.0

A = Arabinose, B = Rhamnose, C = Xylose, D = Mannose, E = Galactose, F = Glucose, G = Glucuronic acid, H = Galacturonic acid. [1]As aditol acetate derivatives; [2]as TMS methyl glycosides derivatives after hydrogen fluoride degradation. ND: not detected.

(F = 7.89, p = 0.0014, ANOVA). The total sugars in one of the boiling water extracts from each of these four sources as well as from the endophyte isolated from the dinitrogen grown association, and the distribution of the sugars in the

Table 2. Total water soluble carbohydrates from the *Azolla-Anabaena* association, the *Anabaena*-free *Azolla*, and the freshly isolated *Anabaena*, and their characterization following fractionation into three major groups

	Association grown on dinitrogen		Association grown on dinitrogen and nitrate		Endophyte-free grown on nitrate		Endophyte-free grown on ammonium		Isolated endophyte	
Fresh wt (g)	11.5		11.6		11.5		11.5			
Total sugars (mg)	11.9	100%	13.4	100%	17.5	100%	21.6	100%	12.5	100%
mg sugar/gFW	1.0		1.2		1.5		1.9			
Fractionation sequence and distribution of sugars (mg)										
80,000 g supernatant	9.9	83%	10.7	80%	15.2	87%	19.2	89%	7.5	60%
Fraction a: 80,000g pellet	2.1	18%	2.6	19%	2.1	12%	2.4	11%	5.0	40%
Fraction b: 0.45 µ Millipore residue	1.2	10%	2.0	15%	2.8	16%	2.4	11%	2.1	17%
Fraction 1 (a+b):	3.3	28%	4.6	34%	4.9	28%	4.8	22%	7.1	57%
0.45 µ Millipore filtrate	8.6	72%	8.7	65%	12.4	71%	16.8	78%	5.4	43%
Fraction 2: Amnicon PM-10 residue	3.6	30%	3.4	25%	3.3	19%	6.0	28%	3.5	28%
Fraction 3: Amnicon PM-10 filtrate	5.0	42%	5.3	40%	9.1	52%	10.8	50%	1.9	15%

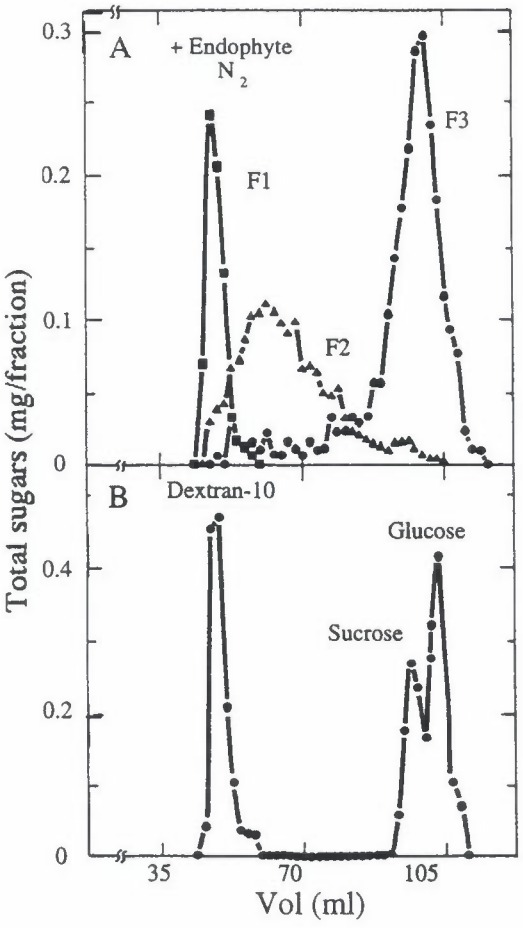

Figure 2. Comparison of representative elution profiles obtained for the three major groups (F1, F2, and F3) after fractionation of the total soluble carbohydrates in boiling water extracts from the dinitrogen grown *Azolla caroliniana* association (A) with those obtained for calibration standards (Dextran-10, sucrose and glucose, each at 1 mg/ml) (B) on a Bio-Gel P-2 column.

individual fractions attained by subsequent treatments, are presented in Table 2. Minor variation was noted in the absolute values obtained for total sugars and their distribution among the fractions, particularly in the distribution between F1 and F2, in the repetitions of this study. However, a greater proportion of the total sugars invariably occurred in F3 of the endophyte-free plants than in this fraction from the association. This was verified for the data shown for F3 in Table 2 ($X^2 = 4.04$; $p < 0.05$). The data shown in Table 2 are a valid representation of our total studies. A comparison of the elution profiles

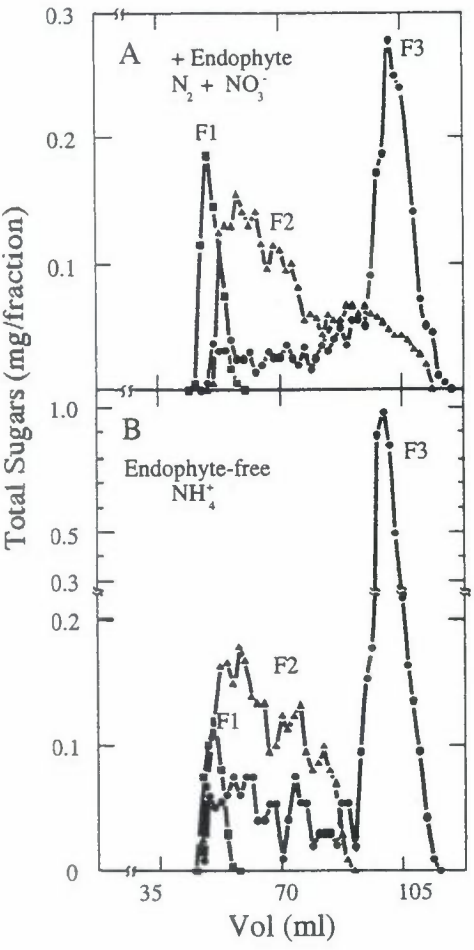

Figure 3. Representative Bi -Gel P2 column elution profiles obtained for the three major groups (F1, F2, and F3) after fractionation of the total soluble carbohydrates in boiling water extracts of the *Azolla caroliniana* association grown on nitrate and dinitrogen (A) and the endophyte-free *A. caroliniana* grown on medium containing ammonium (B).

obtained for F1, F2, and F3 from the dinitrogen grown association (Fig. 2A) with those obtained for calibration standards (Fig. 2B) on a Bio-Gel P-2 column indicates that F1 contains primarily larger polysaccharides and F3 the di- and monosaccharides. As F2 eluted between F1 and F3, it was assumed to contain primarily those oligosaccharides larger than sucrose as well as smaller

polysaccharrides. Due to the retention of fraction b on the 0.45 μ Millipore filter, F1 is assumed to more closely reflect the composition of the 80,000g supernatant (fraction a in Table 2) than it does that of the total. Bio-Gel P-2 column elution profiles for the three fractions (F1, F2 and F3) obtained with extracts of the association grown on medium containing nitrate in the experiment shown in Table 2, and those obtained for the endophyte-free *Azolla* grown on medium containing ammonium in one of the replications of the experiment shown in Table 2, are presented in Fig. 3A and 3B, respectively. In accord with the data shown in Table 2, the Bio-Gel P-2 column elution profiles of the fractions obtained from extracts of the endophyte-free plants grown on medium supplemented with nitrate (not shown) reflected the increase sugar content relative to the association, notably in F3, and generally were similar to those obtained for the endophyte-free plants grown on medium replete with ammonium. The endophyte accounts for about 20% of the dinitrogen grown association's protein (Ray et al., 1978) and necessarily contributes to the soluble carbohydrate fractions obtained from the association, regardless of whether it is grown on dinitrogen alone or in conjunction with a source of combined nitrogen. However, as the total sugars/g FW, and the proportion of mono- and disacharrides (F3), are consistently higher in the endophyte-free plants than in the association (Table 2), the endophyte must simultaneously contribute to the associations total sugars and result in less total sugar per unit of biomass. In order to gain some insight on the endophyte's contribution to the total soluble carbohydrates obtained in extracts of the association, the composition of the soluble carbohydrates extracted from the freshly isolated endophyte was determined. As shown in Table 2, 57% of the total sugars in the extracts of the endophyte occurred in the large polysaccharide fraction (F1). Moreover, from the data in Table 2 it is clear that whereas the endophyte makes a contribution to each fraction shown for the association, its most significant contribution, in regard to the distribution of total sugars, would be to fraction a. This is in fact strongly suggested by the data in Table 2 since fraction a of the association extracts accounted for a greater percentage of the total sugars than did fraction a in the extracts from endophyte-free plants.

Analysis of sugars in individual fractions

After elution from the Bio-Gel P-2 column, F1 and F2 from the dinitrogen grown association were hydrolyzed and partially characterized as their alditol acetate derivatives (Mort and Bauer, 1982). The composition of F1 (Fig. 4A) indicated a predominance of solubilized cell wall components while elevated content of glucose residues in F2 (Fig. 4B) suggested a major contribution from degradation products of storage carbohydrates present in

Azolla and in the endophyte. F3 from the dinitrogen-grown association, the association grown on dinitrogen and nitrate, the endophyte-free *Azolla* grown on combined N as nitrate or ammonium and the endophyte isolated from the dinitrogen grown association were analyzed following TMS-oxime derivatization. Chromatograms of F3 from the association grown on nitrate along with dinitrogen and from the endophyte-free *Azolla* grown on nitrate or ammonium were virtually indistinguishable from those previously presented for the dinitrogen grown association (Peters et al., 1985). In the association and the endophyte-free *Azolla*, sucrose accounted for 50–70% of the total sugars in F3. Fructose and glucose were the major monosaccharides and ribose and fucose were identified. As shown previously (Peters et al., 1988) sucrose was identified as a major constituent of F3 from the isolated endophyte. This finding has been addressed in a study strongly suggesting that the endophyte receives sucrose from the fern (Kaplan and Peters, 1988).

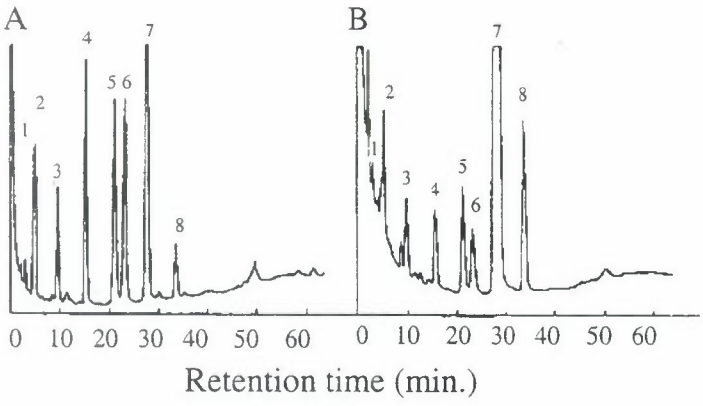

Figure 4. Gas-liquid chromatograms of the alditol-acetate derivatives after hydrolysis of F1 (A) and F2 (B) obtained from the dinitrogen grown *Azolla caroliniana* association. 1. Rhamnose, 2. Fucose, 3. Arabinose, 4. Xylose, 5. Mannose, 6. Galactose, 7. Glucose, 8. Inositol (internal standard).

4. Discussion

There was no marked difference in the content or distribution of carbohydrates in the boiling water extracts obtained from the dinitrogen grown association and those obtained from the association grown on dinitrogen and nitrate (Table 2, Fig. 2A and Fig. 3A). This was not unexpected as growth rates and C/N ratios are comparable for the association grown on dinitrogen alone or

with combined N as nitrate, ammonium or urea in the growth medium (Peters et al., 1981ab, 1982). Moreover, the endophyte retains appreciable nitrogenase activity after growth of the association on, and assimilation of, the combined N source (Peters et al., 1981a; 1982). In contrast, there was a marked difference between the content and distribution of these carbohydrates in the association, regardless of the N source, and that of the endophyte-free plants. The total sugars/gFW and the proportion of the total sugars present as mono- and disacharrides (F3) were consistently higher in the endophyte-free plants than in the association. Hence, although the endophyte necessarily contributes to total sugars of the association, the absence of the endophyte leads to a higher total sugar content. This suggests that in the association the endophyte may well be metabolizing sugars, especially the mono- and disacharrides, produced by the fern and/or that the carbon and nitrogen metabolism in the endophyte-free plants is altered relative to the association. The concept of the endophyte metabolizing sugars is consistent with studies showing that in the association the fern provides the endophyte with sucrose (Kaplan and Peters, 1988). Moreover, incoming sucrose has been shown to be the primary source of reductant in legume nodules (Streeter, 1991; 1995).

Carbon and nitrogen metabolism are linked in photosynthetic organisms. Both processes are dependent upon the organic carbon and energy from photosynthesis. It is to be expected, therefore, that under optimal growth conditions, the two processes will operate in concert. When the rate of nitrogen metabolism is reduced by nitrogen limitation or by a decrease in the amount of, and/or the activity of, the enzymes involved in nitrogen assimilation, an accumulation of carbon storage compounds occurs and there is a concomitant increase in the C/N ratio (Huppe and Turpin, 1994, and references therein). An increase in the C/N ratio also is observed in C-3 plants grown under elevated levels of carbon dioxide (Conroy, 1992 and references therein). The higher C/N of the endophyte-free plants relative to the association suggests that absence of the endophyte causes a shift in the balance between carbon and nitrogen metabolism. The endophyte-free plants grown with nitrate or ammonium as the nitrogen source do exhibit a slower growth rate (Peters et al., 1981b; Peters unpublished observations), a more compact growth habit and a greater root biomass (Peters, 1978) than the dinitrogen grown association. They do not display, however, any symptoms of nutrient deficiency/nitrogen stress and they contain increased concentrations of mono- and disaccharides. The latter suggests that: ammonium produced via the endophyte's fixation of dinitrogen in the leaf cavities may be coupled more efficiently with carbon metabolism than are exogenous sources of combined nitrogen; and, that it is the preferred N source in the association in the presence or absence of an exogenous combined N source. The fern's preference for ammonium produced by the

endophyte has been indicated in prior studies on the input from combined N sources along with that from the fixation of dinitrogen (Peters et al., 1981a).

As sucrose rather than starch is the end product of photosynthesis in rapidly growing plants, its occurrence in the association is consistent with both its growth rate and the fact that the endophyte in the leaf cavity appears to provide a strong sink for the sucrose synthesized by the fern. However, the occurrence of increased concentrations of mono- and disaccharides (especially sucrose) in the endophyte-free plants relative to the association was not expected. Given the slower growth rate of the endophyte-free plants and the absence of a comparable sink for the fern's photosynthesis one might well have expected an increase in the levels of storage carbohydrates and their degradation products (F1 and F2) rather than sucrose (F3). The reason(s) for this phenomenon are not clear. It was beyond the scope of the present study to address whether the absence of the endophyte caused an alteration of the levels or activities of specific enzymes involved in either the uptake and assimilation of exogenous nitrogen sources or those involved in carbon metabolism.

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